SPECIAL THANKS

EMPRESAS COLABORADORAS SEV

VIROLOGÍA

Publicación Oficial de la Sociedad Española de Virología

XIII CONGRESO NACIONAL DE VIROLOGÍA

Madrid 2015

Volumen 18
Número 1/2015
EXTRAORDINARIO

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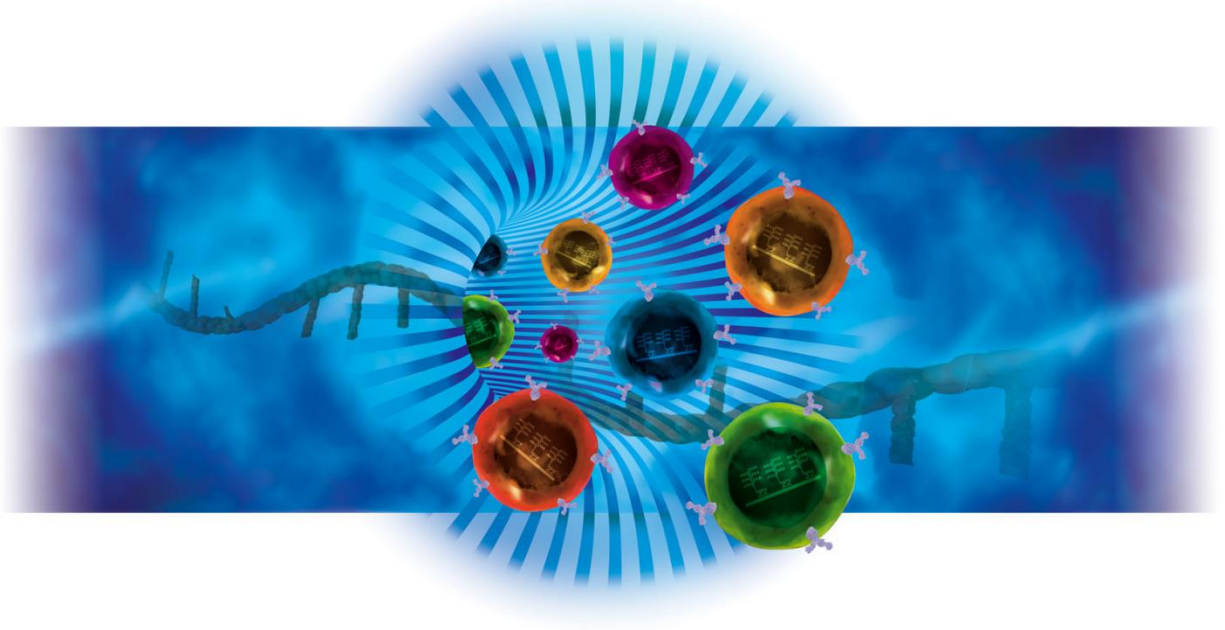
Detect RNA and protein in millions of single cells

Enter a new dimension of single-cell analysis



Detect RNA and protein simultaneously by flow cytometry to:

- See gene expression heterogeneity at the single-cell level
- Compare RNA and protein in the same cell
- Evaluate viral RNA within infected cells
- Detect non-coding RNA in cell subsets
- Analyze mRNA expression levels when antibody selection is limited

Real insight starts with single cells.
View webinar on demand "Detection of RNA with flow cytometry"
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VIROLOGÍA . PUBLICACIÓN OFICIAL DE LA SOCIEDAD ESPAÑOLA DE VIROLOGÍA

13th Spanish National Congress Of Virology

Madrid 2015

XIII CONGRESO NACIONAL DE VIROLOGÍA

Del 7 al 10 de junio de 2015

Auditorio de la Fábrica Nacional de Moneda y Timbre

Real Casa de la Moneda de Madrid

Madrid 2015

Volumen 18
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SEV – Sociedad Española de Virología
Centro de Biología Molecular "Severo Ochoa"
C/ Nicolás Cabrera, 1
28049 Cantoblanco – Madrid
web@sevirologia.es

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13th Spanish National Congress Of Virology

Madrid 2015

XIII CONGRESO NACIONAL DE

VIROLOGÍA

B I E N V E N I D A

El Comité Organizador del XIII Congreso Nacional de Virología (XIII CNV) tiene el placer de comunicaros que éste se celebrará en Madrid, del 7 al 10 de junio de 2015. En el XIII CNV participan conjuntamente la Sociedad Española de Virología (SEV) y la Sociedad Italiana de Virología (SIV), y estará abierto a la participación de virólogos de Latinoamérica. La SEV, la SIV y el Comité Organizador os da la bienvenida.

El Acto Inaugural del Congreso se celebrará en el Ateneo de Madrid el día 7 de Junio y la sede del Congreso será el Ayre Gran Hotel Colón de Madrid y la Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda en las Sesiones de los días 8 al 10 de Junio. El Ayre Gran Hotel Colón y la Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda se ubican junto al famoso Parque del Retiro y el Palacio de Deportes.

Los miembros del Comité Organizador y Científico hemos diseñado un Programa Científico atractivo, contando con la presencia de virólogos españoles y extranjeros de reconocido prestigio, cubriendo los diversos campos de la virología, básicos, aplicados, traslacional y clínicos en sus vertientes humana, animal y vegetal. También se muestra la interacción de la virología con otras áreas de la biología, la medicina, la nanotecnología, la informática, la química o la física. El programa científico ha buscado que estén representadas las distintas áreas de la virología y os sintáis identificados, de tal forma que participéis de forma activa y enviéis comunicaciones. Todos los asistentes podremos discutir sobre los avances, logros y retos en el estudio de los virus, para generar nuevas ideas y promover la investigación entre la comunidad de virólogos, en un Congreso que esperamos sea fructífero y distendido.

Para que los jóvenes tengan una mayor presencia, se ha destinado una Sesión a presentaciones rápidas, no mas de tres minutos, las denominadas "flash presentations" en las que en breve tiempo, como si de un anuncio se tratara, deberéis defender y difundir el gran trabajo que estáis desarrollando.

Agradecemos a los miembros del Comité Organizador y del Comité Científico del XIII Congreso Nacional de Virología su esfuerzo e ilusión en la preparación de este importante Evento, especialmente al personal del Hospital General Universitario Gregorio Marañón y de otras instituciones de Madrid por su ayuda con los temas locales. Hacemos extensivo nuestro agradecimiento al Presidente y a los miembros de la Junta Directiva de la Sociedad Española de Virología por el apoyo que nos han prestado desde el inicio de los preparativos.

También agradecemos a todas las instituciones participantes y empresas colaboradoras en este XIII Congreso Nacional de la Sociedad Española de Virología como patrocinadores, ya que sin su apoyo hubiera sido imposible realizar este Evento tan importante para la virología.

Finalmente, Madrid ofrece los atractivos turísticos de una moderna capital europea. Por lo tanto, sería una gran satisfacción para nosotros si marcarais estas fechas en vuestras agendas.

Deseamos que todos disfrutéis de este Congreso.

Un cordial saludo en nombre del Comité Organizador,

Mª Angeles Muñoz-Fernández,

Presidenta del Comité Organizador del XIII CNV

XIII CONGRESO NACIONAL DE

VIROLOGÍA

13th Spanish National Congress Of Virology

Madrid 2015

WELCOME

The Organizing Committee of the 13th National Virology Congress (CNV) are pleased to announce that the Congress will be held from 7th to 10th June, 2015 at the Ateneo de Madrid where the Opening Session will take place. Whereas the Scientific Sessions from 8th to 10th June will be held at the Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda, the building of which is located next to the famous Retiro Park and Palacio de los Deportes. The 13th CNV will have great pleasure to host the Italian Society for Virology (SIV) together with the Spanish Society for Virology (SEV). The participation of virologists from Latin America will be especially welcom. The SEV, the SIV and the Organizing Committee hope that you will enjoy the Congress in Madrid and exploit the opportunity to interact with your colleagues.

The members of the Organizing and Scientific Committees have planned an attractive Scientific Programme with the presence of Spanish and foreign virologists of recognized prestige that will cover various fields of basic, applied, translational and clinical aspects of human, veterinary and plant virology. The programme will also cover the fruitful interactions of virology with other areas of biology, medicine, nanotechnology, computing, chemistry and physics. We hope that basic and clinical researchers working in any branch of virology will be interested in submitting abstracts and actively participating in this Congress. All together, we will be able to discuss current progress, achievements and challenges in the study of viruses with the aim of generating new ideas and promoting research among the community of virologists, in a Congress that we hope will be both a fruitful and relaxed. To make the participation for young researchers in the scientific sessions easy, short (3 minutes) "flash-presentation" sessions have been scheduled in the Scientific Programme.

We would like to thank the Scientific and Organizing Committees of this 13th National Congress of Virology for the enthusiasm and effort in the preparation of this important event. We are especially grateful to the staff of the Hospital General Universitario Gregorio Marañón and other institutions of Madrid for their help with the local issues. We extend our thanks to the President and the Executive Board of the Spanish Society of Virology for the support to this Congress. Moreover, we gratefully acknowledge the sponsorship of several institutions and companies: the 13th CNV would have not been possible without their support.

Moreover, we believe that Madrid will offer you all the tourist attractions of a modern European capital. Therefore, it would be great pleasure for us if you could mark these dates in your agenda

With my best regards on behalf of the Organizing Committee,

Mª Angeles Muñoz-Fernández

Chairwoman of the Organizing Committee of the 13th CNV.

13th Spanish National Congress Of Virology
Madrid 2015

XIII CONGRESO NACIONAL DE
VIROLOGÍA

ABREVIATURAS

PS: SESIÓN PLENARIA *(Invited plenary lecture)*
P: CONFERENCIAS PLENARIAS
OP: PRESENTACIONES ORALES *(Invited paper)*
CO: PRESENTACIONES ORALES
PO: SESIÓN POSTERS

TOPICS

PS: PLENARY SESSION *(Invited plenary lecture)*
P: PLENARY LECTURE
OP: ORAL PRESENTATIONS *(Invited paper)*
CO: ORAL PRESENTATIONS
PO: POSTERS SESSION

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Eloisa Yuste, Hospital Clinic, Barcelona

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13th Spanish National Congress Of Virology

Madrid 2015

XIII CONGRESO NACIONAL DE

VIROLOGÍA

PROGRAMA / PROGRAMME

DAILY PROGRAMME - REDUCED VERSION

DAILY PROGRAMME - REDUCED VERSION

Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda

C/ del Doctor Esquerdo, 36, 28028 Madrid

ATENEO DE MADRID: Salón de Actos
C/Calle del Prado, 21, 28014 Madrid
Teléfono: 914 29 17 50

Sunday june 7, 2015

AUDITORIUM ATENEO DE MADRID

17:00-17:15	**WELCOME CEREMONY**
17:15-19:45	**Plenary Session I: Frontiers in virology**

Welcome Lectures

Chairpersons: JUAN ORTÍN - RICARDO FLORES

17:15-17:50	*RALF BARTENSCHLAGER
17:50-18:25	*ESTEBAN DOMINGO
18:25-19:00	*NOBUHIRO SUZUKI

The Virologist Conference Senior Award

19:10-19:45	*ANTONIO TENORIO

*Invited Speakers

20:00-22:00	**WELCOME COCKTAIL (Restaurant Ateneo de Madrid)**

Monday june 8, 2015

Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda

8:00-9:00 REGISTRATION

	AUDITORIUM REAL CASA DE LA MONEDA	WHITE ROOM	AUDIOVISUAL ROOM
9:00-10:00	**Plenary Session II:** EMERGING VIRUSES		
	Chairpersons: JAVIER BUESA - ANTONIO ALCAMÍ		
	9:00-9:30 *LUIS ENJUANES		
	9:30-10:00 *FRANCO RUGGERI		
10:00-11:00	**Oral Presentations**		
	Chairperson: RAFAEL DELGADO		
	10:00-10:15 *Mª PAZ SÁNCHEZ		
	10:15-10:30 *ANA NEGREDO		
10:30-11:00	COFFEE BREAK // VISIT POSTERS [GOYA ROOM - EXHIBITION AREA]		
11:00-12:20	**Plenary Session III:** HEPATITIS A, B AND C: BASIC, TRANSLATIONAL AND CLINICAL RESEARCH		
	Chairpersons: JOSEP QUER - PABLO GASTAMINZA		
	11:00-10:20 *ROSA M. PINTÓ		
	11:20-11:40 *MANUEL ROMERO		
	11:40-12:00 *FRANCISCO RODRÍGUEZ-FRÍAS		
	12:00-12:20 *SOFIA PEREZ DEL PULGAR		
12:30-14:00	LUNCH– [TRUSS MADRID]		
14:15-15:00	AUTHOR WORKSHOP: HOW TO GET PUBLISHED Sponsored by the Journal. *Virus Research* FRENSDORFF BRENRING AND ALINA HELSLOOT		
15:00-17:00	**Parallel Session I:** EMERGING VIRUSES AND VETERINARY	**Parallel Session II:** HIV	**Parallel Session III:** PLANT VIRUS
	Chairpersons: ANA M. DOMENECH - JAVIER ORTEGO	**Chairpersons:** MANUEL LEAL - JOSÉ ALCAMÍ	**Chairpersons:** JESÚS NAVAS - VICENTE PALLÁS
	15:00-15:15 MIGUEL ANGEL JIMÉNEZ-CLAVERO	15:00-15:15 MARJORIE PION	15:00-15:15 VERÓNICA TRUNIGER
	15:15-15:30 COVADONGA ALONSO	15:15-15:30 MAURO DI PILATO	15:15-15:30 ARES MINGOT
	15:30-15:45 ANA BELÉN BLAZQUEZ	15:30-15:45 ESTHER BALLANA	15:30-15:45 MIKEL VALLE
	15:45-16:00 CARLOS CASTAÑO-RODRIGUEZ	15:45-16:00 BEATRIZ PACHECO	15:45-16:00 SUSANA RUIZ-RUIZ
	16:00-16:15 LETICIA FRANCO	16:00-16:15 CRISTINA LORCA-ORÓ	16:00-16:15 ELVIRA FIALLO-OLIVÉ
	16-15-16:30 ELENA PASCUAL	16-15-16:30 JOSE LUIS JIMENEZ	16-15-16:30
	16:30-16:45 LILIANNE GANGES	16:30-16:45 JUAN GARCÍA-ARRIAZA	16:30-16:45 INMACULADA FERRIOL
	16:45-17:00 NATALIA BARREIRO PIÑEIRO	16:45-17:00 YOLANDA M. PACHECO	16:45-17:00 VIJI VIJAYAN
17.00-17:30	COFFEE BREAK // VISIT POSTERS [GOYA ROOM - EXHIBITION AREA]		
17:30-19:30	**Parallel Session IV:** ANIMAL VACCINES	**Parallel Session V:** CELL-VIRUS INTERACTION	**Parallel Session VI:** ROLE OF VIRUS IN PEDIATRIC DISEASES (SEV-SEIP) [Joint Session SEV-SEIP]
	Chairpersons: FERNANDO RODRIGUEZ – ALEJANDRO BRUN	**Chairpersons:** COVADONGA ALONSO - ENRIQUE VILLAR	**Chairpersons:** M. ISABEL GONZÁLEZ - MARISA NAVARRO
	17:30-17:45 ALEJANDRO MARÍN-LÓPEZ	17:30-17:45 GRACIELA ALONSO	17:30-17:45 *LUIS PRIETO
	17:45-18:00 MANUEL DURAN FERRER	17:45-18:00 JORDI ARGILAGUET	17:45-18:00 *ROSA RODRIGUEZ-FERNÁNDEZ
	18:00-18:15 NADIA INGLESE	18:00-18:15 LUCÍA BARRADO GIL	18:00-18:15 *CRISTINA CALVO
	18:15-18:30 ANA MARIA FALCON	18:15-18:30 LAURA SANZ-SÁNCHEZ	18:15-18:30 *DANIEL BLAZQUEZ-GAMERO
	18:30-18:45 JOSÉ MIGUEL AVIA	18:30-18:45 DANIEL PÉREZ-NUÑEZ	
	18:45-19:00 EVA CALVO-PINILLA	18:45-19:00 JOSE MARÍA ALMENDRAL	
	19:00-19:15 PAULA LÓPEZ-MONTEAGUDO	19:00-19:15 PILAR GARCÍA-BRONCANO	
	19:15-19:30 JAVIER ORTEGO	19:15-19:30 PABLO RÍOS-MARCO	

*Invited Speakers

19:00-21:00 SEV GENERAL MEETING [BOARDROOM] // POSTERS SESSION

Tuesday june 9, 2015

Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda

	AUDITORIUM REAL CASA DE LA MONEDA	WHITE ROOM	AUDIOVISUAL ROOM
9:00-9:40	**Plenary Session IV:** INNATE IMMUNITY		
	Chairperson: MARIANO ESTEBAN		
	9:00-9:40 *VOLKER THIEL		
9:40-10:40	**Oral Presentations**		
	Chairperson: ANGEL CORBÍ – JAVIER MARTÍNEZ-PICADO		
	9:40-10:00 *JUAN JOSÉ BERLANGA		
	10:00-10:20 *MARIA TERESA COIRA		
	10:20-10:40 *EZEQUIEL RUIZ MATEOS		
10:40-11:15	COFFEE BREAK // POSTERS [GOYA ROOM - EXHIBITION AREA]		
11:15-12:45	**Plenary Session V:** STRUCTURAL ANALYSIS OF VIRUS AND BIOTECHNOLOGY		
	Chairpersons: JOSÉ ESTÉ - MARJORIE PION		
	11:15-11:45 *BEN BERKHOUT		
	11:45-12:15 *MARCO VIGNUZZI		
	12:15-12:45 *GIORGIO PALÚ		
12:45-13:30	**Oral Presentations**		
	Chairperson: FRANCISCO SOBRINO		
	12:45-13:00 *MARGARITA SALAS		
	13:00-13:15 *CARLOS BRIONES		
	13:15-13:30 *JOSÉ ÁNGEL MARTÍNEZ ESCRIBANO		
13:30-15:00	LUNCH – [TRUSS MADRID]		
15:00-17:00	**Parallel Session VII:** VIRAL ENTRY MECHANISMS	**Parallel Session VIII:** SPECIFIC IMMUNITY	**Parallel Session IX:** MICROBIOME AND HEALTH [Joint Session SEV-SEM]
	Chairpersons: JOSÉ A. MELERO - JOSÉ MARÍA ALMENDRAL	**Chairpersons:** MARGARITA DEL VAL - YOLANDA PACHECO	**Chairpersons:** ALBERT BOSCH AND ROSA DEL CAMPO
	15:00-15:15 COVADONGA ALONSO	15:00-15:15 JOSÉ ANTONIO MELERO	15:00-15:30 *ROSA DEL CAMPO
	15:15-15:30 RAFAEL CEÑA-DIEZ	15:15-15:30 SARA MARTIN DELGADO	15:30-16:00 *ESTHER JIMÉNEZ QUINTANA
	15:30-15:45 VIRGINA GONDAR	15:30-15:45 SILVIA LÓPEZ-ARGÜELLO	16:00-16:30 *ALBERTO LÓPEZ BUENO
	15:45-16:00 FLAVIA CARIDI	15:45-16:00 MARTA LOPEZ DE DIEGO	16:30-17:00 *TUIJA KEKARAINEN
	16:00-16:15 CRISTIAN SMERDOU	16:00-16:15 F. XAVIER LÓPEZ-LABRADOR	
	16:15-16:30 SUSANA GUIX	16:15-16:30 MAURO DI PILATO	
	16:30-16:45 FERNANDO MÉNDEZ	16:30-16:45 SILVIA GÓMEZ-SEBASTIAN	
	16:45-17:00 MONTSERRAT DE CASTELLARNAU	16:45-17:00 MARIA TERESA SÁNCHEZ-APARICIO	
17.00-17:30	COFFEE BREAK // VISIT POSTERS [GOYA ROOM - EXHIBITION AREA]		
17:30-19:30	**Parallel Session X:** TEACHING AND DISSEMINATION OF THE VIROLOGY	**Parallel Session XI:** ANTIVIRAL DRUGS	**Parallel Session XII:** REPLICATION MECHANISMS
	Chairpersons: ESPERANZA GÓMEZ-LUCÍA - JOSE A. LÓPEZ-GUERRERO	**Chairpersons:** JULIÁ BLANCO - RAFII MOHAMED	**Chairpersons:** AMELIA NIETO - LUIS MENÉNDEZ
	17:30-17:33 *JOSE ANTONIO LOPEZ-GUERRERO	17:30-17:45 AMELIA NIETO	17:30-17:45 CARMEN RIVAS
	17:33-17:41 *JAVIER MEDINA	17:45-18:00 GLORIA LOZANO	17:45-18:00 LAURA LERMA
	17:41-17:49 *MANUEL SEARA	18:00-18:15 DANIEL SEPÚLVEDA-CRESPO	18:00-18:15 BERNAT BLASCO-MORENO
	17:49-17:57 *ANA DOMÉNECH	18:15-18:30 PALOMA RODRÍGUEZ-RODRÍGUEZ	18:15-18:30 LILIANA CUBAS
	17:57-18:05 *RAFAEL AÑEZ	18:30-18:45 JOSÉ A. DEL CAMPO	18:30-18:45 GINÉS ÁVILA
	18:05-18:13 *MIGUEL ÁNGEL JIMÉNEZ-CLAVERO	18:45-19:00 ANA J PÉREZ-BERNÁ	18:45-19:00 ROCIO COLOMA
	18:13-18:21 *ESPERANZA GÓMEZ-LUCÍA	19:00-19:15 SANDRA FRANCO	19:00-19:15 JENNIFER S. JUNGFLEISCH
	18:21-18:30 *JOSE ANTONIO LOPEZ-GUERRERO	19:15-19:30 ESTER LÁZARO	19:15-19:30 JASMINA VASILIJEVIC
	18:30-19:00 DISCUSIÓN		
	19:00-19:30 GRAN CONCURSO DE EPIDEMIA VIRTUAL		
			*Invited Speakers
20:30	CONGRESS DINNER		

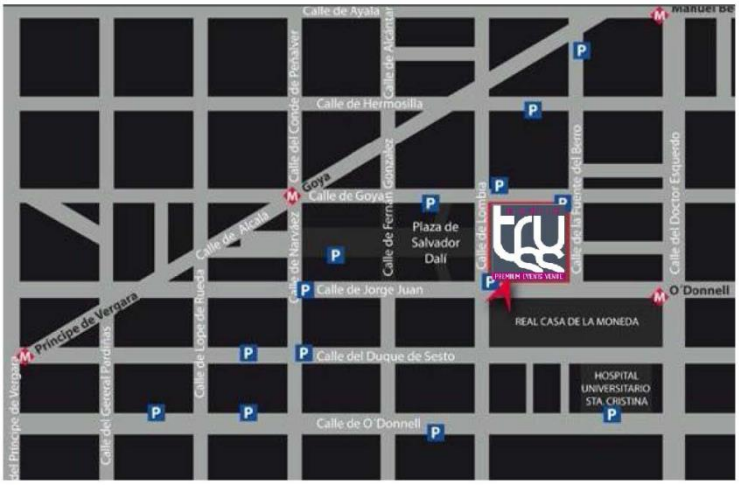
Wednesday june 10, 2015

Fábrica Nacional de Moneda y Timbre - Real Casa de la Moneda

	AUDITORIUM REAL CASA DE LA MONEDA	WHITE ROOM	AUDIOVISUAL ROOM
9:00-10:20	**Plenary Session VI: PLANT VIRUS**		
	Chairperson: JUAN ANTONIO GARCÍA		
	*Invited plenary lecture		
	9:00-9:40 NEW CONCEPTS IN THE BIOLOGY OF MULTIPARTITE VIRUSES *STÉPHANE BLANC *INRA, UMR BGPI, Montpellier, France*		
	9:40-10:20 MEMBRANE REARRANGEMENTS IN PLANT VIRUS RNA REPLICATION *LUISA RUBINO *Institute for Sustainable Plant Protection, CNR, Bari, Italy*		
10:20-11:30	**FLASH PRESENTATIONS**		
	Chairperson: SUSANA ALVAREZ AND JOSÉ LUIS JIMÉNEZ		
11:30-12:00	**COFFEE BREAK // VISIT POSTERS [GOYA ROOM - EXHIBITION AREA]**		
12:00-12:30	**THE VIROLOGIST CONFERENCE YOUNG AWARD**		
	Chairperson: PEDRO MAJANO		
	*Invited plenary lecture		
	TEN YEARS OF HEPATITIS C VIRUS CELL CULTURE INFECTION MODELS *PABLO GASTAMINZA *Centro Nacional de Biotecnologia-Consejo Superior de Investigaciones Cientificas (CNB-CSIC), Madrid*		
12:30-13:15	**CLOSING LECTURE**		
	Chairperson: MIGUEL ÁNGEL JIMÉNEZ-CLAVERO		
	*Invited plenary lecture		
	MECHANISMS OF VIRAL PERSISTENCE IN INSECTS *CARLA SALEH *Institut Pasteur, Viruses and RNA interference Unit, Paris, France*		
13:15-13:30	**CLOSING CEREMONY**		
	Chairperson ALBERT BOSCH AND MANUEL RODRÍGUEZ		
	13:15-13:30 AWARD GIVING CEREMONY		
	13:30-13:45 ANNOUNCEMENT OF THE UPCOMING "XIV CONGRESO NACIONAL DE VIROLOGÍA"		

CLOSING OF THE MEETING

LUNCH-MAP
TRUSS MADRID | PREMIUM EVENTS VENUE (PALACIO DE LOS DEPORTES)



13th Spanish National Congress Of Virology

Madrid 2015

XIII CONGRESO NACIONAL DE

VIROLOGÍA

PROGRAMA / PROGRAMME

SUNDAY JUNE 7, 2015

ATENEO DE MADRID: Salón de Actos
C/Calle del Prado, 21, 28014 Madrid
Teléfono: 914 29 17 50

17:00-17:15

WELCOME CEREMONY

17:15-19:45

Plenary Session (PL1): Frontiers in virology

Welcome Lectures — **Chairpersons: JUAN ORTÍN - RICARDO FLORES**

*Invited plenary lecture

17:15-17:50 **CELL BIOLOGY OF VIRAL REPLICATION CYCLES: A COMPARISON OF HEPATITIS C VIRUS AND DENGUE VIRUS**

***RALF BARTENSCHLAGER**

Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany
Division Virus-associated carcinogenesis, German Cancer Research Center, Heidelberg, Germany

*Invited plenary lecture

17:50-18:25 **VIRUS BEHAVIOR AT EXTREME FITNESS VALUES**

***ESTEBAN DOMINGO**

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid
Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona

18:25-19:00 *Invited plenary lecture

A NEW VIRUS LIFE STYLE: A CAPSIDLESS SSRNA VIROPHAGE HOSTED BY AN UNRELATED NOVEL DSRNA VIRUS

***NOBUHIRO SUZUKI**

Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama 710-0046, Japan
2NARO Institute of Fruit Tree Science, 92 Shimokuriyagawa, Morioka, Iwate 020-0123, Japan

THE VIROLOGIST CONFERENCE SENIOR AWARD

19:10-19:45 *Invited plenary lecture

FRONTIERS IN PUBLIC HEALTH MICROBIOLOGY: A CONTINUING CHALLENGE

***ANTONIO TENORIO**

Arbovirus and Imported Viral Diseases, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid

20:00-22:00 **WELCOME COCKTAIL (Restaurant Ateneo de Madrid)**

AUDITORIUM | WHITE ROOM | AUDIOVISUAL ROOM

08:00-09:00

RECEPTION CASA DE LA MONEDA

REGISTRATION

9:00-10:15

Plenary Session (PL2): Emerging Viruses

Chairperson: Javier Buesa - Antonio Alcamí

9:00-9:35 *Invited plenary lecture

CORONAVIRUS PATHOGENESIS AND PROTECTION

***Luis Enjuanes**

(CNB-CSIC), Madrid

9:35-10:15 *Invited plenary lecture

ZOONOTIC, FOODBORNE AND ENVIRONMENTAL TRANSMISSION IN ROTAVIRUS AND HEPATITIS E VIRUS INFECTION

***Franco Ruggeri**

Dept. of Veterinary public health & food safety, Istituto Superiore di Sanità, Rome, Italy

10:15-11:00

Oral Presentations (OP1)

Chairperson: Rafael Delgado

10:15-10:30 *Invited paper

THE ROLE OF THE NATIONAL REFERENCE LABORATORY REGARDING ARBOVIRUSES

***Mª Paz Sánchez**

Centro Nacional de Microbiología. Instituto de Salud "Carlos III", Madrid

10:30-10:45 *Invited paper

LABORATORY DIAGNOSIS OF ÉBOLA VIRUS DISEASE IN SPAIN. DIAGNOSTICS OF ÉBOLA VIRUS DISEASE SUSPETED CASES

***Ana Negredo**

Centro Nacional de Microbiología. Instituto de Salud "Carlos III", Madrid

10:30-11:00 VISIT POSTERS AND COFFEE BREAK [GOYA ROOM - EXHIBITION AREA]

11:00-12:20

Plenary Session III: Hepatitis A, B and C: Basic, Translational and Clinical Research

Chairperson: Josep Quer - Pablo Gastaminza

11:00-11:20 *Invited plenary lecture

HEPATITIS A VIRUS: NEW PARADIGMS OF AN OLD PATHOGEN

***Rosa M. Pintó**

University of Barcelona, Barcelona

11:20-11:40 *Invited plenary lecture

CONTINUUM OF CARE IN HEPATITIS C: FROM DETECTION TO CURE AND ERADICATION

***Manuel Romero**

Valme University Hospital, University of Seville,

AUDITORIUM | WHITE ROOM | AUDIOVISUAL ROOM

11:40-12:00 *Invited plenary lecture

HEPATITIS B AND D: WHEN EVERY SINGLE DETAIL HAS A HIDDEN MEANING

***Francisco Rodríguez-Frías**

Hospital Universitari Vall d'Hebron, Barcelona(VHIO), CIBERehd

12:00-12:20 *Invited plenary lecture

VIROLOGY OF HEPATITIS C VIRUS INFECTION AFTER LIVER TRANSPLANTATION

***Sofia Perez Del Pulgar**

Hospital Clínic, IDIBAPS, CIBERehd, Barcelona

12:30-14:00 LUNCH [Truss Madrid - c/Jorge Juan, 99]

14:15-15:00 *Invited paper

AUTHOR WORKSHOP: HOW TO GET PUBLISHED
Sponsored by the Journal.*Virus Research*

***Frensdorff Brenring and *Alina Helsloot**

ELS – HJ (ELS-AMS)

15:00-17:00
Parallel Session (PS1): Emerging Viruses And Veterinary

Chairpersons: Ana M. Doménech - Javier Ortego

15:00-15:15

2003-2015: 12 YEARS OF RESEARCH ON MOSQUITO-BORNE EPORNITIC FLAVIVIRUSES IN SPAIN. WEST NILE, USUTU, BAGAZA…AND BEYOND

Miguel Angel Jiménez-Clavero

INIA-CISA, Valdeolmos, Madrid

15:15-15:30

THE ANTIVIARL EFFECT OF INTERFERON INDUCED TRANSMEMBRANE PROTEINS (IFITMS) IN AFRICAN SWINE FEVER VIRUS INFECTION

Covadonga Alonso

I. N. de Investigación y Tecnología Agraria y Alimentaria, Madrid

15:30-15:45

AMINO ACID SUBSTITUTIONS IN THE NON-STRUCTURAL PROTEINS 4A OR 4B MODULATE THE INDUCTION OF AUTOPHAGY IN WEST NILE VIRUS INFECTED CELLS

Ana Belén Blazquez

Departamento de Biotecnología. INIA, Madrid

15:45-16:00

ROLE OF SARS-CoV VIROPORINS E, 3a AND 8a IN VIRUS REPLICATION AND VIRULENCE

Carlos Castaño-Rodriguez

Centro Nacional de Biotecnologia (CNB-CSIC), Madrid

16:00-16:15

VIROLOGICAL AND EPIDEMIOLOGICAL FEATURES OF CHIKUNGUNYA VIRUS INFECTION AMONGST TRAVELERS RETURNING TO SPAIN, 2008-2014

Leticia Franco

Instituto de Salud Carlos III, Madrid

16-15-16:30

HEMAGLUTININ PROTEIN OF PESTE DES PETITS RUMINANTS VIRUS ACTIVATES THE INNATE IMMUNE RESPONSE VIA TOLL-LIKE RECEPTOR 2 SIGNALING

Elena Pascual

INIA-CISA, Valdeolmos, Madrid

15:30-17:00
Parallel Session PS2): HIV

Chairpersons: Manuel Leal - José Alcamí

15:00-15:15

STUDY OF THE PROCESSIGN-BODIES (P-BODIES) ROLE IN THE RESTRICTION AGAINST HIV-1 IN PRIMARY HUMAN T CELLS

Marjorie Pion

H. G. U. Gregorio Marañón, Madrid

15:15-15:30

NEUTROPHIL MIGRATION VIA NFκB ACTIVATION BY MODIFIED VACCINIA VIRUS AS A NOVEL MECHANISM TO ENHANCE HIV-SPECIFIC T CELL RESPONSES

Mauro Di Pilato

Centro Nacional de Biotecnología (CNB-CSIC), Madrid

15:30-15:45

IDENTIFICATION AND CHARACTERIZATION OF THE MOLECULAR PATHWAY LEADING TO SAMHD1-MEDIATED VIRAL RESTRICTION

Esther Ballana

IrsiCaixa, Barcelona

15:45-16:00

INTRACELLULAR FACTORS BLOCKING EARLY STEPS OF THE HIV-1 REPLICATIVE CYCLE IN COMMON MARMOSET LYMPHOCYTES

Beatriz Pacheco

Centro de Biologia Molecular Severo Ochoa. Madrid

16:00-16:15

CHARACTERIZATION AND INHIBITION OF THE HIV-1 NUCLEOCAPSID MATURATION-CONDENSATION STEP

Cristina Lorca-Oró

IDIBAPS, Barcelona

16-15-16:30

PREVENTION OF HIV-1 VAGINAL TRANSMISSION AND MODE OF ANTIVIRAL ACTION BY TOPICAL POLYANIONIC CARBOSILANE DENDRIMER G2-S16 IN HUMANIZED BLT MICE

Jose Luis Jiménez

H.G.U. Gregorio Marañón, Madrid

15:30-17:00
Parallel Session (PS3): Plant Virus

Chairpersons: Jesús Navas - Vicente Pallás

15:00-15:15

STRUCTURAL AND FUNCTIONAL DIVERSITY OF PLANT VIRUS 3´-CAP-INDEPENDENT TRANSLATIONAL ENHANCERS

Verónica Truniger

CEBAS-CSIC, Murcia

15:15-15:30

EXPRESSION AND FUNCTION OF THE TRANS-FRAME P1N-PISPO GENE PRODUCT OF THE POTYVIRUS SWEET POTATO FEATHERY MOTTLE VIRUS (SPFMV)

Ares Mingot

Centro de Investigación Agrigenómica (CRAG), Barcelona

15:30-15:45

STRUCTURE OF FLEXIBLE FILAMENTOUS PEPINO MOSAIC VIRUS BY HIGH RESOLUTION CRYOEM

Mikel Valle

Structural Biology Unit, CIC bioGUNE, Derio, Vizcaya

15:45-16:00

GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE IS CO-OPTED FOR REPLICATION OF CITRUS TRISTEZA VIRUS VIA INTERACTION WITH THE VIRAL-ENCODED PROTEIN p23

Susana Ruiz-Ruiz

IBMCP (UPV-CSIC), Valencia

16:00-16:15

BIOLOGICAL CHARACTERIZATION OF NON-CODING DNA SATELLITES ASSOCIATED TO NEW WORLD BEGOMOVIRUSES

Elvira Fiallo-Olivé

Universidad de Málaga - Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Málaga

AUDITORIUM

16:30-16:45

POSTNATAL PERSISTENT INFECTION WITH CLASSICAL SWINE FEVER VIRUS IN DOMESTIC PIGS AND WILD BOARS: OPENING PANDORA'S BOX

Lilianne Ganges

(CReSA)-(IRTA), Campus de la UAB, Barcelona

16:45-17:00

GETTING IC-TAGGING TO WORK INTO THE ENDOPLASMIC RETICULUM

Natalia Barreiro Piñeiro

Centro Singular de Investigación en Química Biológica y Materiales Moleculares, Santiago de Compostela

WHITE ROOM

16:30-16:45

HEAD-TO-HEAD COMPARISON OF POXVIRUS NYVAC AND ALVAC VECTORS EXPRESSING IDENTICAL HIV-1 CLADE C IMMUNOGENS IN PRIME/BOOST COMBINATION WITH ENV PROTEIN IN NON-HUMAN PRIMATES

Juan García-Arriaza

Centro Nacional de Biotecnología (CSIC), Madrid

16:45-17:00

IMMUNOVIROLOGICAL TRAITS OF HIV SUBJECTS WITH DELAYED INITIATION OF CART AND SUBSEQUENT POOR CD4 RESTORATION. STUDY ON PRE-TREATMENT SAMPLES

Yolanda Pacheco

Hospital Virgen del Rocío, Sevilla

AUDIOVISUALROOM

16:30-16:45

UNRAVELLING THE RNA2-ENCODED POLYPROTEIN CLEAVAGE SITES OF TOMATO-INFECTING TORRADOVIRUSES USING N-TERMINAL PROTEIN SEQUENCING AND A REVERSE GENETICS SYSTEM

Inmaculada Ferriol

University of California Davis –USA

16:45-17:00

ANALYSIS OF TOLERANCE MECHANISMS AND TRADE-OFFS IN PLANT-VIRUS INTERACTIONS

Viji Vijayan

Centro de Biotecnologia y Genomica de Plantas, Madrid

17:00-17:30 VISIT POSTERS AND COFFEE BREAK [GOYA ROOM - EXHIBITION AREA]

17:30-19:30 Parallel Session (PS4): Animal Vaccines

Chairpersons: **Fernando Rodriguez**, **Alejandro Brun**

17:30-17:45

A NOVEL STRATEGY FOR MULTISEROTYPE PROTECTION AGAINST BLUETONGE VIRUS USING MUNS-MI MICROSPHERES AND RECOMBINANT MVA EXPRESSING VP2, VP7 AND NS1 PROTEINS

Alejandro Marín-López

INIA-CISA, Valdeolmos, Madrid

17:45-18:00

TRIAL FOR CHECKING THE PROTECTIVE IMMUNITY OF A COMMERCIAL VACCINE AGAINST THE "NEW VARIANT" OF THE RABBIT HAEMORRHAGIC DISEASE VIRUS

Manuel Duran Ferrer

Laboratorio Central de Veterinaria (MAGRAMA), Madrid

18:00-18:15

SYLVATIC RABIES IN THE NORTH-EAST OF ITALY: MONITORING AND EVALUATION OF THE EFFECTIVENESS OF PROPHYLAXIS IN WORKERS AT RISK AND TRAVELERS

Nadia Inglese

Dept. Molecular Medicine, University of Padua

18:15-18:30

ROLE OF INFLUENZA VIRUS SMALL RNAs CONTROLLING PATHOGENICITY IN VIVO

Ana Maria Falcon

Centro Nacional de Biotecnología (CNB-CSIC), Madrid

18:30-18:45

BLOCKING OF TYPE I IFN PATHWAY BY PESTE DES PETITS RUMINANTS VIRUS (PPRV)

José Miguel Avia

Centro de Investigación en Sanidad Animal (CISA)-INIA, Madrid

INIA-CISA, Valdeolmos, Madrid

17:30-19:30 Parallel Session (PS5): Cell-Virus Interaction

Chairpersons: **Covadonga Alonso**, **Enrique Villar**

17:30-17:45

RNA-SEQ BASED TRANSCRIPTOME ANALYSIS OF THE INTERFERON HOST RESPONSE UPON VACCINIA VIRUS INFECTION

Graciela Alonso

Centro de Biología Molecular Severo Ochoa-CSIC, Madrid

17:45-18:00

A SYSTEM BIOLOGY APPROACH REVEALS GENES AND PATHWAYS INVOLVED IN T-CELL EXHAUSTION AT TISSUE LEVEL

Jordi Argilaguet

Universitat Pompeu Fabra, Barcelona

18:00-18:15

AFRICAN SWINE FEVER VIRUS REPLICATION IS AFFECTED BY THE INHIBITION OF THE PROTEASOME SYSTEM

Lucía Barrado Gil

Instituto Nacional de Investigacion y Tecnología Agraria y Alimentaria (INIA), Madrid

18:15-18:30

CORRELATIVE LIGHT AND ELECTRON MICROSCOPY TO STUDY VIRAL MORPHOGENESIS AND EGRESS

Laura Sanz-Sánchez

Centro Nacional de Biotecnologia CNB-CSIC, Madrid

18:30-18:45

CD2v INTERACTS WITH ADAPTOR PROTEIN AP-1 DURING AFRICAN SWINE FEVER INFECTION

Daniel Pérez-Núñez

Centro Biología Molecular Severo Ochoa (CBMSO), Madrid

17:30-19:30 Parallel Session (PS6): Role of Virus in Pediatric Diseases (SEV-SEIP) [Joint Session SEV-SEIP]

Chairpersons: **Mª Isabel González-Tomé**, **Marisa Navarro**

17:30-17:45 *Invited paper

IMPLICATIONS OF CONTROL OF VIRAL LOAD DURING PREGNANCY AND RISK OF HIV-1 MOTHER-TO-CHIELD TRANSMISSION

***Luis Prieto**

Hospital Universitario de Getafe, Madrid

17:45-18:00 *Invited paper

RSV BRONCHIOLITIS: CHALLEGES IN 2015

***Rosa Rodriguez-Fernández**

Hospital Materno-Infantil Gregorio Marañón, Madrid

18:00-18:15 *Invited paper

NEUROLOGICAL AND SYSTEMIC PARECHOVIRUS INFECTIONS IN CHILDREN

***Cristina Calvo**

Hospital Severo Ochoa, Leganés – Madrid

18:15-18:30 *Invited paper

CONGENITAL CYTOMEGALOVIRUS (cCMV) INFECTION: HOW, WHEN, WHERE

***Daniel Blazquez-Gamero**

Hospital 12 Octubre, Madrid

AUDITORIUM | WHITEROOM | AUDIOVISUAL ROOM

18:45-19:00

ADMINISTRATION OF ANTISERUM FROM MICE VACCINATED WITH MODIFIED VACCINIA ANKARA VIRUS EXPRESSING AFRICAN HORSE SICKNESS VIRUS (AHSV) VP2 PROTEIN CONFERS PROTECTION WHEN ADMINISTERED BEFORE OR AFTER CHALLENGE

Eva Calvo-Pinilla

The Pirbright Institute, Pirbright-United Kingdom

19:00-19:15

BA71ΔfX: A LIVE ATTENUATED VACCINE THAT CONFERS PROTECTION AGAINST HOMOLOGOUS AND HETEROLOGOUS AFRICAN SWINE FEVER VIRUSES

Paula López-Monteagudo

IRTA-CReSA, Barcelona

19:15-19:30

EXPERIMENTAL BLUETONGUE VIRUS 4 SUBUNIT VACCINE DELIVERED TO ANTIGEN PRESENTING CELLS

Javier Ortego

INIA-CISA, Valdeolmos, Madrid

18:45-19:00

THE MAMMALIAN CELL CYCLE REGULATES PARVOVIRUS NUCLEAR CAPSID ASSEMBLY

Jose María Almendral

Centro de Biología Molecular Severo Ochoa, Madrid

19:00-19:15

POLYANIONIC CARBOSILANE DENDRIMERS PREVENT VAGINAL/RECTAL HSV-2ENTRY*IN VIVO*

Pilar García-Broncano

Hospital General Universitario Gregorio Marañón, Madrid

19:15-19:30

RECRUITMENT OF HOST FACTORS BY THE CRE ELEMENT OF THE HEPATITIS C VIRUS

Pablo Ríos-Marco

Consejo Superior de Investigaciones Científicas, Madrid

19:00-21:00 SEV GENERAL MEETING [BOARDROOM] // POSTERS SESSION

AUDITORIUM | WHITE ROOM | AUDIOVISUAL ROOM

09:00-9:45

Plenary Session (PL4): Innate Immunity

Chairperson: **MARIANO ESTEBAN**

9:00-9:45 *Invited plenary lecture

TO SENSE OR NOT TO SENSE VIRAL RNA - ESSENTIALS OF CORONAVIRUS INNATE IMMUNE EVASION

***Volker Thiel**

University of Bern, Switzerland

9:45-10:40

Oral Presentations (OP2): Innate Immunity

Chairperson: **ANGEL CORBÍ**

JAVIER MARTÍNEZ-PICADO

9:45-10:00 *Invited paper

FUNCTIONAL INTERACTION BETWEEN THE EIF2A KINASE GCN2 AND THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

***Juan José Berlanga**

Centro de Biología Molecular Severo Ochoa, Madrid

10:00-10:20 *Invited paper

P56LCK AND PKC INHIBITORS PRESERVE SAMHD1 ANTIVIRAL FUNCTION, INTERFERING WITH HIV-1 REPLICATION

***Maria Teresa Coira**

Instituto de Salud "Carlos III", Madrid

10:20-10:40 *Invited paper

ROLE OF MONOCYTES AND PLASMACYTOID DENDRITIC CELLS IN THE CONTROL AND IMMUNOPATHOGENESIS OF HIV AND HCV INFECTION

***Ezequiel Ruiz Mateos**

Instituto de Biomedicina de Sevilla (IBiS)/Hospital Universitario Virgen del Rocío. Seville

10:40-11:15 **COFFEE BREAK // VISIT POSTERS [GOYA ROOM - EXHIBITION AREA]**

AUDITORIUM | WHITE ROOM | AUDIOVISUAL ROOM

11:15-12:45

Plenary Session (PL5): Structural Analysis Of Virus And Biotechnology

Chairpersons: **JOSÉ ESTÉ - MARJORIE PION**

11:15-11:45 *Invited plenary lecture

HIV-1 EVOLUTION: DRUG-RESISTANCE AND NUCLEOTIDE COMPOSITION

***Ben Berkhout**

Academic Medical Center of the University of Amsterdam-(CINIMA),Holland

11:45-12:15 *Invited plenary lecture

RNA VIRUS POPULATION DYNAMICS IN SEQUENCE SPACE AND FITNESS LANDSCAPES

***Marco Vignuzzi**

Institut Pasteur, Paris, France

12:15-12:45 *Invited plenary lecture

VIRUSES AS TOOLS FOR THERAPEUTIC INTERVENTIONS IN CANCER AND INFECTIOUS DISEASES

***Giorgio Palú**

Department of Molecular Medicine, University of Padova, Padova, Italy

12:45-13:30

Oral Presentations (OC3): Structural Analysis Of Virus And Biotechnology

Chairperson: **FRANCISCO SOBRINO**

12:45-13:00 *Invited paper

BACTERIOPHAGE Ø29: FROM MOLECULAR BIOLOGY TO BIOTECNOLOGY

***Margarita Salas**

Centro de Biología Molecular "Severo Ochoa" (CSIC–UAM) Madrid

13:00-13:15 *Invited paper

STRUCTURAL ANALYIS OF VIRAL AND VIROIDAL RNA BY ATOMIC FORCE MICROSCOPY

***Carlos Briones**

Department of Molecular Evolution, Centro de Astrobiología (CSIC-INTA), Madrid

13:15-13:30 *Invited paper

BREAKING THE BARRIERS TO INCREASE THE PRODUCTIVITY OF BACULOVIRUS-BASED TECHNOLOGIES

***José Ángel Martínez Escribano**

Departamento de Biotecnología, INIA, Madrid

13:30-15:00 **LUNCH** **[Truss Madrid - c/Jorge Juan, 99]**

AUDITORIUM

15:00-17:00
Parallel Session VII:
Viral Entry Mechanisms (PL7)

Chairpersons: JOSÉ A. MELERO, JOSÉ MARÍA ALMENDRAL

15:00-15:15

LIPID COMPONENTS IN AFRICAN SWINE FEVER VIRUS ENTRY AND REPLICATION

Covadonga Alonso

I. N. de Investigación y Tecnología Agraria y Alimentaria, Madrid

15:15-15:30

POLYANIONIC CARBOSILANE DENDRIMERS AS PROMISING MICROBICIDE CANDIDATES AGAINST HSV-2 INFECTION: BROAD-SPECTRUM ACTIVITY AND ACTION MECHANISM

Rafael Ceña-Diez

Hospital General Universitario Gregorio Marañón, Madrid.

15:30-15:45

ROLE OF CLATHRIN AND CLATHRIN ADAPTOR PROTEIN-1 IN HEPATITIS C VIRUS EGRESS

Virgina Gondar

Instituto de Investigación Sanitaria Hospital La Princesa, Madrid

15:45-16:00

THE pH STABILITY OF FOOT-AND-MOUTH DISEASE VIRUS PARTICLES IS MODULATED BY RESIDUES LOCATED AT THE PENTAMERIC INTERFACE AND IN THE N TERMINUS OF VP1

Flavia Caridi

Centro de Biología Molecular "Severo Ochoa" (CSIC), Madrid

16:00-16:15

ANALYSIS OF THE ORIGIN AND INFECTIVITY OF INFECTIOUS MICROVESICLES DERIVED FROM SEMLIKI FOREST VIRUS DEVOID OF CAPSID

Cristian Smerdou

FIMA, Zaragoza

16:15-16:30

TYPE I INTERFERON RESPONSE IS DELAYED IN HUMAN ASTROVIRUS INFECTED CELLS

Susana Guix

University of Barcelona, (INSA-UB), Barcelona, Spain

16:30-16:45

LA PROTEÍNA VP5 JUEGA UN PAPEL ESENCIAL EN LA DISEMINACIÓN DEL VIRUS DE LA BURSITIS INFECCIOSA

Fernando Méndez

Centro Nacional de Biotecnología (CSIC), Madrid

16:45-17:00

A MODEL FOR HEPATITIS A VIRUS TRANSCYTOSIS IN HEPATOCYTES

Montserrat De Castellarnau

Universidad de Barcelona, Barcelona

WHITE ROOM

15:00-17:00
Parallel Session VIII:
Specific Immunity (PL8)

Chairpersons: MARGARITA DEL VAL, YOLANDA PACHECO

15:00-15:15

THE STRUCTURALLY RELATED FUSION PROTEINS OF HUMAN RESPIRATORY SYNCYTIAL VIRUS AND METAPNEUMOVIRUS ARE ANTIGENICALLY AND IMMUNOGENICALLY DISSIMILAR

José Antonio Melero

Instituto de Salud Carlos III, Madrid

15:15-15:30

VIH-1 INDUCE A DEREGULATION IN B-CELL POPULTIONS THROUGH A PARTIAL REGULATORY B-CELL PHENOTYPE IN VITRO

Sara Martin Delgado

Hospital General Universitario Gregorio Marañón, Madrid

15:30-15:45

ENGINEERED THERMOSTABLE EMPTY CAPSIDS OF FMDV FOR IMPROVED VACCINES

Silvia López-Argüello

Centro de Biologia Molecular Severo Ochoa, Madrid

15:45-16:00

ROLE OF INTERFERON STIMULATED GENES IN INFLUENZA VIRUS PRODUCTION AND ANTIVIRAL SIGNALLING

Marta Lopez De Diego

University of Rochester, Rochester, NY, USA

16:00-16:15

EPIDEMIOLOGÍA MOLECULAR DE GRIPE A Y B EN ENFERMEDAD RESPIRATORIA GRAVE Y ESTADO VACUNAL

F. Xavier López-Labrador

(FISABIO-Salud Pública/Universtat de València), (CIBER-ESP), Instituto de Salud Carlos III, Madrid

16:15-16:30

MODIFICATION OF PROMOTER SPACER LENGTH IN VACCINIA VIRUS AS A STRATEGY TO CONTROL THE ANTIGEN EXPRESSION

Mauro Di Pilato

Centro Nacional de Biotecnología (CNB-CSIC), Madrid

16:30-16:45

HIGHLY EFFICIENT INSECT CELL-BASED PLATFORM FOR VIRUS-LIKE PARTICLE VACCINES PRODUCTION USING AN IMPROVED BACULOVIRUS VECTOR

Silvia Gómez-Sebastian

Alternative Gene Expression S.L (ALGENEX), Madrid

16:45-17:00

VIRAL PROTEINS TARGET COMPLEXES IN THE RIG-I LIKE RECEPTOR

Maria Teresa Sánchez-Aparicio

ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI, NY-USA

AUDIOVISUAL ROOM

15:00-17:00
Parallel Session IX
Microbiome And Health (PL9)
[Joint Session SEV-SEM]

Chairpersons: ALBERT BOSCH, ROSA DEL CAMPO

15:00-15:15 *Invited paper

SCIENTIFIC EVIDENCES OF GUT MICROBIOTA IMPLICATIONS IN HUMAN DISEASES

***Rosa Del Campo**

Microbiology Department, University Hospital Ramón y Cajal, Madrid

15:15-15:30 *Invited paper

INITIAL BACTERIAL COLONIZATION: IMPACT ON HUMAN HEALTH

***Esther Jiménez Quintana**

Departamento de Nutrición, Bromatología y Tecnología de los Alimentos. Universidad Complutense de Madrid

15:30-15:45 *Invited paper

METAGENOMIC ANALYSIS OF VIRUSES IN THE HUMAN ORAL CAVITY

***Alberto López Bueno**

Centro de Biología Molecular Severo Ochoa (C.S.I.C.-U.A.M.), Madrid

15:45-16:00 *Invited paper

VACCINE DRIVEN EVOLUTION OF PORCINE CIRCOVIRUSES

***Tuija Kekarainen**

Centre de Recerca en Sanitat Animal (CReSA) - Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Bellaterra

17:00-17:30 VISIT POSTERS AND COFFEE BREAK [GOYA ROOM - EXHIBITION AREA]

AUDITORIUM

17:30-19:30
Parallel Session X:
Teaching and Dissemination of the Virology (PL10)

Chairpersons: ESPERANZA GÓMEZ-LUCÍA, JOSE A. LÓPEZ-GUERRERO

17:30-17:33 *Invited paper

INTRODUCTION

***Jose Antonio Lopez-Guerrero**

Universidad Autónoma de Madrid, Madrid

17:33-17:41 *Invited paper

TEACHING VIROLOGY AT A PREUNIVERSITARY LEVEL

***Javier Medina**

Departamento de Ciencias Naturales, IES ALPAJÉS (Consejería de Educación, Comunidad de Madrid), Aranjuez, Madrid

17:41-17:49 *Invited paper

THE RADIO AS A VIROLOGY INFORMATION DIFFUSOR

***Manuel Seara**

Radio Nacional de España. Prado del Rey, Madrid

17:49-17:57 *Invited paper

VIROLOGY FOR ALL IN FREE AND ONLINE DIVULGATION JOURNALS

***Ana M. Doménech**

Universidad Complutense de Madrid, Madrid

17:57-18:05 *Invited paper

YOUNG VIROLOGISTS TO RECEIVE THE BATON

***Rafael Añez**

Universidad Complutense de Madrid, Madrid

18:05-18:13 *Invited paper

DISSEMINATION OF VIROLOGY THROUGH BLOGS AND OTHER SOCIAL MEDIA

***Miguel Ángel Jiménez-Clavero**

INIA-CISA, Valdeolmos, Madrid

18:13-18:21 *Invited paper

GAMES AS TOOLS FOR TEACHING VIROLOGY

***Esperanza Gómez-Lucía**

Universidad Complutense, Madrid, Madrid

18:21-18:30 *Invited paper

CONCLUSIONS

Jose Antonio Lopez-Guerrero

Universidad Autónoma de Madrid, Madrid

18:30-19:00

DISCUSSION

19:00-19:30

QUIZ VIRTUAL EPIDEMIC

WHITE ROOM

17:30-19:30
Parallel Session XI:
Antiviral Drugs (PL11)

Chairpersons: JULIÁ BLANCO, RAFII MOHAMED

17:30-17:45

CHD1 CHROMATIN REMODELER IS A POSITIVE MODULATOR OF INFLUENZA VIRUS REPLICATION THAT PARALLELS RNAP II DEGRADATION IN THE INFECTED CELLS

Amelia Nieto

Centro Nacional de Biotecnologia (CNB-CSIC), Madrid

17:45-18:00

LOCAL RNA FLEXIBILITY PERTURBATION OF THE IRES ELEMENT INDUCED BY A NOVEL LIGAND INHIBITS VIRAL RNA TRANSLATION

Gloria Lozano

Centro de Biologia Molecular Severo Ochoa, Madrid

18:00-18:15

ANTIVIRAL ACTIVITY OF POLYANIONIC CARBOSILANE DENDRIMERS AGAINST HEPATITIS C VIRUS IN CELL CULTURE

Daniel Sepúlveda-Crespo

H. G.U. Gregorio Marañón, Madrid

18:15-18:30

APTAMERS DESIGN AS ANTIVIRAL AGENTS AGAINST INFLUENZA VIRUS

Paloma Rodríguez-Rodríguez

Centro Nacional de Biotecnologia (CNB-CSIC), Madrid

18:30-18:45

SIMVASTATIN AND METFORMIN INHIBIT CELL PROLIFERATION AND HEPATITIS C REPLICATION IN VITRO, BY DOWNREGULATING TCTP AND INCREASING PTEN

José A. Del Campo

Valme University Hospital. Sevilla

18:45-19:00

HEPATITIS C VIRUS REPLICATION FACTORY STUDIED BY CRYO SOFT-X-RAY TOMOGRAPHY: PLATFORM FOR PHARMACEUTICAL TRIALS OF NEW ANTIVIRAL DRUGS AT CELLULAR LEVEL

Ana J. Pérez-Berná

Sincrotrón ALBA, Barcelona

19:00-19:15

DETECTION OF A SEXUALLY TRANSMITTED HEPATITIS C VIRUS PROTEASE INHIBITOR-RESISTANCE VARIANT IN A HUMAN IMMUNODEFICIENCY VIRUS-INFECTED HOMOSEXUAL MAN

Sandra Franco

Función irsiCAIXA, Barcelona

19:15-19:30

TRANSIENT INCREASES IN THE ERROR RATE CAN OPEN NEW ADAPTIVE PATHWAYS IN AN RNA VIRUS

Ester Lázaro

Centro de Astrobiología (INTA-CSIC), Madrid

AUDIOVISUAL ROOM

17:30-19:30
Parallel Session XII:
Replication Mechanisms (PL12)

Chairpersons: AMELIA NIETO, LUIS MENÉNDEZ

17:30-17:45

MODULATION OF P85 β ACTIVITY BY SUMO

Carmen Rivas

Universidade de Santiago de Compostela (CIMUS-IDIS), Santiago de Compostela

17:45-18:00

EXPRESSION OF PSEUDORABIES VIRUS IE180 PROTEIN UNDER THE CONTROL OF HUMAN TUMOR-SPECIFIC PROMOTERS (hTERT AND CEA): I.- APPLICATION TO OBTAIN CITOLYTIC VECTORS IN TUMOR CELLS

Laura Lerma

Universidad Autónoma de Madrid, Madrid

18:00-18:15

THE EXONUCLEASE XRN1P IS SPECIFICALLY REQUIRED FOR THE TRANSLATION OF BROME MOSAIC VIRUS

Bernat Blasco-Moreno

Universitat Pompeu Fabra, Barcelona

18:15-18:30

IFN-α TREATMENT CAUSES A MASSIVE APOPTOSIS IN IBDV INFECTED CELLS

Liliana Cubas

Centro Nacional de Biotecnología (CSIC), Madrid

18:30-18:45

BIOGENESIS AND DYNAMICS OF TOROVIRUS REPLICATIVE STRUCTURES

Ginés Ávila

Centro Nacional de Biotecnología (CSIC), Madrid

18:45-19:00

STRUCTURAL BASIS OF INFLUENZA VIRUS RNP ACTIVITY

Rocio Coloma

Centro Nacional de Biotecnología (CSIC), Madrid

19:00-19:15

THE DEAD-box HELICASE DHH1 PROMOTES TRANSLATION OF HIGHLY STRUCTURED mRNAS

Jennifer S. Jungfleisch

Universitat Pompeu Fabra, Barcelona

19:15-19:30

INCREASED PATHOGENESIS OF INFLUENZA A H1N1 VIRUS LED BY A PA RESIDUE DETECTED IN A FATAL CASE

Jasmina Vasilijevic

Centro Nacional de Biotecnologia (CNB-CSIC), Madrid

20:30 CONGRESS DINNER

WEDNESDAY JUNE 10, 2015

AUDITORIUM

9:00-10:20

Plenary Session VI:

Plant Virus (PL6)

Chairperson: **JUAN ANTONIO GARCÍA**

9:00-9:40 *Invited plenary lecture

NEW CONCEPTS IN THE BIOLOGY OF MULTIPARTITE VIRUSES

***Stéphane Blanc**

INRA, UMR BGPI, Montpellier, France

9:40-10:20 *Invited plenary lecture

MEMBRANE REARRANGEMENTS IN PLANT VIRUS RNA REPLICATION

***Luisa Rubino**

Institute for Sustainable Plant Protection, CNR, Bari, Italy

10:20-11:30

FLASH PRESENTATIONS

Chairpersons: **SUSANA ALVAREZ AND JOSÉ LUIS JIMÉNEZ**

11:30-12:00 COFFEE BREAK // VISIT POSTERS [GOYA ROOM - EXHIBITION AREA

12:00-12:30

THE VIROLOGIST CONFERENCE YOUNG AWARD

Chairpersons: **PEDRO MAJANO**

*Invited plenary lecture

TEN YEARS OF HEPATITIS C VIRUS CELL CULTURE INFECTION MODELS

***Pablo Gastaminza**

Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (CNB-CSIC), Madrid

12:30-13:15

CLOSING LECTURE

Chairpersons: **MIGUEL ÁNGEL JIMÉNEZ-CLAVERO**

*Invited plenary lecture

MECHANISMS OF VIRAL PERSISTENCE IN INSECTS

***Carla Saleh**

Institut Pasteur, Viruses and RNA interference Unit, Paris, France

13:15-13:30

CLOSING CEREMONY

Chairpersons: **ALBERT BOSCH AND MANUEL RODRÍGUEZ**

13:15-13:30

AWARD GIVING CEREMONY

13:30-13:45 ANNOUNCEMENT OF THE UPCOMING "XIV CONGRESO NACIONAL DE VIROLOGÍA"

CLOSING OF THE MEETING

ABSTRACTS POR SESIONES

ABSTRACTS BY SESSIONS

PLENARY SESSION I (PS I): FRONTIERS IN VIROLOGY

Chairpersons:
JUAN ORTÍN AND RICARDO FLORES
Sunday June 7, 2015
LECTURE ROOM ATENEO DE MADRID

*Invited plenary lecture

17:15-17:50h (P1)

CELL BIOLOGY OF VIRAL REPLICATION CYCLES: A COMPARISON OF HEPATITIS C VIRUS AND DENGUE VIRUS

RALF BARTENSCHLAGER [1,2]

[1]*Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany*

[2]*Division Virus-associated carcinogenesis, German Cancer Research Center, Heidelberg, Germany*

Positive strand RNA viruses replicate in the cytoplasm in distinct membranous replication factories. Thus, a hallmark of this virus class is the induction of profound membrane alterations that fall into two morphological groups: the invagination/spherule type and the double membrane vesicle (DMV) type. These morphotypes are represented by the Dengue virus (DENV) and the hepatitis C virus (HCV), respectively. These viruses belong to the same family, the *Flaviviridae*, because they share a similar genome organization and overall replication strategy. Yet, the interaction between these viruses and their host cell is fundamentally different. While DENV induces an acute lytic infection, HCV replicates persistently with little cytopathogenicity. Moreover, both viruses appear to utilize different cellular pathways and host cell machineries to remodel intracellular membranes in order to build up their replication factories. In my presentation, I will summarize our current state of knowledge how these viruses usurp host cell pathway to achieve efficient replication and virus production and describe an example how this knowledge has been used to develop a highly potent antiviral therapy.

*Invited plenary lecture

17:50-18:25h (P2)

VIRUS BEHAVIOR AT EXTREME FITNESS VALUES

ESTEBAN DOMINGO[1,2], NM BEACH[1], E MORENO[1], C PERALES[1,2,3]

[1]*Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, Madrid, Spain,*

[2]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain*

[3]*Liver Unit, Internal Medicine, Laboratory of Malalties Hepàtiques, Vall d'Hebron Institut de Recerca-Hospital Universitari Vall d'Hebron, (VHIR-HUVH), Universitat Autònoma de Barcelona, Barcelona, Spain*

Fitness is a parameter that captures the overall replicative efficacy of a virus in a given environment. A recent study with hepatitis C virus (HCV) replicating in human hepatoma Huh-7.5 cells has documented that high fitness correlates with resistance to several anti- HCV agents in the absence of recognized resistance mutations in the HCV mutant spectra (Sheldon et al. J. Virol. 88: 12098, 2014). Previous studies have shown that fitness tends to increase when large viral populations are allowed to multiply in a constant environment, and tends to decrease when the virus is subjected to repeated bottleneck

passages. Work with vesicular stomatitis virus and foot-and-mouth disease virus documented stochastic fluctuations when very high or very low fitness values are approached (Novella et al. J. Virol. 73: 1668, 1999; Lázaro et al. PNAS 100: 10830, 2003). In the case of HCV, viral fitness and multidrug resistance reach a plateau (and even decrease slightly) when the number of serial passages in hepatoma cells approaches 200. Interestingly, a pattern of increasing fluctuations in virus titer has been observed between passages 100 and 200, with a trend towards a decrease of specific infectivity (ratio of infectivity to amount of viral RNA). Two non-mutually exclusive mechanisms may contribute to fitness fluctuations: (i) fitness dependence of mutation deleteriousness, and (ii) participation of defective genomes in modulation of fitness levels. Experiments are now in progress to identify the mechanism involved, because fitness variations may impact efficacy not only of monotherapy but also of combination treatments. In particular, in lethal mutagenesis-based antiviral protocols, the advantage of a sequential inhibitor-mutagen administration over the corresponding combination (Perales et al. TIM 20: 595, 2012) was largely lost when applied to high fitness HCV. Recent results suggest that the doses of inhibitor (telaprevir) required to control viral load for an effective ribavirin mutagenesis-driven lethality are higher for high fitness than low fitness HCV. Also, high fitness HCV displays increased resistance to both the inhibitory and mutagenic activity of ribavirin, as compared with low fitness HCV (Perales et al. in preparation). Fitness and its connection with viral load are increasingly perceived as relevant for the management of HCV infections. Some recent clinical data point to low inhibitor sensitivity of HCV, independent of the presence of resistance mutations. Selection of specific mutations is one among other mechanisms of drug resistance that are still largely unexplored. Implications for current antiviral therapies will be discussed.

*Invited plenary lecture

18:25-19:00h (P3)

DENDRITIC EFFECTS: THE ROLE OF DENDRIMERS AS SOFT SUPER-ATOMS IN NANOPERIODIC PROPERTY PATTERNS

R. ZHANG[1], S. HISANO[1], A. TANI[1], H. KONDO[1], S. KANEMATSU[2], NOBUHIRO SUZUKI[1]

[1] *Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama 710-0046, Japan*

[2] *NARO Institute of Fruit Tree Science, 92 Shimokuriyagawa, Morioka, Iwate 020-0123, Japan*

A rapidly growing number of viruses of lower eukaryotes have been reported in the past few decades. These have contributed to further enhance our understanding of virus evolution and diversity. Simultaneously, some unusual viruses have challenged the “common rules” of viruses in sizes and concepts. One such virus group includes the so called dsDNA megaviruses that viruses exceed those of bacterial parasites such as mycoplasmas and in coding capacity (>1.2 Mb) and particle size (>0.5 m). The discovery of giant viruses was followed by the identification of satellite DNA viruses termed “virophage” co-infecting amoeba with helper giant dsDNA viruses.

Virophages have much smaller particles of 50 nm in diameter and dsDNA genomes (18 kb) which encode no DNA or RNA polymerases. Other unusual virus examples include "naked" or "capsidless" RNA viruses that are unable to form virus particles, exemplified by hypoviruses, which were the first to be reported as such a virus from the chestnut blight fungus.

Rosellinia necatrix is a filamentous ascomycete that causes white root rot in diverse perennial crops worldwide. This fungus is one of versatile fungal hosts to many viruses and suitable for studying virus/virus and virus/host interactions. Herewith, we show unique interactions in *R. necatrix* between an as-yet-undescribed positive-strand (+) RNA virus, with properties similar to but distinct from a virophage, and a novel, hosting double-stranded (ds) RNA virus. We found a mixed viral infection in a hypovirulent strain of *R. necatrix*. Co-infecting viruses are tentatively termed yado-kari virus 1 (YkV1) with a (+) RNA genome of approximately 6 kb and yado-nushi virus 1 (YnV1) with a dsRNA genome of approximately 9 kb. YkV1 possesses one single ORF encoding RNA-dependent RNA polymerase (RdRp), while YnV1 has two ORFs, each encoding capsid protein (CP) and RdRp. Immunological and molecular analyses revealed trans-encapsidation of not only YkV1 RNA but also RdRp by the major CP of the other virus, YnV1. Virion transfection assay and previous epidemiological data strongly suggest that YkV1 depends on YnV1 for viability, although it probably encodes functional RdRp. This hypothesis was confirmed by establishing infectious full-length cDNA of YkV1. We propose the term "RNA virophage" for the capsidless (+) RNA virus, YkV1, which highjacks CP of the dsRNA virus, YnV1, for the trans-encapsidation of its genome and RNA polymerase at the replication site.

THE VIROLOGIST CONFERENCE SENIOR AWARD

*Invited plenary lecture

12:00-13:00h (P4)

FRONTIERS IN PUBLIC HEALTH MICROBIOLOGY: A CONTINUING CHALLENGE

ANTONIO TENORIO

Arbovirus and Imported Viral Diseases, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, España

My first contact with public health virology was in 1963, when I received the oral poliovirus vaccine. Twenty years later I was in charge of the last outbreak due to autochthonous transmission of wild poliovirus in Spain. It affected a social group rejecting vaccinations, like everywhere with this and other vaccines. In the meantime, efforts for **controlling the circulation of eradicable viruses** are continuous and have been including new threats and methodologies.

In the nineties, our priority was improving the capability of **virus diagnostics in the Spanish hospitals**, developing innovative molecular tools and transferring methods and knowledge to the National Health System. Now, the majority of them are able to diagnose most of the common viral infections in both immunocompetent and immunodeficient patients.

With the new millennium, new threats suddenly exploded, directly derived from the **globalization**: clusters of imported viral hemorrhagic fevers in different European Countries, old viruses causing epidemics in naïve continents, wild viruses producing new infections in humans, new viral diseases transmitted by imported vectors or reservoirs.

The only way to control these imported infections (including Dengue and Chikunguna in areas with competent vectors) was the construction of **national and international networks, integrating tropical medicine and virology groups.**

On the other hand, Spain could not be the exception in Europe, and wild, zoonotic viruses were also circulating and causing disease in humans. The assembly of different research groups in a **multidisciplinary network** allowed us to detect dozens of viruses in wild life in Spain, some of them infecting humans (West Nile, Lymphocytic Coriomeningitis, or Toscana, but also some *new* viruses), or potentially infecting humans (Crimean-Congo Hemorrhagic Fever or the *new* filovirus Lloviu). Moreover, we had a qualitative change when we started to work with the viruses as ecological entities, fighting for their survival with other viruses or environmental frontiers.

The current Ebola outbreak in West Africa is the last example of a virus emerging from several globalization effects: migration of reservoirs from their original habitats, rapid spread of infected individuals to other countries –mainly war refugees resident in the first infected area–, or to the main cities of each infected country, impoverished health systems and improved communication ways.

To address this new challenge, we need a clear leadership and funding, **joining the efforts of social sciences, epidemiology, ecology and virology**. Otherwise, the new threat could be a virus adapted by serial passage to human species.

PLENARY SESSION (PS II): EMERGING VIRUSES

Chairpersons:

JAVIER BUESA - ANTONIO ALCAMÍ

Tuesday June 8, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

9:00-9:30h (P5)

CORONAVIRUS PATHOGENESIS AND PROTECTION

LUIS ENJUANES,J. L. NIETO-TORRES, M. DEDIEGO, J. M. JIMENEZ-GUARDEÑO, J. A. REGLA-NAVA, C. CASTAÑO-RODRIGUEZ, R. FERNANDEZ-DELGADO, JAVIER CANTON-BAILON, JAVIER GUTIERREZ-ALVAREZ, S. ZUNIGA, AND I. SOLA.

Department of Molecular and Cell Biology.National Center of Biotechnology (CNB-CSIC), Madrid, Spain.

The identification of the genes involved in coronavirus (CoV) virulence and in signaling pathways contributing to pathogenesis has been addressed using SARS- and MERS-CoVs. SARS-CoV non-essential genes have been deleted using a reverse genetics system. Among them, deletion of E gene led to an attenuated phenotype (SARS-CoV-ΔE). The expression of proinflammatory cytokines was reduced

in lungs of mice infected with a mouse adapted SARS-CoV-MA15-ΔE compared to lungs infected with the wild type virus. In infections by SARS-CoV with and without E protein, NF-κB was the only proinflammatory pathway differentially activated. Addition of an inhibitor of NF-κB led to a reduced inflammatory response after SARS-CoV infection and to an increase in mice survival. Therefore, these inhibitors could serve as antivirals. A reduction in neutrophil migration to lung-infected areas was observed in mice infected with SARS-CoV-MA15-ΔE, probably contributing to the lower degree of inflammation detected and to SARS-CoV-ΔE attenuation. SARS-CoV E protein is a viroporin with three domains: amino terminus, transmembrane and carboxy-terminus. The role of the different domains of E protein in SARS-CoV virulence, including its ion channel activity and a PDZ binding domain (PBM) mapping at the most carboxy-terminus of this protein was evaluated. Alteration of the three domains attenuated the virus, and the mechanisms of attenuation have been studied. The attenuated mutants provided long-term protection both in young and elderly mice against the challenge with pathogenic SARS-CoVs. We showed that E protein PBM binds syntenin, activating p38 MAPK and causing lung inflammation and edema. Inhibition of p38 MAPK protected 80% of the mice infected with virulent SARS-CoV. Deletion of E gene in MERS-CoV using a reverse genetics system, led to a replication-competent propagation-defective virus that is a safe vaccine candidate. These data indicated that SARS-CoV and MERS-CoV with E protein deleted or modified are promising vaccine candidates.

*Invited plenary lecture

9:30-10:00h (P6)

ZOONOTIC, FOODBORNE AND ENVIRONMENTAL TRANSMISSION IN ROTAVIRUS AND HEPATITIS E VIRUS INFECTION

FRANCO M. RUGGERI [1]

[1] *Dept. of Veterinary public health & food safety, Istituto Superiore di Sanità, Rome, Italy*

Viruses harbored in the intestinal tract can persist in a harsh environment for long time and are normally shed with feces of infected hosts in large amount. In addition to direct passage between individuals, they can therefore be transmitted via a contaminated environment, including surface waters and foodstuff. Moreover, viruses such as rotavirus and the hepatitis E virus can infect a wide range of animal species, and animal strains can be efficiently transmitted zoonotically adapting replication in human hosts.

Rotavirus is the major cause of acute gastroenteritis in children worldwide and vaccination is showing high efficacy against common human strains in an increasing number of countries. Nonetheless, the large genetic variation of rotavirus makes it unclear if protection is similar for all viral genotypes of animal origin. During 8 years of molecular surveillance of rotavirus in Europe, several thousands of rotavirus strains from infected children were genotyped to investigate shifts in the distribution of common strains and the possible emergence of rare, animal or exotic genotypes. Animal strains were also

investigated from domestic animal species, confirming that different hosts are mostly infected with species-specific genotypes. However, in sporadic cases close genetic relatedness was identified between several genes of human and animal strains, suggesting human infection with animal-derived reassortant rotaviruses. Rotavirus infection of adults was also confirmed, which may be related to strains acquired during travelling or from animal hosts. Animal and human strains were shown to be present in sewage samples highlighting possible risks of dual infection and inter-species gene reassortment.

The hepatitis E virus (HEV) is the cause of sporadic cases of acute human hepatitis in industrialized countries and of large waterborne outbreaks of disease particularly in Asia and Africa. Of the four main genotypes, genotype g3 is largely spread among domestic swine bred throughout Europe, and small outbreaks and an increasing rate of human infections are being reported in several countries in association with raw pork consumption. In addition to farmed swine, also wild boar, deer and other wild animal species are susceptible to HEV infection, and genetic characterization of human and animal g3 and g4 strains are in support of actual risks of zoonotic transmission of infection. The finding of HEV genome in swine feces, liver and meat of pigs at slaughter age, and the large import-export of animals and pork products highlights needs of controlling the spread of infection in Europe.

ORAL PRESENTATIONS (OP I): EMERGING VIRUSES

Chairperson: RAFAEL DELGADO

Wednesday June 8, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited paper

10:00-10:15h OP I(CO1)

THE ROLE OF THE NATIONAL REFERENCE LABORATORY REGARDING ARBOVIRUSES

MP SÁNCHEZ-SECO, [1], A. VÁZQUEZ[1], A. NEGREDO[1], L. FRANCO [1],F. DE ORY[1], A. TENORIO [1]

1. Centro Nacional de Microbiología. Instituto de Salud "Carlos III". Most of the authors (MPSS, AV, AN, LF and FO) are members of ViroRed and (MPSS, AV, AN and LF) RICET networks.

The European Centre for Disease Prevention and Control defines five activities to carry out by a National Reference Laboratory (NRL): Reference diagnostics, Reference resource materials, Monitoring, alert and response, Scientific advice and Collaboration and research. Low prevalence diseases are of special importance for NRLs since their requirement of a high degree of specialization. The National Centre of Microbiology in the Institute of Health "Carlos III" is the NRL for zoonoses.

Many zoonoses are caused by arthropod-borne viruses (arboviruses) and most of them are clear examples of emerging viruses.

The emergency and / or re-emergency of virus diseases may cause the eruption in our environment of very low or zero prevalence diseases. This fact will be, obviously, associated with the emergence

of a new, "weird" or exotic virus. Beyond their actual involvement in Public Health, these situations imply the need for swift action whose first step is the detection of the agent.

In our country, except for Toscana virus, a phlebovirus transmitted by sandflies that produces cases of neurological infection every year, the circulation of arboviruses in man has been highly limited. However, this situation can be reversed at any time. And this possibility should not be forgotten as less probable events as the appearance of the first case of Ebola virus infection out of Africa occurred recently (October 2014) due to the ongoing crisis of West Africa caused by this virus.

In recent years, there have been four situations that deserve special consideration: the first outbreak of West Nile virus in Spain (2010), the first outbreak of Chikungunya virus in a European country (Italy, 2007), the arrival of the virus to the Americas (2013- 2014) with a large increase in the number of viremic patients in our environment coming from that continent and the appearance in Extremadura of ticks carrying the virus causing Crimean-Congo haemorrhagic fever.

Previous work on preparedness is required to be able to respond properly to these challenging situations. Previous development and maintenance of techniques and protocols and specialized personnel capable of dealing with these very rare situations is part of the duties of a NRL.

*Invited paper

10:15-10:30h OP I(CO2)

LABORATORY DIAGNOSIS OF EBOLA VIRUS DISEASE IN SPAIN DIAGNOSTICS OF EBOLA VIRUS DISEASE SUSPECTED CASES

A NEGREDO[1], A VÁZQUEZ[1], L FRANCO[1], JM RUBIO[2], I JADO[2], G FEDELE[2], P ANDA[2,], MP SÁNCHEZ-SECO[1].

1. Laboratory of Arboviruses and Viral Imported Diseases, Centro Nacional de Microbiología. Instituto de Salud "Carlos III", Majadahonda, Madrid, Spain.

2. Health Alert Team, Centro Nacional de Microbiología. Instituto de Salud "Carlos III", Majadahonda, Madrid, Spain.

The Spanish National Center of Microbiology (CNM) is the National Reference Laboratory for zoonosis in Spain. In the current Ebola virus disease (EVD) epidemic in West Africa, the CNM has been assigned as the laboratory in charge of analyzing suspicious sample from EVD patient under investigation.

In the current EVD epidemic, CNM as National Ebola Reference Laboratory have received samples from 45 EVD suspicious patients during 2014. In this group are included travellers from African hotspot area (34 suspicious cases), repatriated suspicious cases from Liberia, Sierra Leone and Mali (2 confirmed cases and 2 suspicious cases), and high risk local contacts (7 suspicious cases). We followed the European Centers for Disease Control and Prevention´s (eCDC) diagnostic algorithm indicating ebolavirus nucleic acid detection on a blood sample as diagnostic procedure. In our case we used a BSL-3 facility to inactivate the clinical samples and after that, RNA extraction was carried

out in BSL-2 conditions. Positive results were confirmed by using a second PCR assay on a different genomic target and we sent the positive samples to a BSL-4 WHO collaborative laboratory (Bernhard Nocht Institute for Tropical Medicine, Hamburg and Germany) for the confirmation of the results. Differential diagnosis was made for malaria infection.

With the exception of the 2 EVD repatriated patients all travellers from Africa were negative (36/38). 22 out of 38 suspected cases were malaria positive patients. We confirm Ebola virus (EBOV) positive cases in samples from 2 repatriated patients and we detected the first case of infection outside Africa ever reported. This case was detected in a nurse assistant who had been in contact with the second repatriated person that came from Sierra Leone in September. In addition, The EBOV RNA concentration in plasma was measured daily from the 3 EBOV positive patients. The 2 EBOV positive repatriated patients died during the critical period of EVD but the third EBOV patients attended in Spain was discharged when RNA was not detected in any biological fluid.

The outbreak of EVD in West Africa has hit our Public Health Systems and coordination among different actors involved in the response has been established. In relation with the Laboratory diagnosis in CNM it was necessary to create an alert team to be able to attend health alert situation the 24 hours/7 days. Laboratory diagnostic have been carried out by this alert team and by Arbovirus and Imported viral disease laboratory. The time of response is less than 24 hours after an alert is received.

PLENARY SESSION III (PSIII): HEPATITIS A, B AND C: BASIC, TRANSLATIONAL AND CLINICAL RESEARCH

Chairpersons:

JOSEP QUER AND PABLO GASTAMINZA

Thursday June 8, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

11:00-11:20h (P7)

HEPATITIS A VIRUS: NEW PARADIGMS OF AN OLD PATHOGEN

R.M. PINTÓ, F.J. PÉREZ, M. DE CASTELLARNAU, L. D'ANDREA, S. GUIX, A. BOSCH

Enteric Virus Laboratory, Departament of Microbiology, University of Barcelona, Barcelona, Spain

Hepatitis is still the most common acute hepatitis worldwide, in spite of efficient available vaccines. In developed countries, the men-having-sex-with-men (MSM) group is particularly susceptible to large outbreaks and foodborne outbreaks also occur frequently due to the global food trade.

The hepatitis A virus (HAV) is the causative agent of the infection and although belongs to the *Picornaviridae* family, it is a very unique picornavirus at the genomic, structural, antigenic and biological levels.

Its capsid crystal structure has recently been described, revealing a much smoother particle compared to other picornaviruses, with higher thermal stability at acid than neutral pH. By the way, HAV has an extremely stable capsid at acid pH and at high biliary salt concentration, features required for its

biological cycle. These particularities may be related to its very special codon usage, shaped by fine tuning translation selection and resulting in a highly regulated ribosome traffic pace. Additionally, there is also a very fine tuning between capsid codon usage and IRES efficiency or in other words HAV has a very inefficient IRES which correlates with an overall slow speed of translation.

The requirement of stability for a virus with long periods in the environment, out of the host body, is ensured by negative selection of mutations affecting capsid rare codons. Many of these rare codons encode for surface residues located in the epitope sites avoiding the emergence of new serotypes. Antigenic variants may, however, arise in particular situations of immune pressure. Overall, codon usage may be considered a genomic constraint to antigenic variability.

Additionally, HAV has adopted several strategies to avoid its elimination from blood. On the one hand, its capsid structure at neutral pH is unable to interact with glycophorin A, a decoy factor present at the erythrocyte membrane, avoiding its clearing from the blood. On the other, it has recently described that HAV exists in a double phenotype: as free particles and enveloped in exosomes. A hypothesis has been proposed regarding HAV exit from hepatocytes. Exocytosis through the basolateral membrane would mainly give rise to enveloped particles in blood, and exit through the apical membrane into the biliar canaliculi would give rise to free particles in feces. The advantage of the occurrence of enveloped particles in blood is the capacity to escape the antibody interaction. These strategies would help in the ultimate fate of an otherwise low replicative virus.

*Invited plenary lecture

11:20-11:40h (P8)

CONTINUUM OF CARE IN HEPATITIS C: FROM DETECTION TO CURE AND ERADICATION

MANUEL ROMERO-GÓMEZ

UCM Digestive Diseases and ciberehd.Valme University Hospital, University of Seville, Sevilla, Spain.

Hepatitis C virus infection is a major health problem affecting to millions of people infected during the second part of the XXth century. Blood transfusions and drug users are the two main risk factors for hepatitis C. A selection of just one clone is usual during the infection process. Butyrophilin family genes are crucial in genotype selection and female gender, IL28B-CC genotype and HCV-genotype 1 are crucial to promote spontaneous viral clearance. Humans are the only one reservoir for the virus and HCV does not integrate on the human genome giving the opportunity for cure of the disease and effective eradication of the outbreak using directed antiviral drugs. Viral genotype and fibrosis stage are two key aspects making decisions on hepatitis C care. UDPS has demonstrated to be useful detecting genotype and subtype o even mixed infections in patients non correctly typed by second generation Lipa®. Transient elastography allow a close monitorization of fibrosis progression. Changes on liver stiffness one year apart classify patients at risk for liver diseases progression and lower survival. Combination of Sofosbuvir

+ Ledipasvir or Parataprevir/r + Ombitasvir + Dasabuvir with or without ribavirin for 8, 12 or 24 weeks reach sustained virological response rate higher than 95% in patients with compensated liver disease genotype 1 or genotype 4. Sofosbuvir + Daclatasvir could be useful in patients infected by genotype 3a but failed in cirrhotics previously non-responders. In patients with previous fail to first-generation protease inhibitor triple therapy or decompensated cirrhosis the combination of sofosbuvir + ledipasvir with ribavirin could achieve SVR in more than 90% and improved liver dysfunction in a large proportion of patients. Eradication of the virus means blocking transmission of the virus and cure of the disease in non-cirrhotics. In patients with cirrhosis sustained virological response is associated with decreased risk of liver dysfunction and improved survival for all causes. A residual risk of liver cancer remained and surveillance is required.

*Invited plenary lecture

11:40-12:00h(P9)

HEPATITIS B AND D: WHEN EVERY SINGLE DETAIL HAS A HIDDEN MEANING

FRANCISCO RODRIGUEZ FRÍAS

Unitat Patologia Hepàtica. Serveis de Bioquímica i Microbiologia (Unitat Virologia). Hospital Universitari Vall d'Hebron, Barcelona, Spain. Institut Recerca Vall d'Hebron (VHIO), CIBERehd

The hepatitis B (HBV) and delta (HDV) viruses are surprising mainly for the extraordinary compactness of their small genomes: HBV 3.2 kb DNA, HDV 1.7 kb RNA, the smallest known animal viruses, both are responsible for chronic liver disease, recognized as "incurable" HBV has infected a third of the world population from whom, 300 million are chronic carriers, and responsible for more than half of the liver cancers worldwide. Besides, HDV, which infectivity depends on the HBV envelope, infects about 15 million HBV carriers ,being responsible for the most severe form of hepatitis). The molecular biology of these two agents is absolutely amazing. HBV viral cycle, despite being a DNA virus, includes a reverse transcription step and the viral genome remains in the infected cell nucleus as a "mini-chromosome" (known as cccDNA). In fact in an HBV infected hepatocyte four different forms of the viral genome can coexist: as a mini-chromosome; as inserted into the host genome; as "incomplete" DNA molecule in secreted virions and as RNA pre-genome in "intracellular previrions". Like a coin with four faces?. The HBV-DNA is completely coding and almost 70% of it has overlapping genes, a “Guinness record of genome compactness”. Among all coded regions, the HBV X protein (154 aa) is associated to an incredible variety of interactions with hepatocyte mechanisms and being considered oncogenic. Despite the availability of highly efficient antiviral treatments inhibiting viral replication, none of them is able to resolve the infection, therefore any individual who has been infected with HBV infected cannot be considered as cured, 2000 million people¡¡¡. His occasional and small “roommate”, the HDV, is even more surprising, its tiny 1.7 kb circular RNA genome has a high rate of self-complementarity (74%) and a single coding region, inhibiting HBV production despite

needing HBV activity to produce envelope particles. The HDV genome has a single coding region, however, two proteins (Delta antigens) are detected as a result of a multicodification mechanism (RNA editing). The question is: are these two proteins enough to control and enslave HBV infection?. Multiple posttranslational modification mechanisms of the delta antigen (isoprenylation, methylation, acetylation and phosphorylation) could give them a huge multifunctionality similar to the multiple social functions that can have a person who dresses in different ways. "the suit defines the function". The new in-depth technologies to study viral quasispecies by massive sequencing are reporting surprises that represent real challenges on both viruses biology.

*Invited plenary lecture

12:00-12:20h (P10)

VIROLOGY OF HEPATITIS C VIRUS INFECTION AFTER LIVER TRANSPLANTATION

SOFÍA PÉREZ-DEL-PULGAR

Liver Unit, Hospital Clínic, IDIBAPS, CIBERehd, Barcelona, Spain.

End-stage liver disease due to chronic hepatitis C virus (HCV) infection is the leading indication for liver transplantation (LT) in the Americas, Europe, and Japan. Unfortunately, infection of the graft is universal in patients with detectable viral load at the time of LT. Furthermore, the course of recurrent HCV infection is accelerated after LT, with approximately 30% of patients developing chronic hepatitis and liver cirrhosis within 5 years after LT. The LT setting is a unique model to study the pathogenesis of HCV infection for several reasons: (1) tissue and serum samples can be obtained before and after HCV infection; (2) infection can be monitored from the beginning and thus, it is possible to obtain data on HCV kinetics and host factors; (3) hepatitis C recurrence after LT is a rapidly progressive disease and patients with mild or very severe hepatitis recurrence can be well characterized. Studies on early kinetics have shown that HCV replication starts a few hours after reperfusion of the graft and that HCV-RNA concentrations increase within the first days after LT, despite the presence of anti-HCV antibodies. The main source of HCV infection is circulating virions. Nevertheless, some data suggest that HCV present in extrahepatic compartments may contribute to hepatitis C recurrence. Escape from neutralizing antibodies and efficient entry into hepatocytes play a major role in reinfection of the liver graft. Indeed, HCV receptor levels at the time of LT may modulate early HCV kinetics and hepatitis C recurrence is associated with increased levels of claudin-1 and occludin at the tight-junctions. Recent estimates show that the proportion of infected hepatocytes ranges from 1 to 54% and correlates with viral load. Some studies have shown that infection is not random. Clustering of HCV-positive hepatocytes suggest a localized mechanism of intrahepatic propagation and control (cell-to-cell transmission and innate immune responses, respectively) and may have implications for HCV therapy. On the other hand, the quasispecies population changes significantly after LT, most likely because of the strong immunosuppression and the need to adapt to a new environment.

However, there are no conclusive data supporting the role of HCV quasispecies composition and disease outcomes. Treatment of recurrent HCV infection after LT is often compromised by adverse effects and limited efficacy of interferon-based therapies. The advent of direct-acting antivirals, particularly in interferon-free regimens, is very likely to improve the prognosis and outcome of patients with severe hepatitis C recurrence.

PLENARY SESSION IV (PSIV): INNATE IMMUNITY

Chairperson: MARIANO ESTEBAN

Tuesday June 9, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

9:00-9:45h (P11)

TO SENSE OR NOT TO SENSE VIRAL RNA – ESSENTIALS OF CORONAVIRUS INNATE IMMUNE EVASION

V OLKER THIEL [1,2]

[1] *Institute of Virology and Immunology, Federal Department of Home Affairs, Bern, Switzerland*

[2] *Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Switzerland*

An essential function of innate immunity is to distinguish self from non-self and receptors have evolved to specifically recognize viral components and initiate the expression of antiviral proteins to restrict viral replication. Coronaviruses are RNA viruses that replicate in the host cytoplasm and evade innate immune sensing in most cell types, either passively by hiding their viral signatures and limiting exposure to sensors or actively, by encoding viral antagonists to counteract the effects of interferons. Since many cytoplasmic viruses exploit similar mechanisms of innate immune evasion, mechanistic insight into the direct interplay between viral RNA, viral RNA-processing enzymes, cellular sensors and antiviral proteins will be highly relevant to develop novel antiviral targets and to restrict important animal and human infections.

ORAL PRESENTATIONS (OP II): INNATE IMMUNITY

Chairperson:
ANGEL CORBÍ AND
JAVIER MARTÍNEZ-PICADO

Tuesday June 9, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited paper

9:45-10:00h OP II(CO3)

FUNCTIONAL INTERACTION BETWEEN THE eIF2α KINASE GCN2 AND THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

J. DEL PINO, J.J. BERLANGA

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain

The reversible phosphorylation of the α-subunit of eukaryotic translation initiation factor 2 (eIF2α) is a well-characterized mechanism of translational control in response to a wide variety of cellular stresses, including viral infection. In mammalian cells there are four eIF2α kinases which specifically phosphorylate this factor at serine 51 in response to different forms of stress: PKR, GCN2, HRI and PERK. Beside PKR, GCN2 participates in

the cellular response against viral infection by RNA viruses with central nervous system tropism, such as Sindbis virus, through its activation by viral RNA. PKR has been involved in the innate antiviral response against HIV-1, although this antiviral effect is very limited due to the distinct mechanisms evolved by the virus to counteract PKR action. Here we report that infection of human cells with HIV-1 conveys the proteolytic cleavage of GCN2 and that purified HIV-1 and HIV-2 proteases produce direct proteolysis of GCN2 in vitro, abrogating the activation of GCN2 by HIV-1 RNA. Moreover, the HIV-1 protein Tat interacts with PKR and inhibits its in vitro activity by acting as a competitive inhibitor due to binding to the same site of eIF2α on the kinase. We have assayed in vitro eIF2α kinase activity of GCN2 and the other two eIF2α kinases, HRI and PERK, in the presence of Tat, and we have observed a reduction of eIF2α phosphorylation in all cases. On the other hand, although the limited sequence similarity between eIF2α and the HIV-1 integrase, we have found that this viral protein is able to inhibit GCN2 eIF2α kinase activity in vitro. These significant findings suggest that cleavage of GCN2 by HIV-1 protease, and competitive inhibition by Tat and the viral integrase, could represent distinct mechanisms of HIV-1 to counteract GCN2 antiviral activity.

*Invited paper

10:00-10:20h OP II(CO4)

p56^{Lck} AND PKCθ INHIBITORS PRESERVE SAMHD1 ANTIVIRAL FUNCTION, INTERFERING WITH HIV-1 REPLICATION

M.R. LÓPEZ-HUERTAS[1], M. BERMEJO[1], S. RODRÍGUEZ-MORA[1], E. MATEOS[1], JOE HEDGPETH[2], JOHN SWINDLE[2], J. ALCAMÍ[1], M. COIRAS[1]

[1]. Inmunopatología del SIDA, Instituto de Salud Carlos III, Madrid, Spain

[2]. Complegen, Inc., Seattle, WA

BACKGROUND. HIV-1 cannot presently be eradicated due to the existence of latently infected cells that persist even with antiretroviral therapy (ART). HIV-1 may infect resting and activated CD4+ T cells but only replicates in activated cells. Among other mechanisms, the inactivation of SAMHD1, an antiviral factor linked to innate immunity, is essential to overcome HIV-1 restriction. SAMHD1 activity is greatly dependent on cell cycle progression, as cyclin A2/CDK1 are responsible for SAMHD1 phosphorylation at T592 (pSAMHD1) and subsequent inactivation. CD4+ T cell activation strongly relies on kinases such as p56^{Lck} and PKC theta (θ). PKCθ is selectively expressed on CD4+ lymphocytes and its activation is mediated by the lymphocyte-specific tyrosine kinase p56^{Lck}, which is required for PKCθ translocation to the plasma membrane, initiating a cascade of events that culminates in the activation of essential factors for HIV-1 replication such as AP-1, NFAT, and NF-κB, as well as in SAMHD1 inactivation. We hypothesized that p56^{Lck} and PKCθ inhibitors could thwart HIV-1 replication using as

mechanism the preservation of SAMHD1 antiviral function.

MATERIAL & METHODS. PBMCs were isolated from healthy donors. pSAMHD1 at T592 was determined by immunoblotting. Retrotranscription and proviral integration was analyzed by qPCR. PKCθ inhibitors CGX1079 and CGX0471 were provided by Complegen. Dasatinib (BMS-354825, Sprycel), an inhibitor of tyrosine kinases such as $p56^{Lck}$, was provided by Bristol-Meyers Squibb.

RESULTS: 1) PHA/IL-2 induced pSAMHD1 in PBMCs and full HIV-1 replication. 2) CGX1079, CGX0471 and Dasatinib interfered with HIV-1 retrotranscription and proviral integration. This interference was not related to viral entry. 3) CGX1079 and CGX0471 slightly reduced T-cell proliferation, partially protecting SAMHD1 from T592 phosphorylation. These inhibitors also interfered with NF-κB, NFAT, and AP-1 activity. 4) Dasatinib completely abrogated T-cell proliferation, impeding SAMHD1 phosphorylation. This was the major mechanism of action of Dasatinib as it showed no effect on viral transcription. 5) Activation ex vivo of PBMCs from patients with chronic myeloid leukemia on treatment with Dasatinib for several years showed that in vivo treatment with Dasatinib prevented SAMHD1 phosphorylation.

CONCLUSIONS: SAMHD1 plays a central role in HIV-1 replication and in the establishment of viral reservoirs, thereby representing a major target for therapeutic intervention. PKCθ and $p56^{Lck}$ inhibitors, able to preserve SAMHD1 antiviral function, could be promising as adjuvant of ART to reduce the reservoir size. Dasatinib is the first compound currently used in clinic that has been described to preserve the antiviral function of an innate factor such as SAMHD1.

*Invited paper

10:20-10:40h OP II(CO5)

ROLE OF MONOCYTES AND PLASMACYTOID DENDRITIC CELLS IN THE CONTROL AND IMMUNOPATHOGENESIS OF HIV AND HEPATITIS C VIRUS INFECTION

E. RUIZ-MATEOS[1]

[1]*Instituto de Biomedicina de Sevilla (IBiS)/Hospital Universitario Virgen del Rocío. Seville. Spain.*

Plasmacytoid dendritic cells (pDCs) are able to sense viral and bacterial infections through Toll Like Receptors (TLRs)-7 and 9, respectively. pDCs activation produces vast amounts of type I interferon (IFN-I). This activation also induces pDC maturation, expressing co-stimulatory molecules and TRAIL (TNF-Related Apoptosis Inducing Ligand) that mediates CD4+ T-cell apoptosis. In the HIV-infection scenario we have shown that these functions are associated with the spontaneous control of the virus. This occur in less than 1% of HIV-infected subjects, the so called HIV elite controllers (EC), who are able to maintain undetectable viral loads during a long period of time in the absence of antiretroviral treatment. These mechanisms also account for hepatitis C virus (HCV) infection. We have shown lower HCV viral loads and others, superior rates of HCV virus spontaneous clearance in HIV elite controllers. The understanding of the mechanisms and characteristics of the pDCs in this extraordinary subjects who

are able to control these two infections, may be very interesting for the development of immunotherapeutic strategies for HIV and HCV infection.

On the other hand, in most of the HIV-infected subjects the control of viremia is possible thanks to combined antiretroviral treatment (cART). However, despite viral load remains undetectable, survival of patients on cART is ten year lower than the general population. Most of the patients died because of non AIDS events (NAEs), such as, hepatic complications in HCV co-infected patients, cardiovascular disease, tumours and other age-related diseases, caused by low grade systemic chronic inflammation due to the activation of the innate immune system. Using multiparametric flow cytometry we are observing that after TLR-2, TLR-4 and TLR-7-specific stimulation the polyfunctionality of monocytes, i.e.: production of cytokines at the same time per cell, of HIV-infected patients on suppressive cART is higher than both, elderly (85 years old) and healthy subjects with the same age, pointing out to specific HIV-related dysregulation of the monocyte function contributing to the long-term development of NAEs. In the same way, aberrant IFN-I production of pDCs also occurs on cART which contributes to immune system dysregulation and has been associated with cardiovascular diseases development.

The double-edged sword of pDCs and monocytes/macrophages in the control and pathogenesis of HIV and HCV-infection will help to both: the better understanding of the physiological function of these components of the innate immune system and to the development of immunotherapeutic strategies for the treatment of chronic viral infections.

PLENARY SESSION V (PS V): STRUCTURAL ANALYSIS OF VIRUS AND BIOTECHNOLOGY

Chairpersons:

JOSÉ ESTÉ AND MARJORIE PION

Tuesday June 9, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

11:15-11:45h (P12)

HIV-1 EVOLUTION: DRUG-RESISTANCE AND NUCLEOTIDE COMPOSITION

B. BERKHOUT

Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Evolution of the human immunodeficiency virus type 1 (HIV-1) was studied in diverse settings. First, we will discuss how HIV-1 gains resistance to the $1^{st}/2^{nd}/3^{rd}$ generation of peptidic entry inhibitors. We describe for the first time the evolution of drug-dependent virus variant in a patient. We discuss the cascade of more and more difficult molecular resistance mechanisms against the three generations of inhibitor. We also demonstrate that resistance against the optimized peptides is extremely difficult to achieve and that it comes at a prize: a significant loss of HIV-1 fitness. Although the therapeutic power of these improved peptides is enormous, also for non-HIV viruses that use similar entry/fusion strategies, the need for drug

injection remains a significant disadvantage for this drug class.

HIV-1 evolution was also studied from a quite different perspective: the profoundly biased nucleotide composition of the single-stranded viral RNA genome. HIV-1 RNA is A-rich (more than 36%) and C-poor (less than 17%). Other (retro)viruses have found other particular niches in sequence space. For instance, the retrovirus HTLV-I RNA is C-rich and G-poor and the coronavirus RNA is U-rich and C-poor. We will discuss the evolutionary pressures that may have shaped these genomes, but also the functional consequences, e.g. in codon usage. Using a novel phylogeny-instructed mutagenesis (PIM) strategy, we recently generated HIV-1 constructs with an increased and decreased A-count in a small genome segment. The properties of these virus variants will be presented.

*Invited plenary lecture

11:45-12:15h (P13)

RNA VIRUS POPULATION DYNAMICS IN SEQUENCE SPACE AND FITNESS LANDSCAPES

M. VIGNUZZI

Institut Pasteur, Paris, France

RNA viruses form highly diverse populations or quasispecies. Recent advances in sequencing technologies now makes it feasible to characterize the genetic structure of these populations and monitor their evolution in space and time as they adapt to a host environment. We use NGS approaches, *in vitro* and *in vivo*models of virus evolution, and mathematical modeling to examine several aspects of RNA virus biology. We provide evidence that evolutionary trajectories of RNA viruses may be inherently predictable and we show that phenotype is determined by the group contribution of minority variants. Finally, we show how multi-dimensional reduction of deep sequence data may be used to generate maps of sequence space, with which we can generate empirical fitness landscapes to better monitor virus populations during adaptive walks.

*Invited plenary lecture

12:15-12:45h (P14)

VIRUSES AS TOOLS FOR THERAPEUTIC INTERVENTIONS IN CANCER AND INFECTIOUS DISEASES

G. PALÙ

Department of Molecular Medicine, University of Padova, Padova, Italy

After a short overview of the field, a few approaches will be described of preclinical and clinical deployment of viral vectors for new advanced molecular therapies of cancer, AIDS and allied genetic disorders. Details will be reported on: i) appropriate viral vector design, ii) suitable strategies of HIV gene knock-down, iii) metabolic and immune-inflammatory treatment of cancer and iv) gene editing of stem cells derived from somatic lines.

ORAL PRESENTATIONS (OP III): STRUCTURAL ANALYSIS OF VIRUS AND BIOTECHNOLOGY

Chairperson: FRANCISCO SOBRINO
Tuesday June 9, 2015
AUDITORIUM REAL CASA DE LA MONEDA

*Invited paper

12:45-13:00h OPIII (CO6)

BACTERIOPHAGEø29. FROM MOLECULAR BIOLOGY TO BIOTECHNOLOGY

MARGARITA SALAS

Centro de Biología Molecular "Severo Ochoa" (CSIC–UAM) Madrid.

The *Bacillus subtilis* phage ø29 has a linear, double-stranded DNA (19.275 bp) with a protein, named terminal protein (TP), covalently linked to the 5' DNA ends. In the presence of the TP-DNA template a molecule of TP primes the initiation of ø29 DNA replication by formation of the TP-dAMP initiation complex catalyzed by the phage DNA polymerase. Then, the same polymerase catalyzes chain elongation in a highly processive way coupling polymerization to strand displacement. These two properties of the ø29 DNA polymerase, high processivity and strand displacement capacity, together with a high fidelity, make of this enzyme an outstanding polymerase to amplify DNA. In fact, ø29 DNA polymerase has been commercialized to amplify both circular and linear genomic DNA. In addition, we have improved the ø29 DNA polymerase performance by fusion of DNA binding motifs.

*Invited paper

13:00-13:15h OPIII(CO7)

STRUCTURAL ANALYSIS OF VIRAL AND VIROIDAL RNA USING ATOMIC FORCE MICROSCOPY

CARLOS BRIONES

Department of Molecular Evolution, Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid, Spain.

Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain.

RNA is a macromolecule of paramount importance in biology. Apart from storing heritable information in RNA viruses and viroids, RNA plays key roles in the flow of genetic information, including the control of gene expression, the initiation of cap-independent translation by Internal Ribosome Entry Site (IRES) elements, and the catalysis of the peptidyl transferase reaction within the ribosome. Additionally, different RNA molecules function as efficient catalysts (ribozymes), natural RNAs (riboswitches) as well as *in vitro* selected ones (aptamers) specifically recognise molecular targets, and certain RNAs provide a structural scaffold in ribonucleoprotein aggregates.

RNA molecules adopt specific three-dimensional structures critical to their function, and different physicochemical techniques are currently used to analyse RNA structure. Among them, Atomic Force Microscopy (AFM) is a nanotechnology-based technique belonging to the group of Scanning Probe Microscopies, whose nanometre resolution in air and liquid environments is optimal for the visualisation of RNA molecules of different

lengths, as well as RNA-RNA or RNA-protein complexes adsorbed on flat surfaces (1). AFM does not require any staining or coating of the imaged molecule, thus minimising its structural disruption. Currently, AFM is used in different fields of virology (2).

We have set up AFM technique to analyse the native structure of viral and viroidal RNA molecules. First, the Mg^{2+}-dependent folding of the Hepatitis C Virus (HCV) IRES element has been investigated. A sharp structural switch was monitored in a HCV IRES-containing, 574 nt-long RNA molecule (3) when Mg^{2+} concentration increased from 2 to 4 mM. This conformational rearrangement was hindered by the presence of the microRNA miR-122. The competing effect of Mg^{2+} and mir122 allowed envisaging a model for the long-range RNA-RNA interaction within HCV IRES in its natural sequence context (4).

We are also analysing the 3D structure of genomic viroid RNAs belonging to the families *Pospiviroidae* (PSTVd, 359 nt-long) and *Avsunviroidae* (ELVd and PLMVd, 332-351 nt-long) in different ionic conditions. Our AFM images confirm the main features of their previously known rod-like and multibranched secondary structures, respectively (5), and provide information on viroid tertiary structure. This talk will summarise our main results and challenges ahead.

1. Hansma et al. (2004). *Curr. Opin. Struct. Biol.* 14, 380.

2. Kuznetsov et al. (2010). *Nucleic Acids Res.* 38, 8284.

3. Beguiristain et al. (2005). *Nucleic Acids Res.* 33, 5250.

4. García-Sacristán et al. (2015). *Nucleic Acids Res.* 43, 565.

5. Flores et al. (2014). *Annu. Rev. Microbiol.* 68: 395.

*Invited paper

13:15-13:30h OPIII (CO8)

BREAKING THE BARRIERS OF BACULOVIRUS-BASED PRODUCTION TECHNOLOGIES

J.M. ESCRIBANO

Departamento de Biotecnología, INIA, Madrid, Spain

Baculoviruses are widely used in different biotechnology applications including pest control pests and production of adeno-associated viruses or recombinant proteins. The use of baculovirus vectors in research about protein function and structure or to industrial production of different recombinant proteins has grown in recent decades. Since the development of the baculovirus vector expression system (BEVS) in the ’80s, thousands of recombinant proteins, ranging from cytosolic enzymes to membrane-bound proteins, have been successfully produced in baculovirus-infected insect cells. During more than 30 years of continuous improvements in the BEVS, only a modest 30-40% increase in productivity was achieved. Two bottle necks limited the industrial use of the BEVS, the maximum production yields reached in comparison to the most optimized systems (milligrams *vs* grams per liter), and the frequent partial proteolysis found as a consequence of the damages induced in insect cells during infection by the vector (apoptosis). Recently (Gómez-Sebastian et al., 2014), a baculovirus vector expression cassette containing rearranged baculovirus-derived

genetic regulatory elements has been described. This newly designed expression cassette reduces the above mentioned limitations in production of the BEVS (400% increase), and also improved protein integrity by prolonging cell viability as a consequence of a virus-induced apoptosis delay with respect to a standard baculovirus vector. Baculoviruses modified with this expression cassette have been used for efficient production of vaccines based on virus-like particles and surface glycoproteins. In addition, during the last decade, several research groups and companies have been using insects as living biofactories in combination with baculovirus vectors for recombinant protein production. The most widely used insects are *Bombyx mori* and *Trichoplusia ni*Lepidoptera. Insects offer unique advantages of productivity, scalability and success with difficult-to-express proteins. A considerable number of recombinant proteins have been produced in insects as living bioreactors and in most cases those were produced at levels never reached in insect cells, obtaining yields of grams per liter of extract, comparable to the most productive systems based on bacteria, yeast or mammalian cells. These insect-based production methods are currently used for production of diagnostic reagents and several subunit vaccines for animal health, produced in these biofactories, are under development and will be marketed in the near future. In conclusion, baculovirus vectors are excellent allies for the biotechnological and pharmaceutical companies in the production of biologics and recent advances configured the BEVS as one of the most cost-efficient and productive method to develop the next generation vaccines.

PLENARY SESSION VI (PS VI): PLANT VIRUS

Chairperson: JUAN ANTONIO GARCÍA

Wednesday June 10, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

9:00-9:40h (P15)

NEW CONCEPTS IN THE BIOLOGY OF MULTIPARTITE VIRUSES

A SICARD[1], J-L ZEDDAM[1,2], M YVON[1], Y MICHALAKIS[3], S GUTIERREZ[1] AND S BLANC[1]#.

1. INRA, UMR BGPI, Montpellier, France.

*2. IRD**, UMR RPB, Montpellier, France***

3. CNRS, UMR MIVEGEC 5290, Montpellier, France.

Multipartite viruses are characterized by a genome composed of two or more nucleic acid segments, each encapsidated individually. A classical view in virology assumes that the viral replication cycle occurs within individual cells, where the whole viral genome information is replicated, and is then reiterated in successively infected cells during host invasion. In the context of multipartite viruses, this view implies that at least one copy of each of the genome segments must repeatedly enter together in individual cells for successful infection. Because one or more genome segments may be missing in numerous susceptible cells, thus aborting infection, these viral systems are believed to bear an enormous cost, which drastically increases with the number of segments constituting the viral

genome. It has even been concluded that multipartite viruses with an elevated number of segments (as for example member species of the family *Nanoviridae*) appear so costly that they should not have evolved and should thus not exist!

To address this apparent paradox, we have experimentally tested the thus far undisputed assumption that the segments of a multipartite virus must be together within individual cells for the system to be functional. For this, we used the nanovirus *Faba bean necrotic stunt virus*, which genome is composed of 8 ssDNA segments, each encapsidated individually. Our results indicate that the various segments are not always together within individual cells and yet, that the system scattered over several distinct cells appears functional. This observation has several important implications. First, it questions the cost that has always been attributed to multipartite viral systems, where gathering a copy of each segments in single cells was though to be mandatory. Second, it demonstrates that the replication cycle of a virus is not necessarily "cell-autonomous" and that the spatial unit of a virus replication cycle can be, in some cases, an ensemble of interconnected cells within which the various part of viral genetic information are obviously communicating.

*Invited plenary lecture

9:40-10:20h (P16)

MEMBRANE REARRANGEMENTS IN PLANT VIRUS RNA REPLICATION

L RUBINO

Institute for Sustainable Plant Protection, CNR, Bari, Italy

Positive-strand RNA viruses constitute a large group of infectious agents causing major plant, animal and human diseases. Genome replication occurs in association with host cell membrane structures derived from the endoplasmic reticulum (ER) (picornaviruses, potyviruses, comoviruses, nepoviruses and bromoviruses) or from the limiting membrane of organelles such as lysosomes or endosomes (alphaviruses), vacuoles (cucumoviruses), mitochondria (nodaviruses, some tombusviruses, carmoviruses, ampeloviruses and maculaviruses), peroxisomes (several tombusviruses) and chloroplasts (tymoviruses and some marafiviruses). Viral proteins are involved in targeting the replication complex to the specific intracellular membranes. Intracellular membranes are normally modified to form vesicular structures with a narrow neck through which the interior of the vesicles communicates with the cytosol. A variety of observations indicates that, indeed, virus replication takes place in the closed environment of the vesicles, including co-localization of virus replicase and virus RNA progeny with cell membranes and strong dependance of viral synthesis on lipid metabolism. Confinement of the virus replication complexes in closed environments represents an advantage for

the viral RNA, which is protected from degradation of host ribonucleases and recognition of host defence reactions. Vesiculation of the target cellular membrane in natural hosts and in the yeast *Saccharomyces cerevisisae*, an alternative model host for studying virus replication, is well documented for nodaviruses (animal viruses) and bromo- and tombusviruses (plant viruses). *Flock house virus* (FHV, genus *Nodavirus*, family *Nodaviridae*) protein A is a transmembrane protein that contains N-terminal signals targeting the outer membrane of mitochondria and elicits the formation of vesicular structures. *Brome mosaic virus* (BMV, genus *Bromovirus*, family *Bromoviridae*) replication occurs on the ER membranes, in spherules containing genomic RNA, the 2a replicase protein and the 1a virus RNA replication factor. The 1a multifunctional protein has RNA capping and helicase functions, and directs targeting and assembly of the replication complex on the ER membranes. The replication of members of the genus *Tombusvirus* (family *Tombusviridae*) has been studied in plant and yeast cells. *Carnation Italian ringspot virus* p36 protein contains the determinants for targeting the replication complex to the outer membrane of mitochondria; the p33 of several other tombusviruses contains sequences necessary to localize virus replication on the limiting membrane of peroxisomes.

THE VIROLOGIST CONFERENCE SENIOR AWARD

Chairperson: JUAN ANTONIO GARCÍA

Wednesday June 10, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

12:00-12:30h (P17)

TEN YEARS OF HEPATITIS C VIRUS CELL CULTURE INFECTION MODELS

PABLO GASTAMINZA LANDART

Laboratorio de Infección por el Virus de la Hepatitis C

Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (CNB-CSIC)-Madrid (SPAIN)

Since its identification as the etiologic agent for the non-A, non-B hepatitis in 1989, hepatitis C virus (HCV) could not be robustly cultured in vitro. In fact, to date and despite enormous efforts from the scientific community, it is not possible to efficiently culture primary virus isolates from HCV-infected patients. In 2005, several laboratories reported the possibility of producing infectious hepatitis C virus from cloned cDNA. This recombinant genotype 2a strain was generated by Dr. Takaji Wakita from a consensus sequence of the viruses circulating in a Japanese patient with Fulminant Hepatitis (JFH-1). Transfection of in vitro transcribed, full-length genomic RNA, produced, for the first time, infectious HCV virions with relatively high efficacy in the supernatants of the transfected cells.Ever since, new infectious molecular clones capable of producing infectious virions from different HCV genotypes have been developed. By

recapitulating every aspect of the viral lifecycle, these unique tools have been instrumental for the study of basic aspects of HCV infection in cell culture, especially those that were not recapitulated in other experimental systems such as replicons and HCV glycoprotein-pseudotyped retroviral vectors. In this sense, our contribution to the field throughout these years involves the description of biophysical and ultrastructural features of infectious HCV virions as well as the identification of cellular determinants that mediate infectious HCV assembly and secretion. In recent years, we have focused our attention in the study of host-virus interactions to better understand the molecular and cellular mechanisms underlying different aspects of HCV infection. In addition, our group has taken advantage of cell-based screening assays to identify novel molecules with antiviral potential, with the aim of developing new tools to dissect the virus life cycle and with the hope of identifying new ways of tackling this important pathogen.

CLOSING LECTURE

Chairperson:

MIGUEL ÁNGEL JIMÉNEZ-CLAVERO

Wednesday June 10, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited plenary lecture

12:30-13:15h (P18)

MECHANISMS OF VIRAL PERSISTENCE IN INSECTS

MARIA CARLA SALEH

Institut Pasteur, Viruses and RNA interference Unit, Paris, France.

The establishment and maintenance of persistent viral infections is widely debated and remains largely misunderstood. We show that in *Drosophila*, persistence is achieved through a mechanism involving reverse transcription of non-retroviral RNA virus and the RNA interference pathway. Fragments of diverse RNA viruses are reverse transcribed early during infection, resulting in DNA forms embedded within LTR-retrotransposon sequences. Inhibition of reverse transcription hinders the appearance of these DNA forms and is accompanied by increased viral titers and cell death. These viral/retrotransposon-DNA chimeras produce transcripts that are processed by the RNAi machinery. Knocking down RNAi components in persistently infected cells shifts the equilibrium from persistent to acute infection. Our results reveal an unanticipated physiological function for retrotransposons and propose a role in RNAi-mediated immune protection for parasitic viral insertions into host genomes.

Parallel Session I:

EMERGING VIRUSES AND VETERINARY

Chairpersons:

ANA M. DOMENECH AND JAVIER ORTEGO

Monday June 8, 2015

AUDITORIUM REAL CASA DE LA MONEDA

15:00-15:15h (CO 9)

2003-2015: 12 YEARS OF RESEARCH ON MOSQUITO-BORNE EPORNITIC FLAVIVIRUSES IN SPAIN. WEST NILE, USUTU, BAGAZA…AND BEYOND

M. A.JIMÉNEZ-CLAVERO[1], E. PEREZ-RAMÍREZ[1], F. LLORENTE[1], J. FERNÁNDEZ-PINERO[1], A. VÁZQUEZ[2], M. P. SÁNCHEZ-SECO[2], J. FIGUEROLA[3], R. C. SORIGUER[3], S. RUIZ[4], R. VILLALBA[5], C. GÓMEZ-TEJEDOR[5], M. AGÜERO[5], A. TENORIO[2].[1].

[1]INIA-CISA, Valdeolmos, Spain; [2]CNM-ISCIII, Majadahonda, Spain; [3]Doñana Biological Station, CSIC, Seville, Spain; [4]Servicio de Control de Mosquitos, Diputación de Huelva, Spain; [5]Laboratorio Central de Veterinaria, Algete, Spain.

In 1999 an African virus, West Nile virus, unexpectedly emerged in New York, and spread relentlessly throughout America, causing tens of thousands of encephalitis cases in humans and horses, and uncountable (probably millions) deaths in wild birds, in one of the most remarkable episodes of virus emergence one can mention. The virus still persists and has become endemic in wide areas in the Americas.

Meanwhile in the Old World the virus produced sporadic, self-limited outbreaks with little or no human affection. WNV was long known in Europe and the Mediterranean where sporadic outbreaks had been observed since the 1950's, although ceased in the 1970's and 80's, to re-emerge in the 90's. Since then the virus increased its incidence and geographic spread steadily, up to 2008 where a recrudescence of the epidemiological situation was observed in most of Europe, still lingering.

In Spain, little was known about WNV until 2003. That year, the EVITAR (Vector and Rodent-Borne Viral Diseases) network started its activity. Since then a systematic collaborative work conducted in an interdisciplinary way (the "One Health approach") produced an outstanding advance in the knowledge of WNV and other epornitic flaviviruses in our country. Serosurveys in wild birds, horses and humans in Southern Spain evidenced WNV sylvatic circulation for the first time in 2004, while monitoring of mosquito populations in that area identified WNV and many other flaviviruses, some new to science, including a new WNV genetic lineage, while others, (e.g. Usutu virus), had known zoonotic potential. The group developed new technologies to detect the virus and antibodies to it, and toinvestigate the feeding preferences of mosquito species found relevant for flavivirus transmission, thus unveiling transmission pathways potentially leading to WNV spillover to humans and horses in the area, eventually occurring in 2010 in Cádiz. Members of the network isolated WNV for the first time in Spain in 2007 from affected golden eagles, while in 2010 they isolated another flavivirus, Bagaza virus, new to Europe, in an encephalitis outbreak affecting wild birds in Cádiz. Since then, dozens of flavivirus isolates, from Spain and elsewhere, were characterized by full

genome sequencing, establishing new phylogenetic relationships, and determined their virulence and host competence using newly established or optimised bird and mammal models of flavivirus infection.

The "core" of the EVITAR network, represented by those signing this work, still pursues high quality research on vector-borne zoonotic diseases of public health importance in our country.

15:15-15:30h (CO 10)

THE ANTIVIRAL EFFECT OF INTERFERON INDUCED TRANSMEMBRANE PROTEINS (IFITMs) IN AFRICAN SWINE FEVER VIRUS INFECTION

MUÑOZ-MORENO, R.[1,2], C. MARTÍNEZ-ROMERO[2], I. GALINDO[1], L. BARRADO-GIL[1] M. A. CUESTA-GEIJO[1], M. TAMAYO[1], A. GARCÍA-SASTRE[2] AND C. ALONSO[1].

[1] *Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain*

[2] *Present address: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA*

Rapid spread of African swine fever virus across Eastern Europe in the recent years resulted in emergence of the disease in a number of EU countries including the Baltic Republics and Poland*. The interferon-induced transmembrane (IFITM) protein family is a group of antiviral restriction factors that impair flexibility and inhibit membrane fusion restricting viral progression at early infection. While IFITMs are widely known to inhibit several single-stranded RNA viruses, there are limited reports available regarding their effect in double stranded DNA viruses. We have analyzed a possible antiviral function of IFITMs against a double stranded DNA virus: The African swine fever virus (ASFV). This virus has been shown to be inhibited by other IFN-response gene such as MxA (Netherton et al, 2009). Infection by ASFV is IFN (interferon)-sensitive and induces IFITMs expression in vitro. Then, we investigated whether IFITMs expression could impair viral infection. Expression of IFTM1, 2 and 3 reduced virus replication, with IFITM2 and 3 having an impact on viral entry/uncoating. We will discuss the potential mechanisms to inhibit virus infection induced by IFITMs.

*Animal Disease Notification System: Outbreaks per Disease. European Comission,pp. 8.

15:30-15:45h (CO 11)

AMINO ACID SUBSTITUTIONS IN THE NON-STRUCTURAL PROTEINS 4A OR 4B MODULATE THE INDUCTION OF AUTOPHAGY IN WEST NILE VIRUS INFECTED CELLS

A.B. BLÁZQUEZ[1], M.A. MARTÍN-ACEBES[1,2], J.C. SAIZ[1]

[1]*Departamento de Biotecnología. INIA, Madrid, Spain*

[2] *Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, Madrid,Spain*

West Nile virus (WNV) is a neurotropic mosquito-borne flavivirus responsible for outbreaks of meningitis and encephalitis, for which no vaccines or antivirals for human use are available. Autophagy is a catabolic mechanism that sequesters cytoplasmatic components for degradation. The autophagic pathway can

be upregulated to cope with diverse forms of cellular stress, including viral infections. In the case of flaviviruses, the autophagic pathway can play multifaceted roles during the infection of these pathogens that include rearrangements of cellular lipid metabolism, contribution to viral maturation, or involvement in the early steps of the infection. Whereas the activation of autophagy in cells infected with other flaviviruses is well known, the interaction of WNV with the autophagic pathway still remains unclear and there are reports describing opposite findings obtained even analysing viral strains with a common origin. To clarify this controversy, we first analysed the induction of autophagic features in cells infected with a panel of WNV strains. WNV was determined to induce autophagy in a strain dependent manner. We observed that all WNV strains or isolates analyzed, except one (NY99), upregulated the autophagic pathway in infected cells. Even more, interestingly, a variant derived from this NY99 isolated from a persistently infected mouse (B13) also increased LC3 modification and aggregation. Complete genome sequencing of this B13 variant revealed only two non-synonymous nucleotide substitutions when compared to parental NY99 strain. These nucleotide substitutions introduced one amino acid replacement in NS4A and other in NS4B. Using genetically engineered viruses we showed that introduction of any one of these replacements, alone or in combination, was sufficient to upregulate the autophagic pathway. Thus, in this work we have shown that naturally occurring point mutations in the viral non-structural proteins NS4A and NS4B confer WNV with the ability to induce the hallmarks of autophagy such as LC3 modification and aggregation. Even more, the differences on the induction of an autophagic response observed among WNV variants in infected cells did not correlate with alterations on the activation of the unfolded protein response (UPR), suggesting an uncoupling of UPR and autophagy during flavivirus infection. The findings here reported could help to improve the knowledge of the cellular processes involved on flavivirus-host cell interactions and contribute to the design of effective strategies to combat these pathogens.

15:45-16:00h (CO 12)

ROLE OF SARS-CoV VIROPORINS E, 3a AND 8a IN VIRUS REPLICATION AND VIRULENCE

C. CASTAÑO-RODRIGUEZ[1], JL. NIETO-TORRES[1], ML. DEDIEGO [1], C. VERDIÁ-BÁGUENA[2], JM. JIMENEZ-GUARDEÑO[1], JA. REGLA-NAVA[1], R. FERNANDEZ-DELGADO[1], VM. AGUILELLA VM[2], L. ENJUANES[1]

[1]*Department of Molecular and Cell Biology, National Biotechnology Centre (CNB-CSIC), Darwin 3, Universidad Autonoma de Madrid, 28049 Madrid, Spain.*

[2]*Department of Physics, Laboratory of Molecular Biophysics. Universitat Jaume I, 12071 Castellón, Spain.*

SARS-CoV has three viroporins: 3a, E and 8a. We have engineered recombinant SARS-CoV (rSARS-CoV) variants missing each of these proteins. Their analysis has shown that none of them are essential for virus replication and that proteins E and 3a are relevant in virulence. Interestingly, a virus lacking both E and 3a genes could not

be rescued suggesting that a complementation between proteins E and 3a is required for virus viability. This hypothesis is supported by the fact that the two proteins co-localize in the cell. These two viroporins share at least two activities: PDZ binding motif (PBM) and ion channel (IC), which are virulence factors in SARS-CoV. In the present work we studied whether 3a and E protein IC activities are responsible for this complementation. To this end, rSARS-CoV in which IC activities of E protein or 3a protein are knocked out, were firstly engineered. In order to generate rSARS-CoV without E or 3a protein IC activity (rSARS-CoV-EIC$^-$, rSARS-CoV-3aIC$^-$), the amino acids involved in their IC activity were determined. E protein has a single transmembrane domain (TMD) while 3a protein has three TMDs. The conductance of synthetic peptides of each of the TMDs and full-length proteins with native or mutant sequences was measured in artificial membranes and amino acids involved in 3a and E proteins IC activity were identified. Then, rSARS-CoV-3aIC$^-$ and rSARS-CoV-EIC$^-$ viruses were constructed. Evaluation of rSARS-CoV-EIC$^-$ pathogenicity in BALB/c mice showed that E protein IC activity is a virulence factor. Furthermore, E protein IC activity is required for inflammasome activation, which triggers the expression of proinflammatory cytokines leading to edema accumulation and ARDS. In contrast, 3a protein IC activity was not essential for SARS-CoV virulence. Therefore, the complementation between proteins E and 3a does not seem to be mediated by the IC activities of these proteins, which have different relevance in virus virulence. We suggest that the PBMs of proteins E and 3a could be responsible for the complementation. Experiments to define the basis of this complementation are being performed.

16:00-16:15h (CO 13)

VIROLOGICAL AND EPIDEMIOLOGICAL FEATURES OF CHIKUNGUNYA VIRUS INFECTION AMONGST TRAVELERS RETURNING TO SPAIN, 2008-2014

L FRANCO[1,2,3], MD FERNÁNDEZ[†1,4], M BANGERT[†,1, 5] F DE ORY[1,2], A POTENTE[1],L HERNANDEZ[1], F LASALA[1], L HERRERO[1] , F MOLERO[1], A I NEGREDO[1,3], MP SÁNCHEZ-SECO[1,2,3] AND SNS HOSPITALS.

† Equally contribution
1-Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid-Spain
2-VIRORED,CYTED
3-Red de Investigación Cooperativa en Enfermedades Tropicales (RICET),RETICS
4- Pasteur Institute, Dakar-Senegal
5-European Programme for Public Health Microbiology Training (EUPHEM) ECDC, Stockholm-Sweden

Chikungunya is endemic in some parts of Africa, Southeast Asia and in the Indian subcontinent. In late 2013, the first documented autochthonous transmission of chikungunya virus (CHIKV) was reported in the Caribbean island of Saint Martin and since then the infection has spread quickly in countries and territories in the Caribbean region, North, Central and South America. With the ongoing outbreak in the Caribbean, CHIKV is increasingly becoming a European public health threat. Transmission of CHIKV to humans occurs through bites of *Aedes aegypti* and *Ae. albopictus* mosquitoes. In Europe *Ae.albopictus* is established primarily around the Mediterranean basin and has

been demonstrated its competency for virus transmission. This resulted in locally-acquired infections in Italy (Emilia Romagna, 2007), and in France (Var and Montpellier, 2010 and 2014 respectively). In this study, we analyzed six years of imported chikungunya infections in Spain. During the study period (2008-2014), a total of 1311 suspected chikungunya infections were studied and more than half were in 2014 alone. From 2008 to 2013, 30 laboratory-confirmed (PCR, IgM/IgG) chikungunya infections were imported whereas in 2014 there were 195 confirmed cases. The presence of IgM or IgG antibodies against chikungunya was performed by immunofluorescence on 830 out 1311 suspected patients with 188 positive for IgM (22.6%) with or without IgG testing. Molecular diagnosis (RT-PCR) was performed on 452 out 1311 suspected patients (34.4%) with viral genome detected in 35 (7.7 %). Majority of chikungunya cases with known travel history in the period 2008-2013 reported travel to Asia whereas in 2014 the main travel destination were the Americas (Dominican Republic, Haiti and Venezuela). Virus sequencing revealed that all samples from Americas felt into Asian genotype (Caribbean Clade), although ECSA/Indian Ocean genotype was detected in cases imported from Asia and Africa. Most of the positive samples in 2014 clustered around May and October, the activity period for the vector present in Spain. We have described a 6.5 fold increase of imported chikungunya infections from the whole period 2008-2013 (30 cases) compared to 2014 (195 cases), the highest number recorded in Spain. Chikungunya is an emerging public health threat to Spain because the conditions for autochthonous transmission are met: presence of competent vector and a large number of travelers returning from affected areas like the Caribbean and northern South America.

16-15-16:30h (CO 14)

HEMAGGLUTININ PROTEIN OF *PESTE DES PETITS RUMINANTS* VIRUS ACTIVATES THE INNATE IMMUNE RESPONSE VIA TOLL-LIKE RECEPTOR 2 SIGNALING

E. PASCUAL[1], S.R. WATTEGEDERA[2], C. SANTIAGO[3], V. MARTÍN[1], G. ENTRICAN[2] AND N. SEVILLA[1]

[1] *Centro de Investigación en Sanidad Animal (CISA)-INIA, Madrid, Spain*

[2] *Moredun Research Institute, Edinburgh, Scotland*

[3] *Centro Nacional de Biotecnología-CSIC, Madrid, Spain*

Toll-like receptors (TLR) are a family of proteins expressed in almost all cell types that act as sentinels of the host innate immune system. TLR family comprises both membrane and intracellular receptors that recognize different types of pathogen associated molecular patterns (PAMPs) leading to the production of pro-inflammatory cytokines and also stimulating the development of long-lasting adaptive immunity. TLR2 is located in the cell surface and, although it was initially thought to act as a bacterial sentinel, it has been shown to recognize a number of viral glycoproteins. In this study we sought to characterize the role of TLR2 in the activation of the immune response by *peste des petits ruminants virus* (PPRV), a morbilivirus of the *Paramixoviridae*family

that causes an acute, highly contagious disease in goats and sheep.

Using 293 cells stably expressing human TLR2 but lacking any other TLR we found that inactivated virus of the vaccine strain Nigeria/75 of PPRV induces IL-8 production in a dose-dependent manner. That activation is only observed in cells expressing TLR2 and is greatly reduced when the receptor is blocked by the pretreatment with a specific antibody. We identified hemagglutinin (H) as the responsible of TLR2 activation by performing the same assays with recombinant, mammalian-expressed purified protein. The exogenous addition of H to the cell culture induces high levels of IL-8 only in TLR2-expressing cells. In order to assess whether TLR2 signaling can also be induced by PPRV in antigen presenting cells, we isolated ovine dendritic cells from peripheral blood monocytes and evaluated cytokine production upon stimulation with either inactivated virus or purified H protein by RT-qPCR. In both cases, we observed a significant increase in IL-8 production, suggesting activation by TLR2 engagement. The involvement of these results on the host immune mechanisms in the control of PPRV infection will be discussed.

16:30-16:45h (CO 15)

POSTNATAL PERSISTENT INFECTION WITH CLASSICAL SWINE FEVER VIRUS IN DOMESTIC PIGS AND WILD BOARS: OPENING PANDORA'S BOX

L. GANGES[1*], S. MUÑOZ-GONZÁLEZ[1], O. CABEZÓN[1,2], N. RUGGLI[3], M. PEREZ-SIMÓ[1], J. A. BOHÓRQUEZ[1], R. ROSELL[1,4], I. MARCO[2], S. LAVÍN[2], A. SUMMERFIELD[3] AND M. DOMINGO[1,5]

[1] *Centre de Recerca en Sanitat Animal (CReSA), Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus de la UAB, Bellaterra, Barcelona, Spain*

[2] *Wildlife Health Service (SEFaS) from the Veterinary School of the Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain*

[3] *Institute of Virology and immunology (IVI), Mittelhäusern, Switzerland*

[4] *Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, (DAAM), Generalitat de Catalunya, Spain*

[5] *Departamento de Sanitat i d'Anatomia Animals, Facultat de Veterinària, UAB, Bellaterra-Barcelona, Spain*

It is well established that trans-placental transmission of classical swine fever virus (CSFV) during mid-gestation can lead to persistently infected offspring. The aim of this work was to evaluate the ability of CSFV to induce viral persistence upon early postnatal infection in wild boars and domestic pigs. Ten new-born domestic piglets and fifteen new-born wild boar were infected intranasally within the first 10 hours after birth with the Catalonia 01 strain (Cat01, CSFV of moderate virulence). Viral replication, innate and specific immune responses were evaluated. During six weeks after postnatal infection (duration of the experiment), most of the piglets remained clinically healthy, despite

persistent high virus titres in the serum, organs and body secretions. The levels of viral RNA detected were similar in both, domestic pigs and wild boar. The clinical signs recorded were also similar, with some temperature peaks above 40 ºC mainly during the first 14 days post infection. Approximately 50 percent of infected animals (pigs and wild boar) were persistently infected without any clinical signs at the end of the experiment. Importantly, these animals were unable to mount any detectable CSFV-specific humoral immune response. Four weeks after infection, PBMCs from the persistently infected seronegative piglets were unresponsive to both, specific CSFV and non-specific PHA stimulation in terms of IFN-γ-producing cells. In the case of wild boar, heterogeneous results were observed in the INF-γ-producing cells after CSFV and PHA stimulations. Although scarce, IFN-γ-producing cells were detected upon CSFV stimulation in three out of nine inoculated wild boar at week 4. Furthermore, high level of IFN-γ-producing cells was detected after PHA stimulation in four of the infected animals. However, a decrease or lack of IFN-γ-producing cells from week 4 to 6 against Cat01 CSFV or PHA was observed. These results suggested the development of a state of immunosuppression in these postnatally persistently infected animals. Taken together, we provided the first data demonstrating the feasibility of generating a postnatal persistent CSFV infection, which has not been shown for other members of the Pestivirus genus yet. Since serological methods are routinely used in CSFV surveillance, persistently infected animals might go unnoticed. In addition, the induction of persistent infection in wild boar provides new insights towards understanding possible mechanism of maintenance of CSFV in the European countries. These experiments were approved by the Ethics Committee for Animal Experiments of the Autonomous University of Barcelona (UAB) according to existing national and European regulations.

16:45-17:00h (CO 16)

GETTING IC-TAGGING TO WORK INTO THE ENDOPLASMIC RETICULUM

N. BARREIRO PIÑEIRO[1], I. LOSTALÉ SEIJO[1], J. BENAVENTE MARTÍNEZ[1], J. MARTÍNEZ COSTAS[1]

[1] *Laboratorio de Virología Molecular, Centro Singular de Investigación en Química Biológica y Materiales Moleculares, Santiago de Compostela, Spain*

Viral factories or viroplasms are structures where viral components are recruited to assemble the viral progeny. A single nonstructural protein named muNS is responsible for the formation of avian reovirus viroplasms. Our previous characterization of muNS demonstrated that a little domain (muNS-Mi) is able to form spherical structures (microspheres) in transfected cells[1]. We developed a molecular tagging system (IC-tagging) that targets proteins to cytoplasmic muNS-derived microspheres that can be easily purified by physical methods[2]. Thus, we can produce In Vivo muNS microspheres decorated with any IC--tagged protein which have been successfully used to immunize mice against a viral infection (Bluetongue virus, BTV)[3]. The surface of enveloped viruses present glycoproteins

which have acquired specific post-translational modifications due to their pass through the endoplasmic reticulum (ER), like glycosylation and disulfide bonds. In an attempt to create a method to produce microspheres that might be used as vaccines against enveloped viruses, we adapted the IC-tagging system to work inside the ER. Our aim is to produce microspheres inside the ER that are able to capture IC-tagged viral-derived proteins that might acquire their post¬translational modifications while being incorporated in the microspheres, thus resembling the surface of enveloped viruses. To do this, we introduced a signal peptide at the N-terminus of the muNS-Mi sequence, promoting the entrance of the protein into the endoplasmic reticulum. The targeted protein was able to form microspheres inside the organelle, although some unspecific aggregation was also observed. Eliminating a glycosylation site in the muNS-Mi sequence increased the microsphere formation efficiency while drastically reducing the aggregation. Additionally, we showed that the IC-tagged proteins can be successfully loaded onto the microspheres, raising the possibility of developing biologically-generated microspheres as particulate subunit vaccines against enveloped viruses.

1. Brandariz-Nuñez, A., Menaya-Vargas, R., Benavente, J. & Martinez-Costas, J. Avian reovirus microNS protein forms homo-oligomeric inclusions in a microtubule-independent fashion, which involves specific regions of its C-terminal domain. J. Virol. 84, 4289–301 (2010).

2. Brandariz-Nuñez, A., Menaya-Vargas, R., Benavente, J. & Martinez-Costas, J. A versatile molecular tagging method for targeting proteins to avian reovirus muNS inclusions. Use in protein immobilization and purification. PLoS One 5, e13961 (2010).

3. Marín-López, A. et al. VP2, VP7, and NS1 proteins of bluetongue virus targeted in avian reovirus muNS-Mi microspheres elicit a protective immune response in IFNAR(-/-) mice. Antiviral Res. 110, 42–51 (2014).

Parallel Session II: HIV

Chairpersons:

MANUEL LEAL AND JOSÉ ALCAMÍ

Monday June 8, 2015

WHITE ROOM

15:00-15:15h (CO 17)

STUDY OF THE PROCESSIGN-BODIES (P-BODIES) ROLE IN THE RESTRICTION AGAINST HIV-1 IN PRIMARY HUMAN T CELLS

P GIL MARTIN[1], R CORREA-ROCHA[2], M.A MUÑOZ-FERNANDEZ[1] AND **M PION**[1, 2]

[1]Laboratory of Molecular ImmunoBiology, Hospital General Universitario Gregorio Marañón; Instituto de Investigación Sanitaria del Gregorio Marañón. C/ Dr. Esquerdo 46, 28007 Madrid, Spain; [2]ImmunoRegulation Laboratory, Hospital General Universitario Gregorio Marañón; Instituto de Investigación Sanitaria del Gregorio Marañón. C/ Maiquez, 9, 28009 Madrid, Spain

Naive and resting CD4+ T cells are restrictive in cases of HIV infection, while activation of these cells induces a permissive state in infection. Several restriction factors have already been described as acting in the early stages of the viral cycle. However, these restriction factors were generally studied in cell line or during the replication of HIV. Only a few works have described the very first steps of HIV infection in primary human cells. It is interesting to note that HIV-1 Gag protein may be found associated to P-bodies

proteins, which are cytoplasmic structures in which siRNA-related silencing occurs. There is some debate as to whether this union could be related to the replicative capacity of the virus but nothing has been done about whether this union could have an impact at the first steps of the viral cycle in primary CD4+ T cells. The aim of this study is to investigate whether P-bodies could constitute a new restrictive factor during the early stages of the viral cycle in primary human cells.

In the study, we analyzed the presence and the intracellular localization of several proteins related to the P-bodies in naive and in activated CD4+ T cells by confocal microscopy. Moreover, the presence of the viral RNA into P-bodies was analyzed by molecular assays after only 4 hours of infection.

We first demonstrated that presence and intracellular distribution of P-bodies-related proteins were altered comparing non-activated or activated CD4+T cells. Furthermore, the presence of the HIV-1 genome linked to Ago2, which is one of the P-bodies proteins associated to the RNA silencing process, was detected after just 4 hours of infection.

The study shows for the first time that during the very early stages of HIV infection in non-activated CD4+ T cells, the virus can be found associated to P-bodies proteins, which may explain in part the restriction of these cells against HIV infection.

15:15-15:30h (CO 18)

NEUTROPHIL MIGRATION VIA NFκB ACTIVATION BY MODIFIED VACCINIA VIRUS AS A NOVEL MECHANISM TO ENHANCE HIV-SPECIFIC T CELL RESPONSES

M. DI PILATO[1], E. MEJÍAS-PÉREZ[1], M. ZONCA[2], B. PERDIGUERO[1], C. GÓMEZ[1], M. TRAKALA[3], J. NIETO[1], J. L. NÁJERA[1], C. O. S. SORZANO[4], C. COMBADIÈRE[5], G.PANTALEO[6], L. PLANELLES[2] AND M. ESTEBAN[1]

[1]. Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología, Madrid, Spain
[2]. Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, Madrid, Spain
[3]. Grupo de División Celular y Cancer, Centro Nacional de Investigaciones Oncológicas,Madrid, Spain
[4]. Biocomputing Unit, Centro Nacional de Biotecnología, Madrid, Spain
[5]. INSERM UMR_S 945, Faculté de Médecine Pitié-Salpétrière, Laboratoire Immunité et Infection, Paris, France
[6]. Division of Immunology and Allergy, Department of Medicine, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland

Neutrophils are antigen-transporting cells that generate vaccinia virus (VACV)-specific T cell responses, yet how VACV modulates neutrophil recruitment and its significance in the immune response are unknown. We generated an attenuate d VACV strain (NYVAC) that expresses HIV-1 clade C antigens but lacks three specific viral genes (*A52R*, *K7R*, *B15R*). We found that these genes act together to inhibit the $NF_{\kappa}B$ signaling pathway. Triple ablation in modified virus restored NFκB function in macrophages. After virus infection of mice, $NF_{\kappa}B$ pathway activation led to expression of several cytokines/chemokines that increased the migration of neutrophil

populations (N_α and N_β) to the infection site. N_β cells displayed features of antigen-presenting cells (APC) and activated virus-specific CD8 T cells. Enhanced neutrophil trafficking to the infection site was responsible for increasing the T cell response to HIV vector-delivered antigens. These results identify a mechanism for poxvirus-induced immune response and alternatives for vaccine vector design.

15:30-15:45h (CO 19)

IDENTIFICATION AND CHARACTERIZATION OF THE MOLECULAR PATHWAY LEADING TO SAMHD1-MEDIATED VIRAL RESTRICTION

E. BALLANA[1], R. BADIA[1], E. RIVEIRA-MUÑOZ[1], A. RUIZ[1], J. TORRES-TORRONTERAS[2], E. PAULS[1], B. CLOTET[1], R. MARTÍ[2], JA ESTÉ[1]

[1]. AIDS Research Institute - IrsiCaixa, Hospital Germans Trias i Pujol, Badalona

[2]. Mitochondrial Pathology Laboratory, Institut de Recerca Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona

Monocytes are refractory to HIV infection and only become susceptible to infection after differentiation into macrophages, due to deactivation of the restriction factor SAMHD1 by phosphorylation. The aim of the present work was the identification and characterization of cell signaling events leading to host SAMHD1 activation/deactivation. Therefore, we have evaluated the contribution of cyclin-dependent kinases (CDK) and their corresponding activating cyclins in the control of SAMHD1-mediated viral restriction in monocyte derived macrophages (MDM). First, we have shown that SAMHD1 deactivation is controlled by CDK, which in turn, control cell activation and proliferation. RNA interference of CDK2 but not CDK1, CDK4 or CDK5 suppressed SAMHD1 phosphorylation and blocked viral replication specifically at the level of proviral DNA formation. Importantly, we also found that CDK6 inhibition blocks SAMHD1 phosphorylation probably through the control of CDK2, as RNA interference or pharmaceutical blockade of CDK6 by palbociclib, a potent and selective CDK6 inhibitor, led to reduced CDK2 activation, concomitant with reduced SAMHD1 phosphorylation and blockade of HIV-1 reverse transcription and virus replication. The antiviral effect of these inhibitors disappeared when SAMHD1 was abrogated using the HIV-2 viral protein X (Vpx), demonstrating the specificity of the mechanism of action of these compounds. Knockdown of the cyclin partners of CDK1, CDK2 and CDK6 showed that cyclin D3, the catalytic partner of CDK6, has a major impact in SAMHD1 phosphorylation, dNTP levels and HIV-1 reverse transcription and replication. Finally, we investigated the effect of p21, a member of the Cip/Kip family of cyclin-dependent kinase inhibitors (CDKIs) specifically controlling cell cycle progression through binding and activation of cyclin-CDK1 or –CDK2 complexes. siRNA-induced downregulation of p21 strongly enhanced the phosphorylation of SAMHD1 followed by an increase in HIV-1 proviral DNA formation and virus replication, indicating that p21 affects SAMHD1-mediated HIV-1 restriction and further delineating the cellular pathway involved in SAMHD1-mediated viral restriction. Thus, overall our results indicate a

fundamental role of CDK2 and the CDK6-cyclin D3 complex in SAMHD1-mediated virus restriction in MDM during GO to G1 transition. The present study suggest also that agents targeting cell proliferation may limit HIV-1 infection and hypothetically, might prevent the proliferation of persistently infected cells, offering new possibilities for intervention.

15:45-16:00 h (CO 20)

INTRACELLULAR FACTORS BLOCKING EARLY STEPS OF THE HIV-1 REPLICATIVE CYCLE IN COMMON MARMOSET LYMPHOCYTES

B. PACHECO[1,2,3], L. MENÉNDEZ-ARIAS[1] AND J. SODROSKI[2,3,4]

[1]*Department of Virology and Microbiology, Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain*

[2]*Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA*

[3]*Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA*

[4]*Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA*

The presence of several barriers to HIV-1 replication in cells of many species narrows the viral tropism to humans and chimpanzees. The limited species tropism of HIV-1 is due to two types of host factors: 1) factors that are required for HIV-1 replication, but that exhibit species-specific changes that do not allow efficient use by HIV-1; and 2) dominant-acting factors that block replication in many hosts. The latter, also known as restriction factors, are part of the so-called intrinsic antiviral immunity. Altogether, intracellular restriction factors can act as a powerful barrier to stop viral replication. However, viruses have developed mechanisms that can antagonize restriction factors. Some of the restriction factors known to block replication of HIV-1 and other lentiviruses are TRIM5alpha, APOBEC3G, BST2, SAMHD1, and the recently discovered Mx2. However, several lines of evidence suggest the existence of additional restriction factors that block replication of lentiviruses.

To date, lentiviruses infecting New World monkeys have not been described. In addition, New World monkeys are apparently resistant to infection by known lentiviruses. We have been studying the susceptibility of common marmosets to HIV-1 infection and observed the presence of early post-entry blocks to HIV-1 infection in peripheral blood lymphocytes (PBLs) and B lymphocytic cell lines (B-LCLs). The blockades present in these cells are dominant and phenotypically different from each other. In PBLs, the blockade occurs at the level of reverse transcription, reducing the accumulation of early and late transcripts, as reported for TRIM5alpha. However, we have found that marmoset TRIM5alpha doesn't block HIV-1. In contrast, the restriction factor present in B-LCLs blocks HIV-1 replication at a later step. Additionally, we have generated a few capsid mutants that are able to escape restriction in the marmoset B-LCLs. Our results suggest that the restriction factors responsible for the blocks present in marmoset PBLs and B-LCLs are different. We propose the existence of at least two new restriction factors able to block HIV-1 infection in marmoset cells. The nature of these restriction factors is currently under investigation.

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16:00-16:15h (CO 21)

CHARACTERIZATION AND INHIBITION OF THE HIV-1 NUCLEOCAPSID MATURATION-CONDENSATION STEP

C. LORCA-ORÓ[1], S. LYONNAIS[1], S.K. SADIQ[2], C. LOPEZ-IGLESIAS[3], J.M. GATELL[1], A. MEYERHANS[2,4], G. MIRAMBEAU[1]

[1].AIDS Research group, IDIBAPS, Barcelona, Spain
[2]. Infection Biology Unit, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain
[3]. Cryo-Electron Microscopy, Scientific and Technological Centers, University of Barcelona, Barcelona, Spain
[4]. Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

The HIV-1 nucleocapsid protein (NCp) is highly conserved and plays multiple roles within the HIV life cycle. The NC domain of the Gag precursor recruits full-length genomic RNA to direct viral assembly. There is also an interaction with a cellular machinery for viral budding and an interplay with the protease for viral maturation leading within the viral core to condensed RNA and mature NCp (NCp7), via two intermediates (NCp9 and NCp15). Here, we focus on the effect of NC-RNA condensation and its inhibition on maturation. The aim of the study is to characterize the mechanism of action of potential NC inhibitors (NCIs).

A HIV-1 mutant that cannot lead to NCp9 and NCp7 (blocked at the NCp15 step) is not able to form a condensed NC and is also deficient to form a conical capsid. In vitro, with purified components, NCp15 is not able to condense a circular ssDNA template whilst NCp9 and NCp7 can. RNA condensation appears much faster within a HIV-1 particle than previously thought: particles at the final phase of budding are already imaged with a condensed NC at their centre. NCp15 in vitro processing by HIV-1 protease occurs within minutes if RNA is bound to it. This can be explained by an original mechanism where protease appears sequestrated by the NCp15-bound RNA complex, inducing a significantly faster turnover. Such a mechanism is consistent with a model, based on polymer physics, of enhanced protease diffusion within the complex.

NCIs have been recently highlighted as potential antiviral drugs. Members of this class, designed to dock within the NCp hydrophobic pocket, efficiently inhibit in vitro NCp7, NCp9 and NCp15 coating upon large single-stranded nucleic acids. We investigated their antiviral effects by two-round infectivity assays using free viruses in TZM-bl cells or chronically HIV-infected ACH-2 cells. Antiviral activity was stronger during the late steps of HIV replication and was within the µmolar range. Transmission electron microscopy showed that NCIs affect maturation. HIV particles, still in contact with the cell membrane, display aberrant morphologies, for both nucleocapsid and capsid, when cells are treated with NCIs. This effect suggests a defective HIV-1 NC assembly and

maturation as the main mechanism of action for NCIs.

The spatiotemporal coordination for HIV assembly, budding and maturation requires thousands of NC-RNA interactions. Disrupting these interactions by a NCI has multimodal effects. Here we provide the first proof of concept for an effect on HIV maturation.

The AIDS Research group, IDIBAPS, as member of the THINPAD consortium (thinpad.unisi.it) has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No 601969.

16-15-16:30h (CO 22)

PREVENTION OF HIV-1 VAGINAL TRANSMISSION AND MODE OF ANTIVIRAL ACTION BY TOPICAL POLYANIONIC CARBOSILANE DENDRIMER G2-S16 IN HUMANIZED BLT MICE

D. SEPÚLVEDA-CRESPO[1,2], Mª J. SERRAMÍA[1,2], R. GÓMEZ[3], F. J. DE LA MATA[3], J. L. JIMÉNEZ[2,*], Mª A. MUÑOZ-FERNÁNDEZ[1,2,*]

[1].*Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain.Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.Spanish HIV HGM BioBank, Madrid, Spain.*

[2].*Plataforma de Laboratorio, Hospital General Universitario Gregorio Marañón, Madrid, Spain. IISGM, Madrid, Spain. CIBER-BBN, Madrid, Spain*

[3].*Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, Alcalá de Henares, Madrid, Spain. CIBER-BBN, Madrid, Spain*

The HIV infection remains after decades one of the most aggressive epidemics around the world. Only in sub-Saharan Africa heterosexual transmission represent the 80% of the new infections, being the 50% of these new infections in women. Therefore, the development of a safe, effective, and low-priced topical microbicide to prevent the sexual transmission of HIV-1 is urgently needed. The emerging field of nanotechnology and its different nanosystems (i.e., dendrimers) play an important role in addressing this challenge.

Polyanionic carbosilane dendrimer G2-S16 has demonstrated potent and a broad-spectrum anti-HIV-1 activity *in vitro*. However, its antiviral activity in humanized (h)-BLT (bone marrow-liver-thymus) mice and its mode of action has not been completely elucidated. In this work, we focused on a preliminary efficacy study of vaginally applied G2-S16 on h-BLT mice. We also assessed the mechanism of antiviral of action on the inhibition of HIV-1 infection through a panel of different *in vitro* antiviral assays.

Topical vaginal administration of 3% G2-S16 prevented HIV-1_{JR-CSF} transmission in h-BLT mice in 84% without irritation or vaginal lesions. Our results also suggest that G2-S16 exerts anti-HIV-1 activity at an early stage of viral replication, as a virucidal agent and as an inhibitor of viral entry blocking the gp120/CD4 interaction. Moreover, we demonstrate the dendrimer's capability to provide a barrier to infection for long periods and to inhibit cell-to-cell HIV-1 transmission, confirming its multifactorial and non-specific ability.

This study represents the first demonstration indicating that HIV-1 vaginally infects humanized BLT mice and that transmission of the virus can be efficiently blocked by vaginally applied G2-S16. These results obtained in h-BLT mice provide a strong step forward in the development of G2-S16-based vaginal microbicides to prevent vaginal HIV transmission in humans.

16:30-16:45h (CO 23)

HEAD-TO-HEAD COMPARISON OF POXVIRUS NYVAC AND ALVAC VECTORS EXPRESSING IDENTICAL HIV-1 CLADE C IMMUNOGENS IN PRIME/BOOST COMBINATION WITH ENV PROTEIN IN NON-HUMAN PRIMATES

J. GARCÍA-ARRIAZA[1], B. PERDIGUERO[1], J. HEENEY[2], M. SEAMAN[3], D. MONTEFIORI[4], C. LABRANCHE[4], N. YATES[4], X. SHEN[4], G. TOMARAS[4], G. FERRARI[4], K. E. FOULDS[5], A. MCDERMOTT[5], S. KAO[5], M. ROEDERER[5], N. HAWKINS[6], S. SELF[6], J. YAO[7], P. FARRELL[7], S. PHOGAT[7], J. TARTAGLIA[7], S. W. BARNETT[8], B. BURKE[8], A. CRISTILLO[9], D. WEISS[9], C. LEE[10], K. KIBLER[11], B. JACOBS[11], B. ASBACH[12], R. WAGNER[12], S. DING[13], G. PANTALEO[14] AND M. ESTEBAN[1]

[1]Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.[2]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK. [3]Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA.[4] Duke University, Durham, USA. [5] Vaccine Research Center, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, USA.[6] Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, USA. [7] Sanofi Pasteur, Swiftwater, USA.[8] Novartis Vaccines, Cambridge, USA.[9] Advanced BioScience Laboratories, Inc., Kensington, USA. [10] Global Solutions for Infectious Diseases, San Francisco, USA.[11] The Biodesign Institute at Arizona State University, Tempe, USA.[12]University of Regensburg, Regensburg, Germany.[13] EuroVacc Foundation, Lausanne, Switzerland. [14] Division of Immunology and Allergy, Department of Medicine, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland.

We have compared the HIV-1-specific cellular and humoral immune responses elicited in rhesus monkeys immunized with two poxvirus vectors (NYVAC and ALVAC) expressing the same HIV-1 antigens from clade C, Env gp140 as a trimeric cell released protein and Gag-Pol-Nef as Gag-induced virus-like particles (VLPs) (referred as NYVAC-C and ALVAC-C). The immunization protocol consisted of two doses of the corresponding poxvirus vector plus two doses of a combination of the poxvirus vector and a purified HIV-1 gp120 protein from clade C. This immunogenicity profile was also compared to that elicited by the RV144 trial vaccine regimen consisting of two doses of the ALVAC vector expressing HIV-1 antigens from clades B/E (ALVAC-vCP1521) plus two doses of a combination of ALVAC-vCP1521 and HIV-1 gp120 protein from clades C or B/E. The results showed that immunization of macaques with NYVAC-C stimulated more potent HIV-1-specific $CD4^+$ and $CD8^+$ T-cell responses than those induced by ALVAC-C. Furthermore, NYVAC-C induced a trend toward higher levels of binding IgG antibodies against clade C HIV-1 gp140, gp120 or MuLV gp70-scaffolded V1/V2 and toward best cross-clade binding IgG responses against HIV-1 gp140 from clades A, B and group M consensus, compared to

ALVAC-C. Most of these binding IgG responses were directed against the V3 loop in all immunization groups. Additionally, NYVAC-C and ALVAC-C also induced similar levels of HIV-1 neutralizing antibodies and antibody-dependent cellular cytotoxicity (ADCC) responses. Interestingly, binding IgA antibodies against HIV-1 gp120 or MuLV gp70-scaffolded V1/V2were absent or very low in all immunization groups.Overall, these results provide a comprehensive survey of the immunogenicity of NYVAC versus ALVAC expressing HIV-1 antigens in non-human primates and indicate that NYVAC may represent an alternative candidate to ALVAC in the development of a future HIV-1 vaccine.

16:45-17:00h (CO 24)

IMMUNOVIROLOGICAL TRAITS OF HIV SUBJECTS WITH DELAYED INITIATION OF cART AND SUBSEQUENT POOR CD4 RESTORATION. STUDY ON PRE-TREATMENT SAMPLES

I ROSADO[1], M LEAL[1], YM PACHECO[1]

[1]*Laboratory of Immunovirology, Clinic Unit of Infectious Diseases, Microbiology and Preventive Medicine, Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital /CSIC /University of Seville, Seville, Spain.*

One out of four HIV-infected subjects with delayed initiation of combined antiretroviral therapy (cART) (below 200 CD4 cells/µL) does maintain low levels of CD4 T cells (below 250 CD4 cells/µL) despite a subsequent long-term effective treatment. These HIV-infected subjects are at an increased risk of clinical progression and death, and no therapeutic alternative is yet available. At the post-treatment time point, when these patients have no restored CD4 levels and persist with very low CD4 T cell counts, they show critical immunovirological alterations. Thus, they finally show increased T-cell activation, senescence and apoptosis, lower thymic function, increased Treg frequency and their virus are more likely X4-tropic. However, whether these factors are cause or consequence of the persistence of low CD4 T cell counts is unknown.

To date, no previous study has focused on their potential early immunovirological alterations. This information could be crucial to help to prematurely recognize these patients by clinicians and for better understanding subjacent mechanism of such failure. Our more recent research address pre-treatment samples from HIV-infected subjects with late diagnosis, focusing on groups of subjects with later poor CD4 restoration and good CD4 restoration, in response to cART. Importantly, at the onset of the cART initiation, both groups had similar, but low, CD4 levels and viral loads (and were also matched by age and sex). With a very complete study, including immunophenotyping of several cell types (monocytes, T cells, dendritic cells,…), an exhaustive profile of soluble markers, and novel *omics* approaches (such as metabolomics and transcriptomics), we are getting involved in a very innovative and interesting project which is yielding relevant information. This is a major collaborative project of the *Spanish AIDS Research Network* (*RIS*); belonging to the WP3 "*Immunological damage and reconstitution*", inside the research programme "*HIV immunopathogenesis and*

vaccines". Samples for the study have been obtained from the *Spanish HIV Biobank*.

Parallel Session III: PLANT VIRUS

Chairpersons:

JESÚS NAVAS AND VICENTE PALLÁS

Monday June 8, 2015

AUDIOVISUAL ROOM

15:00-15:15h (CO 25)

STRUCTURAL AND FUNCTIONAL DIVERSITY OF PLANT VIRUS 3´-CAP-INDEPENDENT TRANSLATIONAL ENHANCERS

V. TRUNIGER[1], M. MIRAS[1], A.M. RODRÍGUEZ-HERNÁNDEZ[2], C. ROMERO-LÓPEZ[3], A. BERZAL-HERRANZ[3], M.A. ARANDA[1]

[1]. *Grupo de Patología Vegetal. Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC). Apdo. correos 164, 30100 Espinardo, Murcia, Spain*

[2]. *Centro de Investigación en Química Aplicada (CIQA). Consejo Nacional de Ciencia y Tecnología (CONACYT). Blvd. Enrique Reyna Hermosillo 140, 25294, Saltillo, Coahuila, México.*

[3]. *Instituto de Parasitología y Biomedicina López-Neyra. Consejo Superior de Investigaciones Científicas (IPBLN-CSIC). Av. Conocimiento s/n. 18016. Armilla (Granada). Spain*

Viral mRNAs have evolved numerous mechanisms to recruit the host translational machinery, allowing them to compete with host mRNAs and avoid defence mechanisms that act at the level of translation. Thus, while most plant-encoded mRNAs contain a 5´-cap and a poly(A)-tail that act synergistically to stimulate translation, ~80% of known positive-strand RNA plant viruses lack one or both of these features in their genomic and subgenomic RNAs. Some of them contain in their 3´-UTRs RNA elements able to enhance their cap-independent translation (3´-CITEs). We have shown that cap-independent translation of *Melon necrotic spot virus* (MNSV, family *Tombusviridae*, genus Carmovirus) RNA is controlled *in cis* by a 3´-CITE (Truniger et al., 2008.*Plant J.* 56:716-727). Remarkably, MNSV 3´-CITEs are diverse, including at least Mα5TE, M264TE and CXTE (Truniger et al., 2008; Miras et al., 2014, *New Phytol.* 202:233-246). The CXTE has been acquired by recombination with an Asiatic isolate of *Cucurbit aphid-borne yellows virus* (CABYV; family *Luteoviridae*, genus Polerovirus), suggesting that 3´-CITEs are modular, interchangeable structural elements. Here we show that CABYV Asiatic and European isolates have two different translational enhancers. For all different 3´-CITEs that we have identified, we analysed their secondary structure and showed that their modes of action differ. While M□5TE depends on the eukaryotic translation initiation factor eIF4E, the other four are eIF4E-independent, conferring translational competence to RNAs in the absence of this factor. On the other hand, we showed that the translation enhancer activity of all five 3´-CITEs depends on the presence of the 5´-UTR *in cis*. For the M□5TE we provided evidence that viral RNA circularization is achieved by long-distance interactions between the 5´- and 3´-ends based on sequence complementarity. This interaction involves nucleotides of the 3´-CITE that have two complementary nucleotide stretches at the 5´end, one in the first 20 nucleotides of the 5´-UTR, the other at the beginning of the coding region of the first ORF. For the

M□5TE, perfect complementarity with the 5´end sequence is required *in vivo* for its translational enhancer activity, and it is therefore essential for virus viability.

15:15-15:30h (CO 26)

EXPRESSION AND FUNCTION OF THE TRANS-FRAME P1N-PISPO GENE PRODUCT OF THE POTYVIRUS *SWEET POTATO FEATHERY MOTTLE VIRUS* (SPFMV)

A. MINGOT[1], A. VALLI[2], B. RODAMILANS[3], D. SAN LEÓN[3], D.C. BAULCOMBE[2], J.A. GARCÍA[3], J.J. LÓPEZ-MOYA[1]

[1]*Center for Research in Agricultural Genomics CRAG, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, Cerdanyola del Vallès, 08193-Barcelona, Spain*

[2]*Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, United Kingdom*

[3]*Centro Nacional de Biotecnología CNB, CSIC, Darwin 3, 28049-Madrid, Spain*

Potyviruses (genus *Potyvirus*, family *Potyviridae)* are plus strand RNA viruses with a genomic organization similar to *Picorna*-like viruses. The genome of potyviruses is characterized by the presence of a large ORF yielding a polyprotein, later autoproteolitically processed into several mature gene products (P1, HCPro, P3, 6K1, CI, 6K2, VPg-NIa, NIb and CP). In addition, a short ORF named PIPO can be found embedded within the P3 region in another frame, starting at a conserved G1-$_2$A$_{6-7}$ motif. This coding sequence is able to generate an essential P3N-PIPO product. Recently, another additional ORF named PISPO was identified *in silico* within the P1 region in the genome of *Sweet potato feathery mottle virus* (SPFMV) and several related sweet potato potyviruses. Expression of this ORF could result in a putative new gene product P1N-PISPO.

The presence of the P1N-PISPO protein has been investigated in *Ipomoea batatas* plants infected with a Spanish isolate of SPFMV. The genome sequence of this isolate was assembled from NGS data, showing that the expected trans-framed PISPO sequence was present, preceded by a G_2A_6 domain. The predicted size for the P1N-PISPO protein was 72.7 KDa. Analysis of the viral gene products present in infected plant tissues was performed using LC-MS/MS after separation in SDSPAGE, focusing in products >50KDa. Detected viral peptides corresponded to proteins such as CI (72 KDa) and HCPro (52.1 KDa), all of them translated from the viral ORF.Moreover, peptides corresponding to the P1 protein were detected from both the N-terminal portion (11 different peptides, 39% coverage), before the frameshifting signal and therefore common for P1 and P1N-PISPO, and in the C-terminal part (2 peptides exclusive for P1, 10% coverage). Interestingly, four peptides exclusive of PISPO, in its unique ORF (21.3% coverage), were also found. These results indicated that both products P1 and P1N-PISPO were expressed in SPFMV infected plants.

To determine the possible function of the P1N-PISPO product during infection, constructs adequate for transient expression were prepared and tested for RNA silencing suppressor (RSS) activity. While in other potyviruses the RSS function is associated to HCPro, our results showed RSS activity for the P1N-PISPO product. The mode of action of this new RSS compared to other RSS from members of the *Potyviridae* family will be discussed.

(Work funded by Mineco grant AGL2013-42537-R. A. Mingot received FPI fellowship BES-2011-045699).

15:30-15:45h (CO 27)

INHALED DELIVERY OF PEGYLATED DENDRIMERS: PHARMACOKINETICS AND THERAPEUTIC POTENTIAL

X. AGIRREZABALA[1], F.E. MÉNDEZ[2], G. LASSO[3], M.A. SÁNCHEZ-PINA[2], M.A. ARANDA[2] AND M. VALLE[1]

[1] *Structural Biology Unit, CIC bioGUNE, Derio, Spain*
[2] *Dpto. de Biología del Estrés y Patología Vegetal, CEBAS-CSIC, Murcia, Spain*
[3] *Department of Biochemistry and Molecular Biophysics, Columbia University, New York, USA*

Flexible filamentous viruses belonging to the *Alphaflexiviridae* family cause from severe to mild diseases in agricultural crops, often reducing yield and crop quality. Viral particles from the Alphaflexiviridae group are formed by a (+)ssRNA molecule encapsidated by single capsid protein (CP) monomers arranged in helical symmetry. *Pepino mosaic virus* (PepMV) is a flexible filamentous plant virus belonging to the genus Potexvirus included in the family *Alphaflexiviridae* which genome consists of a ~6.4 kb (+)ssRNA and encodes five proteins.

In sharp contrast with rigid plant viruses such as *Tobacco mosaic virus*, little is known about the structure of flexible filamentous plant viruses at high resolution. Their intrinsic flexibility precludes high-resolution studies by fiber diffraction or Xray crystallography, and reported cryoEM structures stay at resolutions above 1 nm (Kendall et al., 2012, Virology 436 p.173). A clear exception is the case of the CP from Papaya mosaic virus (PaMV) whose Xray structure was reported. Nevertheless, the atomic data for PaMV CP was obtained with purified protein, not from virions, and lacking around 20% of the protein mass at its C-terminal domain (Yang et al., 2012, J. Mol. Biol. 422 p. 263).

We report here the structure of intact PepMV virions by cryoEM at 4*Å resolution.* The results and the resolution achieved allowed the modeling of the CP, the (+)ssRNA and their relative interactions. The CP was modeled starting with the CP from PaMV, clearly showing a similar folding of the α-helical domain for the *Alphaflexiviridae* family. The ssRNA is allocated and protected in a continuous groove of high electropositive potential built up by the CP in helical arrangement. The CP polymerize through a flexible N-terminal arm providing the structural basis for the flexibility of the virus. Interestingly, the overall structure and organization of CP from PepMV is similar to the organization of nucleoproteins from the *Bunyaviridae* family, a group of enveloped (-)ssRNA viruses. Common features include: the folding and arrangement of the α-helical main domain; the groove for the ssRNA; the N-terminal arm for polymerization; and the relative position between all these elements. Although structural homology between viruses revealed by their atomic structures is common, in the current case, the different nature of capsid protein and nucleoprotein might have profound evolutionary implications.

15:45-16:00h (CO 28)

GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE IS CO-OPTED FOR REPLICATION OFCITRUS TRISTEZA VIRUS VIA INTERACTION WITH THE VIRAL-ENCODED PROTEIN P23

S. RUIZ-RUIZ[1], R. SPÀNO[1], S. DAVINO[1], L. NAVARRO[2], P. MORENO[2], L. PEÑA[1], R. FLORES[1]

[1]*Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia, Spain*

[2]*Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain*

Citrus tristeza virus (CTV), genus *Closterovirus*, family *Closteroviridae,* is an important pathogen that has killed more than 50 million citrus trees in Spain. CTV genome is a positive-sense RNA of approximately 19.3 kb organized in 12 open reading frames (ORFs) potentially coding for at least 17 proteins. One of them, p23, encoded by the 3'-terminal ORF, has no homologues in other closteroviruses and, therefore, is distinctive. CTV-p23 has also peculiar features: i) it is an RNA-binding protein of 209 amino acids with a putative Zn-finger domain and some basic motifs, ii) it accumulates mainly in the nucleolus and Cajal bodies, and in plasmodesmata, and iii) it mediates many functions including the asymmetric accumulation of CTV RNA strands, the intracellular suppression of RNA silencing, and the induction of some CTV syndromes when expressed from the virus, and of CTV-like symptoms and enhancement of systemic infection when expressed ectopically as a transgene in several *Citrus* spp. To search for host interactors of p23, an initial yeast two-hybrid (Y2H) screening of an expression library of *Nicotiana benthamiana* (in which at least one CTV isolate replicates and incites symptoms) led to the identification of the cytoplasmic glyceraldehyde 3-phosphate dehydrogenase (GAPDH), further confirmed in 1-by-1 Y2H tests. Bimolecular fluorescence complementation assays *in planta* corroborated the previous results and provided new insights. Briefly, p23 interacts with itself in the nucleolus, Cajal bodies and plasmodesmata, and with GAPDH (in the cytoplasm forming aggregates) and in plasmodesmata. This latter interaction was preserved in a p23 deletion mutant affecting the C-terminal domain, but not in two other deletion mutants affecting the Zn-finger domain and one internal basic motif. Most importantly, qRT-PCR and RNA gel-blot hybridization showed that virus-induced gene silencing of GAPDH mRNA resulted in a significant decrease in CTV titer. Altogether these data suggest that, paralleling the situation observed in a tombusvirus (Wang and Nagy, *Cell Host and Microbe* 2008), CTV co-opts GAPDH through p23 to convert the host cell into a viral factory. The finding that two very different viruses co-opt for their replication the same host protein (GAPDH) suggests that this protein is endowed with an intrinsic feature, possibly its RNA-binding ability, that facilitates the process.

16:00-16:15h (CO 29)

BIOLOGICAL CHARACTERIZATION OF NON-CODING DNA SATELLITES ASSOCIATED TO NEW WORLD BEGOMOVIRUSES

E.FIALLO-OLIVÉ, R. TOVAR, J. NAVAS-CASTILLO

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga - Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Estación Experimental "La Mayora", 29750 Algarrobo-Costa, Málaga, Spain

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) cause serious diseases in a number of economically important crops, mostly in tropical and subtropical regions. They are plant ssDNA viruses that are transmitted by the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae). Begomoviruses have been shown to be helper viruses for a number of distinct DNA satellites, including betasatellites and alphasatellites. During a survey in Cuba, we found two malvaceous species, *Malvastrum coromandelianum* and *Sidastrum micranthum*, infected with bipartite begomoviruses associated with ssDNA molecules of a quarter the size of the begomoviral genome components. These molecules shared some genetic features with betasatellites and ToLCV-sat such as an A-rich region, but also contained nucleotide stretches of begomoviral origin, presumably the remains of recombination events involved in their origin (Fiallo-Olivé *et al.*, 2012). In this work we have developed infectious clones of two ssDNA satellites, from *M. coromandelianum* and *S. micranthum*, respectively. Agroinoculation of satellites together with their helper begomoviruses showed that satellites were replicated in *Nicotiana benthamiana* plants and in their natural malvaceous hosts. Replication of these satellites is also supported by other geminiviruses, including the monopartite New World begomovirus *Tomato leaf deformation virus*. Our results confirmed that these molecules are indeed satellites of New World bipartite begomoviruses and constitute a novel class of such subviral agents.

16-15-16:30h (CO 30)

INTERFERENCE OF SINGLE AND DUAL BIOTIC STRESSES ON HOST DNA METHYLATION PATHWAYS

E.M. TORCHETTI[1], M. PEGORARO[2], B. NAVARRO[1], M. CATONI[3], E. NORIS[2], F. DI SERIO[1]

[1].*Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, UOS Bari, Italy*

[2].*Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Torino, Italy*

[3].*Sainsbury Laboratory, University of Cambridge, Cambridge, UK*

DNA methylation (DM) pathways play major roles in preservation of genome integrity, transposon stability and regulation of gene expression. In plants, DM has also been involved in responses to abiotic and biotic stresses, including defense against geminiviruses (GV), a large group of viruses with a single-stranded DNA genome that replicates in the nucleus forming minichromosomes associated with cellular histones. It is proposed that host DM machinery impairs viral accumulation in the infected tissues by targeting GV DNA for methylation. In contrast, whether DM is involved in the molecular interplay between plants and nuclear replicating viroids, which are infectious non-protein-

coding RNAs frequently inducing severe diseases in plants, is still unclear. Viroid RNAs are targeted by host enzymes involved in DM pathways, but whether the genes implicated in this pathways are differentially regulated in response to viroid infection is unknown. In addition, whether DM pathways may differentially target host and GV DNA depending on the presence or absence of a nuclear infecting viroid is also not known. To further explore the interference of single and dual infections by nuclear replicating infectious agents, we have developed an experimental system based on tomato plants infected by the geminivirus *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and/or the nuclear-replicating *Potato spindle tuber viroid* (PSTVd). DNA methylation profiles of TYLCSV DNA and of two host genomic targets were tested as molecular sensors of host DM under stress conditions. Moreover, expression of genes involved in DM was investigated at transcriptional level by quantitative RT-PCR assays. Our data show that both TYLCSV and PSTVd interfere with the regulation of most host genes involved in DM pathways and, interestingly, that the plant response to a single stress strongly differs from that to dual stresses, with synergistic effects.

16:30-16:45h (CO 31)

UNRAVELLING THE RNA2-ENCODED POLYPROTEIN CLEAVAGE SITES OF TOMATO-INFECTING TORRADOVIRUSES USING N-TERMINAL PROTEIN SEQUENCING AND A REVERSE GENETICS SYSTEM

I. FERRIOL[1], D. MARQUES DA SILVA[2], M. TURINA[3], E. J. ZAMORA-MACORRA[4], B. W. FALK[1]

[1] *Plant Pathology Department, University of California Davis, 95616, Davis, CA, USA*

[2] *CAPES Foundation, Ministry of Education of Brazil, Brasilia-DF, 70040-020, Brazil*

[3] *Istituto per la Protezione Sostenibile delle Piante, Sez. di Torino, CNR, Turin, Italy*

[4] *Colegio de Postgraduados-Campus Montecillo, 56230, Texcoco, Mexico*

In a time span of just two decades, a new group of RNA plant viruses, the torradoviruses, has been discovered affecting tomatoes and other plant species. The genus *Torradovirus* includes three species: i) *Tomato torrado virus* (ToTV), first found in Europe, and afterward in Central America and Australia; ii) *Tomato marchitez virus* (ToMarV) (also called *Tomato apex necrosis virus*,ToANV), present in Mexico; and iii) *Lettuce necrotic leaf curl virus* (LNLCV) infecting lettuce in the Netherlands. Six new tentative species have been discovered: i) tomato chocolate spot virus(ToChSV) and tomato chocolàte virus(ToChV), both found infecting tomato in Guatemala; ii) tomato necrotic dwarf virus (ToNDV), which infected tomato in California in the mid-80s; iii) cassava torrado-like virus (CsTLV) infects cassava in Colombia; iv) motherwort yellow mottle virus (MYMoV) infects motherwort in Korea; and v) carrot torradovirus 1 (CTV-1),

which infects carrot in UK. All known tomato-infecting torradoviruses are transmitted by whiteflies. Torradoviruses have a bipartite genome consisting of two single-stranded plus-sense RNAs. RNA1 is ca. 7 kb and has one open reading frame (ORF), which encodes replication-associated proteins including the protease, helicase and RNA-dependent RNA polymerase (RdRp). RNA2 is ca. 5 kb and has two ORFs. The ORF1 in RNA2 is unique for torradoviruses, but the functions of its encoded protein are still unclear. The ORF2 has coding regions for a putative movement protein and the three capsid proteins. Little is known about the functions of torradovirus proteins, their replication and polyprotein strategies. Here, we developed an agroinoculation system for the tomato-infecting torradovirus,ToANVthat can trigger infection in *Nicotiana benthamiana*, tomato and tomatillo plants, and will allow us to elucidate torradovirus protein functions. To better understand the tomato-infecting torradovirus polyprotein processing, the cleavage sites in the RNA2 ORF2-encoded proteins of two tomato-infecting torradoviruses (ToANV and ToChSV) were determined by N-terminal sequence analysis. These results showed that the amino acid at the -1 position of the cleavage sites is a Gln (Q). Amino acid sequence comparison of different isolates of ToANV confirmed that this Gln (Q) is also conserved among different isolates of ToANV, and among other members of the genus *Torradovirus*. Finally, site-directed mutagenesis of the RNA dependent RNA polymerase and protease abolished the replication and polyprotein processing of ToANV.

16:45-17:00h (CO 32)

ANALYSIS OF TOLERANCE MECHANISMS AND TRADE-OFFS IN PLANT-VIRUS INTERACTIONS

V. VIJAYAN& I. PAGAN

Centro de Biotecnologia y Genomica de Plantas (UPM-INIA), Madrid

Viruses are important selective forces for their hosts. As a consequence, hosts have developed a variety of mechanisms to prevent or limit virus infection (Resistance), and to reduce the effect of virus infection in the host fitness (Tolerance). Although resistance has been extensively studied, comparatively less is known about which, and how specific, are the host tolerance mechanisms. Because biological fitness cannot be maximized in every situation, mechanisms that increase tolerance to infection by a given virus may come at the cost of reduced tolerance upon infection with other viruses with different life-history traits, i.e., tolerance trade-offs. However, how tolerance is achieved and the potential trade-offs of tolerance to different viruses have been seldom analyzed.

We have analyzed trade-offs in tolerance to infection by *Cucumber mosaic virus* (CMV) and *Turnip mosaic virus* (TuMV) in their natural host *Arabidopsis thaliana.* In *Arabidopsis*, tolerance to CMV is achieved by resource reallocation from vegetative to reproductive structures of the plant. Such phenotypic plasticity is a characteristic of accessions with longer life cycles and slower growth rates. However, this tolerance mechanism might not be effective against more virulent viruses, as TuMV, which may not give the plant

enough time to reallocate resources. To address this subject, 19 accessions for which tolerance to CMV has been previously analyzed were challenged against TuMV. In each accession, virus multiplication, virulence (as effect of virus infection on plant's seed production), effect of TuMV infection on plant growth, and length of vegetative and reproductive periods were quantified. This data was compared with values obtained upon CMV infection.

Results indicated that TuMV multiplication was generally similar in the 19 accessions analyzed. Accessions with shorter life cycles and faster growth rates were more tolerant to TuMV infection than those with larger life cycles and slower growth rates. Therefore, these results were at odds with response of the same accessions to CMV infection, and are compatible with trade-offs of tolerance to TuMV and CMV infection. Interestingly, infected plants of the accessions that were tolerant to TuMV infection showed shorter vegetative periods than the corresponding control individuals, suggesting that this could be an alternative method of achieving tolerance.

In summary, our results provide evidence of tolerance trade-offs in plantvirus interactions, and suggest that *Arabidopsis* plants may achieve tolerance by mechanisms other than resource reallocation.

Parallel Session IV: ANIMAL VACCINES

Chairpersons:

FERNANDO RODRIGUEZ AND

ALEJANDRO BRUN

Monday June 8, 2015

AUDITORIUM REAL CASA DE LA MONEDA

17:30-17:45h (CO 33)

A NOVEL STRATEGY FOR MULTISEROTYPE PROTECTION AGAINST BLUETONGE VIRUS USING muNS-Mi MICROSPHERES AND RECOMBINANT MVA EXPRESSING VP2, VP7 AND NS1 PROTEINS

A. MARÍN-LÓPEZ[1], I.OTERO-ROMERO[2], F. DE LA POZA[1], R. MENAYA-VARGAS[2], E. CALVO-PINILLA[1], J. BENAVENTE[2], J. M. MARTÍNEZ-COSTAS[2] AND J. ORTEGO[1]

[1]. Centro de Investigación en Sanidad Animal, INIA-CISA, Valdeolmos,Madrid, Spain.

[2]. Centro de Investigación en Química Biológica y Materiales Moleculares (CIQUS), Universidad de Santiago de Compostela, Spain.

Recent worldwide outbreaks ofbluetongue virus (BTV) reveal the necessity of controlling and preventing this hemorrhagic disease that affects ruminants. One of the most effective measures against infection is vaccination. The inactivated BTV vaccines that are now being used in Europe are effective in preventing outbreaks of BTV but they are serotype-specific and secondary effects have been associated with repetitive inoculation of aluminum-containing adjuvants. There are 27 known and two further putative BTV serotypes. Consequently, the need to develop, multiserotype, safer and more efficacious vaccines with differential diagnostic capability have re-ignited the interest in

developing improved vaccination strategies against BTV. We have engineered a subunit BTV vaccine candidate based on proteins VP2, VP7, and NS1 of BTV-4 incorporated into avian reovirus (ARV) muNS-Mi microspheres with potent intrinsic adjuvant activity (MS-VP2/MS-VP7/MS-NS1) and recombinant modified vaccinia virus Ankara (rMVA) expressing VP2, VP7 and NS1 proteins from BTV-4 (rMVA -VP2/ rMVA -VP7/ rMVA-NS1).

IFNAR(-/-) mice immunized with MS-VP2/MS-VP7/MS-NS1 in a homologous prime-boost vaccination generated significant humoral and cellular immune response. Immunized mice were fully protected against a homologous challenge with a lethal dose of BTV-4 and partially cross-protected against a heterologous challenge with a lethal dose of BTV-1.

The combination of these two antigen delivery systems, microspheres and rMVAs, in a heterologousprime-boost vaccination strategy maintained the induction of significant levels of neutralizing antibodies. Interestingly, this strategy elicited a stronger cellular immune response than the homologous immunization with microspheres, and fully protected immunized IFNAR(-/-) mice against homologous and heterologous challenges with lethal doses of BTV-4 and BTV-1. These results support the strategy based on microspheres in combination with rMVAs expressing BTV antigens as a promising multiserotype vaccine candidate against BTV.

17:45-18:00h (CO 34)

TRIAL FOR CHECKING THE PROTECTIVE IMMUNITY OF A COMMERCIAL VACCINE AGAINST THE "NEW VARIANT" OF THE RABBIT HAEMORRHAGIC DISEASE VIRUS

M. DURÁN FERRER, A. SÁNCHEZ SÁNCHEZ, J.I. VARO JIMÉNEZ, ELENA SAN MIGUEL IBÁÑEZ, R. VILLALBA MARTÍNEZ, ISABEL GONZALO PASCUAL, F. GARCÍA PEÑA, M. AGÜERO GARCÍA

Laboratorio Central de Veterinaria. MAGRAMA. Algete, Madrid. Spain.

Rabbit haemorrhagic disease is an acute, highly contagious and fatal viral disease caused by a member of the genus Lagovirus and family Caliciviridae that affects wild and domestic members of species *Oryctolagus cuniculus*. Many strains of RHDV with distinct epidemiological and genetic characteristics appear to circulate in nature. Although a single serotype has been described until now, three major subtypes have been reported: RHDV, antigenic variant RHDV*a* and antigenic variant RHDV*b*. The RHDV*b*, also called "RHDV new variant", infects farmed adult individuals previously immunized with vaccines against the other subtypes and singularly induces clinical disease in young rabbits.

The study presented here checks the efficacy of a vaccine temporarily licensed in Spain for clinically protecting 36-39-days-old New Zealand White rabbits (vaccinated 7 days before), against a wild strain of the RHDV*b*. The experimental design followed the European Pharmacopoeia (8.0 version) and minimized the number of individuals due to animal welfare reasons.

Experimental groups were as follows: vaccinated group (VG, n=12), control group 1 (CG-1, n=7), control group 2 (CG-2, n=6) and witness group (WG, n=5). VG was vaccinated following the manufacturer instructions (0.5 ml/SC route). Seven days after, the VG and the CG-1 were challenged (0.2 ml/IM) with full dose of virus (100 x LD_{50}) and the CG-2 with a reduced dose (1 x LD_{50}). The inoculum consisted of diseased young rabbit liver diluted homogenates that were characterized as RHDV new variant by a specific rt-RT-PCR previously developed (Rocha et al., 2013). The WG did not received any intervention.

The animals were clinically monitored during 7 days (CG-1, CG-2) and 13-14 (VG and WG) after challenge, respectively, and the survivors euthanized and subjected to necropsy. Sera and liver samples were taken for antibodies (ELISA, HI test), antigen (ELISA, HA test) and nucleic acid detection (rt-RT-PCR), as well as for histological studies.

The trial resulted valid: 100% (7/7) morbidity and mortality in the CG-1; 50% (3/6) morbidity and mortality in the CG-2; 100% (5/5) survival in the WG; and the vaccine showed its efficacy for protecting 100% (12/12) of young rabbits against the clinical disease.

18:00-18:15h (CO 35)

SYLVATIC RABIES IN THE NORTH-EAST OF ITALY: MONITORING AND EVALUATION OF THE EFFECTIVENESS OF PROPHYLAXIS IN WORKERS AT RISK AND TRAVELERS

N. INGLESE[1], C. SALATA[1], P. DE BENEDICTIS[2], G. CARPENÈ[3], R. MEL[3], M. BEVILACQUA[4], S. SCHMORAK[4], P. ROSSI[5], E. PAGANI[5], P. MULATTI[2], G. PALÙ[1], L. BONFANTI[2], F. MUTINELLI[2], P. D'AGARO[6], D. SANTON[6], T. GALLO[7], S. MARANGON[2], P.TOMAO[8],N. VONESCH[8].

[1]. University of Padua, Department of Molecular Medicine, Padua, Italy
[2]. Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Italy
[3]. UlSS1di Belluno, Servizio di Igiene e Sanità Pubblica, Belluno, Italy
[4]. Azienda Sanitaria dell'Alto Adige, Comprensorio Sanitario di Merano, Servizio Igiene e Sanità Pubblica, Merano, Bolzano, Italy
[5]. Azienda Sanitaria dell'Alto Adige, Comprensorio Sanitario di Bolzano, Laboratorio Aziendale di Microbiologia e Virologia, Bolzano, Italy
[6]. University of Trieste, Department of Medical, Surgical and Health Sciences; I.R.C.C.S. "Burlo Garofolo", Trieste, Italy
[7]. Azienda per servizi sanitari n.4 (ASS4), "Medio Friuli", Dipartimento di Prevenzione, Udine, Italy.
[8]. INAIL,Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro SETTORE RICERCA - Dipartimento di Medicina del Lavoro, Monteporzio Catone , Rome, Italy

Rabies is a global zoonotic disease that occurs in developing and developed countries, producing consistently fatal encephalitis in humans and animals. Rabies virus infects mammals through infected saliva via bites or scratches, although atypical exposures have been documented. In late 2008, wildlife rabies re-emerged in Northeastern Italy in an area bordering Slovenia, spread to Veneto region (Belluno province) and to the autonomous province

of Trento and Bolzano. Since then, 287 animal cases have been detected in wild and domestic animals; the last one has been diagnosed in a red fox in February 2011. No human cases have been reported linked to the recent epidemic and Italy has been declared as free from rabies in February 2013. Several oral fox vaccination campaigns accompanied by efficacy monitoring and extensive surveillance of territories affected by the epidemic have been implemented together with education and preventive vaccination of workers at risk of viral exposure (i.e. forestry and wildlife workers, veterinarians, shelters 'operators and laboratory personnel). The aim of this work was the evaluation of the rabies antibodies level and persistence in workers at risk of exposure and travelers. A total of 347 serum samples were collected: 169 after pre-exposure prophylaxis and 178 after post-exposure prophylaxis performed with different immunization schedules. All sera have been tested to detect rabies virus anti-glycoprotein antibodies by a commercial quantitative indirect ELISA (Platelia TM Rabies II kit; Biorad) and with the reference method FAVN (Fluorescent Antibody Virus Neutralization), according to the procedure recommended by the WHO. The results on the protection level, persistence of antibodies and the comparison between the ELISA and FAVN test will be discussed.

18:15-18:30h (CO 36)

ROLE OF INFLUENZA VIRUS SMALL RNAs CONTROLLING PATHOGENICITY IN VIVO

J. VASILIJEIVC[1, 2], G. GÓMEZ[1], A. NIETO[1, 2] AND A. FALCÓN[1, 2]

[1].*Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain*

[2]*Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (Ciberes), Spain.*

Influenza virus particles contain small RNAs (svRNAs) corresponding to large internal deletions of viral segments generated during in vitro serial passages, which are known as defective interfering particles (DIs). The presence of DIs, potentiate the immune response both in cell cultures and animal models, possible through recognition of double-stranded RNA by different receptors activating antiviral signaling cascades. Accordingly, a single dose of the DIs given several days before inoculation, protects elderly mice and reduces a severe and fatal disease to subclinical and mild infection, probably through activation of antiviral response. Recently the presence of svRNAs in pandemic pH1N1 infected patients has been reported, but at present nobody has evaluated their possible correlation with pathogenicity in humans.

We have analyzed differential virulence among pandemic pH1N1 circulating viruses, comparing the pathogenicity of a virus from a fatal case (F), with a virus that caused mild symptoms (M), both of them without any known co-morbid condition. Higher rate of replication in cell cultures and increased mortality in infected mice was found in the F virus. The high-throughput sequence of virions from these

two strains and six more pandemic isolates showed the presence of small viral RNAs (svRNAs) in all samples, but interestingly the fatal virus contained much lower amounts of these svRNAs. These svRNAs possess the 5' and 3' ends of the parental RNA segments and most have large, single, central deletions. They have been found for all segments, but the majority of them belong to PB2 and PB1 segments.

Activation of innate immune response has been evaluated in M or F virus-infected human lung epithelial cells. This study showed that F virus induces lower amounts of type I interferon and interferon stimulated genes than M virus.

Recombinant viruses with the individual changes found in the F virus were obtained. That one having D529N mutation in the PA polymerase subunit produced less amount of svRNAs in cell culture and was even more pathogenic than the original F isolate in the mice. In addition, deep sequencing of RNA from lungs of mutant PA D529N or control virus-infected mice has been performed and we are now evaluating their differential genome expression profile.

These results suggests that a specific residue in the viral polymerase, found in a virus isolated from a fatal case, is responsible for svRNAs synthesis and establishes a correlation between amount of svRNAs and virus pathogenicity in the mice model and possibly in humans.

18:30-18:45h (CO 37)

BLOCKING OF TYPE I IFN PATHWAY BY PESTE DES PETITS RUMINANTS VIRUS (PPRV)

M. AVIA, G. RANGEL, E. PASCUAL, V. MARTÍN AND N. SEVILLA

Centro de Investigación en Sanidad Animal (CISA)-INIA, Madrid, Spain.

Peste des petits ruminants virus (PPRV) is the causative agent of an economically significant and highly contagious disease of small ruminants, peste des petites ruminants (PPR). PPRV belongs to the *f*amily *Paramyxoviridae*, genus *Morbillivirus*,which includes important pathogens as Measles virus (MeV) in humans or Rinderpest virus (RPV) in animals. The P gene of *Morbillivirus* encodes for the P protein, and also for three non-structural proteins: the V and W proteins, that are produced by co-transcriptional insertion of additional G residues into a fraction of the mRNAs transcribed from the P gen, and the C protein, that results from translation of an alternative open reading frame. Previous studies with MeV and RPV have shown that these non-structural proteins play a role in blocking IFN pathway signaling. In the case of PPRV only V protein seems to interfere with both type-I and type-II IFN signaling pathways. However, there is no evidence of the inhibitory activity of C, P and W proteins.

To improve our understanding of the mechanisms involved in the ability of PPRV proteins to block IFN action, we have studied the inhibition of the activation of IFN stimulated response elements (ISRE), using luciferase reporter assays, by V, C, P

and W proteins. First, we have shown that ICV'89 and India/94, two virulent PPRV strains, and the vaccine strain Nigeria/75, are highly effective in blocking the action of type I IFN by significantly reducing the activation through the ISRE promoter. Thereafter, we have cloned and sequence for the first time the PPRV W protein, needed for subsequent assays. *In vitro* experiments demonstrated that V appears to be the dominant inhibitor of IFN signaling. The W effect was weaker than the inhibition observed with the V protein but still significant. Finally, P protein seems to weakly block IFN activity and C shows no blocking effect on the stimulation through the ISRE promoter. The effect of each protein in STAT1/2 phosphorylation and/or nuclear translocation is currently under study. In summary, this study highlights the ability of PPRV proteins to block the IFN response as a mean to control the host immune response.

18:45-19:00h (CO 38)

ADMINISTRATION OF ANTISERUM FROM MICE VACCINATED WITH MODIFIED VACCINIA ANKARA VIRUS EXPRESSING AFRICAN HORSE SICKNESS VIRUS (AHSV) VP2 PROTEIN CONFERS PROTECTION WHEN ADMINISTERED BEFORE OR AFTER CHALLENGE

EVA CALVO-PINILLA [1], FRANCISCO DE LA POZA [2], PETER MERTENS[1], JAVIER ORTEGO [2], JAVIER CASTILLO-OLIVARES [1].

[1]. The Pirbright Institute, Surrey, United Kigndom.

[2]. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA-CISA, Madrid, Spain

African horse sickness is a fatal viral disease spread by *Culicoides* biting midges that causes high mortality in naive populations of horses. The disease is endemic in sub-Saharan Africa, but outbreaks also occur in North Africa, Asia and Europe, leading to massive economic losses to the equine industry. Previous studies in our group showed that active immunisation with a recombinant modified vaccinia Ankara virus expressing VP2 (MVA-VP2), the major virus neutralisation antigen of AHSV serotype 4, induced virus neutralising antibodies and complete protection against lethal challenge in interferon alpha receptor gene knock-out mice (IFNAR -/-) and horses. In addition, passive transfer of MVA-VP2 antiserum was found to protect mice and significantly decrease levels of viral replication when administered 1h before AHSV challenge.

We have extended these studies to further characterise protective role of the antibody responses induced by MVA-VP2 vaccination. Thus, recipient mice were transferred with antiserum or splenocytes derived from MVA-VP2 vaccinated mouse donors and then challenged with virulent AHSV-4.The protective immunity of the passively immunised mice was compared with that of MVA-VP2 vaccinated and unvaccinated animals. Antiserum recipients showed high protection against disease, with 100% survival rates even in mice that were immunised 48 h after challenge and statistically significant reduction in viraemia in comparison with the control groups. On the other hand, mice that received splenocytes from MVA-VP2 vaccinates, showed a small reduction in viraemia and a 40% survival rate. Results of these experiments show the potential of administration of MVA-VP2 hyper immune

serum as an emergency treatment for AHSV.

19:00-19:15h (CO 39)

BA71ΔFX: A LIVE ATTENUATED VACCINE THAT CONFERS PROTECTION AGAINST HOMOLOGOUS AND HETEROLOGOUS AFRICAN SWINE FEVER VIRUSES

P. LÓPEZ-MONTEAGUDO[1], A. GALLEI[2], A. LACASTA[1], M.J. NAVAS[1], S. PINA[1], F. ACCENSI[1], J.M. RODRÍGUEZ[3], M.L. SALAS[3], F. RODRÍGUEZ[1]

[1]Centre de Recerca en Sanitat Animal (CReSA) - Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus UAB, 08193 Bellaterra, Barcelona, Spain

[2] Boheringer Ingelheim…

[3]Centro de Biología Molecular Severo Ochoa (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), Madrid, Spain

The absence of safe and efficient vaccines against African swine fever virus (ASF), difficult its control. Experimental immunizations with Live Attenuated Viruses (LAV) have demonstrated to induce efficient protective immune responses, albeit most of the times circumscribed to homologous ASFV challenges. The main objective of our work was to explore the protective potential of BA71ΔfX: a genetically modified LAV deficient on a key virulence factor (factor X). BA71ΔfX was obtained by homologous recombination from the virulent BA71 and was grown is Cos-1 cells[1]. Groups of 8 weeks old Large x White male pigs were intramuscularly inoculated once with different amounts of BA71ΔfX and 28 days later were subjected to intramuscular lethal challenge using different ASFV stains. PBS-inoculated pigs were always used as controls in our assays. While 10^3pfu of BA71cos (BA71 passed ten times in Cos cells) killed 100% of the pigs within a week, the same dose of BA71Δfx did not provoke any clinical signs compatible with ASF. Interestingly, 2 out of the six pigs immunized with this low dose of BA71ΔfX survived the lethal challenge with the homologous virulent BA71, corresponding with those showing strong antibody and specific T-cell responses (by an IFN-ELISPOT). The protection afforded by BA71ΔfX was dose-dependent since increasing 30 times the vaccine dose ($3x10^4$pfu) yielded 100% of protection against the BA71 lethal challenge and more importantly, also against the heterologous E75 lethal challenge[2]. In consonance with these results, all pigs intramuscularly inoculated with 10^6pfu of BA71Δfx also survived, in this occasion showing no viremia at any time after challenge. Aiming to extend these studies to the virus strain currently circulating in Europe, a final experiment was set up. Thus, a group of 10 pigs were intramuscularly inoculated with 10^6pfu of BA71ΔfX and then challenged with a lethal dose of Georgia 2007. In contrast with control pigs dying by day 7 post-challenge, all BA71ΔfX-immunized pigs survived Georgia 2007. While 4 of them suffered short viremia and fever peaks after challenge, the other 6 remained clear of virus and clinical signs compatible with ASF throughout the infection. In spite of these impressive protection results, further work is needed to increase the safety of our vaccine since transmission to sentinel pigs has been occasionally recorded when using 10^6pfu of BA71ΔfX. In conclusion, BA71ΔfX has demonstrated to confer very solid protection against experimental challenge

with lethal homologous and heterologous ASF viruses.

19:15-19:30h (CO 40)

EXPERIMENTAL BLUETONGUE VIRUS 4 SUBUNIT VACCINE DELIVERED TO ANTIGEN PRESENTING CELLS

D. LEGISA[1], A. MARÍN-LOPEZ[2], M. PEREZ-AGUIRREBURUALDE[1], F. GONZALEZ[1], V. RUIZ[1], A. WIGDOROVITZ[1], J. ESCRIBANO[3], J. ORTEGO[2]. M. DUS SANTOS[1].

[1]. Instituto de Virología, INTA-Castelar. Buenos Aires. Argentina,

[2]. Centro de Investigación en Sanidad Animal, INIA-CISA, Valdeolmos, Madrid, Spain.

[3]. Departamento de Biotecnología, INIA, Madrid, Spain

Bluetongue virus (BTV), the causative agent of bluetongue disease (BT) in domestic and wild ruminants, is worldwide distributed and it is included in the unified OIE list of notifiable terrestrial and aquatic animal diseases. A total of 27 serotypes have been described so far, and several outbreaks have been reported along with their associated economic loss. Vaccination is critical for controlling the spread of BTV. In the last years, subunit vaccines, viral vector vaccines and reverse genetic-based vaccines have emerged as new alternatives to conventional inactivated or attenuated vaccines. In this study, we developed an experimental subunit vaccine against BTV4, with the benefit of targeting the recombinant BTV protein to antigen-presenting cells. The VP2 protein from an Argentine BTV4 isolate was expressed alone or fused to the Antigen Presenting Cell Homing (APCH) molecule, in the baculovirus insect cell expression system. This molecule was previously described as capable to target fused antigens to Antigen Presenting Cells enhancing humoral and cellular immune responses. The immunogenicity of VP2 and APCH-VP2 recombinant proteins was evaluated in guinea pigs, cattle and IFNAR(-/-) mice. Titers of specific neutralizing antibodies in guinea pigs and cattle immunized with VP2 or APCH-VP2 were high and similar to those induced by a conventional BEI-inactivated vaccine. Even more, similar titers were reached for treatments including BEI-inactivated vaccine, VP2- and the APCH-VP2-based vaccines, although a four-fold lower antigenic mass was used in the APCH-VP2 group. The immunogenicity of recombinant proteins was further studied in the IFNAR(-/-) mouse model. Purified VP2 and APCH-VP2 proteins were inoculated without use of adjuvant, in order to do not mask immunogenicity. Here, the fusion of VP2 to APCH enhanced the cellular immune response and the neutralizing activity induced by VP2.

Parallel Session V:
CELL-VIRUS INTERACTION

Chairpersons:
COVADONGA ALONSO AND
ENRIQUE VILLAR
Monday June 8, 2015
WHITE ROOM

17:30-17:45h (CO 41)

RNA-SEQ BASED TRANSCRIPTOME ANALYSIS OF THE INTERFERON HOST RESPONSE UPON VACCINIA VIRUS INFECTION

G. ALONSO[1], B. HERNÁEZ[1,], J.M. ALONSO-LOBO[1], D. AGUIRRE DE CARCER[1], A. RASTROJO[1], C. FISCHER[2], S. SAUER[2], B. AGUADO[1] AND A. ALCAMI[1].

[1]*Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid, Spain.*

[2]*Max-Planck-Institute for Molecular Genetics, Berlin, Germany.*

Evasion of interferon (IFN)-mediatedantiviral immunityis critical for a successful virus infection. So poxviruses have evolved diverse molecular strategies to counteract IFN host response activity at different levels. In the case of VACV, one of these is to encode a soluble IFN-α/β binding protein (IFNBP) which located at cell surface binds type-I IFN molecules with high affinity, preventing their interaction with the host cell receptor.

In the present work, we have dissected by RNA-Seq the viral modulation of the IFN-based host response at the transcriptional level. RNA from VACV-WR infected murine L929 cells was extracted at 0, 4 and 9 h post-infection in the absence or presence of IFN-alpha and/or recombinant purified IFNBP. To examine the immunomodulatory activity of the IFNBP, the transcriptome analysis from cells infected witha VACV-WR deletion mutant lacking the IFNBP was included. RNA libraries were prepared and paired-end sequencing performed using the Illumina HiSeq system. After removal of low quality reads, over 100M high quality reads per sample were obtained, which could be mapped either to the VACV or *mus musculus* strain C57/BL6 reference genomes. Then, analysis of differential gene expression and GO pathway enrichment analysis were performed to reveal the molecular mechanisms of action.

We could validate the experiment identifying the expected transcriptional changes after IFN-induced signalling in the transcriptome from IFN-treated cells. The addition of recombinant IFNBP to cells prior to IFN completely reverted these IFN-induced changes to basal levels found in untreated cells. The addition of recombinant IFNBP to cell cultures did not result in significant activation of any cellular pathway, in spite of the IFNBP attaching to the cell surface. Finally, to detect and analyze those changes in host gene expression after viral infection of cultures, treated or not with IFN, analysis of differential gene expression and GO pathway enrichment analysis were performed and will be discussed.

17:45-18:00h (CO 42)

A SYSTEM BIOLOGY APPROACH REVEALS GENES AND PATHWAYS INVOLVED IN T-CELL EXHAUSTION AT TISSUE LEVEL

J ARGILAGUET[1], A ESTEVE-CODINA[3], M PEDRAGOSA[1], C PELIGERO[1], S HEATH[3] AND A MEYERHANS[1,2]

[1]*Infection Biology Group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Dr. Aiguader 88 08003, Barcelona, Spain (jordi.argilaguet@upf.edu)*

[2]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain*

[3] *Statistical Genomics, Centro Nacional de Análisis Genómico, Barcelona, Catalonia, Spain.*

Viral infections can be fundamentally categorized as acute or persistent according to their temporal relationships with their hosts. In an acute infection, virus-specific T-cells become activated, proliferate, and differentiate into effector T-cells, allowing the virus elimination within a few weeks. By contrast, persistent infections, such as those caused by HIV and HCV, are not resolved and develop when T-cells become exhausted, i.e. differentiate into a state with poor effector function to avoid immunopathology. There is a broad knowledge of T-cell-intrinsic mechanisms involved in the dysfunction of virus-specific effector lymphocytes during chronic infections. However, the establishment of a persistent infection is the result of the interactions between multiple immune cell populations, and the T-cell-extrinsic mechanisms involved in the induction of T-cell exhaustion are still poorly understood.

We used the Lymphocytic Choriomeningitis Virus (LCMV)-infection mouse model system, which enables us to follow the dynamics of the virus infection and the corresponding host responses with a systems biology approach *in vivo*. Acute or persistent infections were established by inoculating mice with different LCMV-virus doses, and spleen-specific transcriptomes of mice with different infection outcomes were determined. Here, we present the results obtained through a bioinformatic analysis that allows us to describe the kinetics of the main biological processes induced in response to acute and persistent virus infections. We have identified a set of co-regulated genes linked to the virus-specific T-cell effector response during an acute infection, and we describe how this biological process is fragmented into several ones during a persistent infection at the time of exhaustion appearance. Hub genes temporally related to these processes were characterized, representing control points of the main biological pathways involved in infection fate at tissue level. Moreover, we used a free web-based software (digital cell quantification, DCQ) that combines genome-wide gene expression data with an immune cell compendium to infer changes in the quantities of immune cell types from the transcriptome profiles obtained from spleens. This allowed us to predict the immune cell subpopulations likely involved in the previously characterized biological processes induced in response to an acute and persistent virus infections.

18:00-18:15h (CO 43)

AFRICAN SWINE FEVER VIRUS REPLICATION IS AFFECTED BY THE INHIBITION OF THE PROTEASOME SYSTEM

L. BARRADO GIL[1], I. GALINDO [1], M.A. CUESTA-GEIJO[1], R. MUÑOZ-MORENO[1,2] AND C. ALONSO [1]

[1] *Departament of Biotechnology, Instituto Nacional de Investigación Agraria y Alimentaria (INIA), Madrid, Spain*

[2] *Present address: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA.*

Ubiquitination has a central role in a variety of cellular processes, such as control of cell division, signal transduction, transcriptional regulation, immune response, endocytosis, cellular trafficking, and cell survival control. Many viruses manipulate the proteasome system for their advantage. Some of them encode proteins that can modify the host's ubiquitin machinery, and other viruses even encode their own ubiquitinating or deubiquitinating enzymes.

ASFV encodes a gene with high homology with the E2 or ubiquitin conjugating (UBC) enzyme. UBCv is expressed throughout ASFV infection and accumulates at late times post infection. The presence of a viral ubiquitin conjugating enzyme in the virions implies that the ubiquitin-proteasome pathway could play an important role during ASFV infection.

We found that the proteasome inhibitor MG132 blocked a post entry step in ASFV replication. In the presence of MG132, ASF viral genome replication, late gene expression and viral production were severely reduced.Moreover,we excluded that virus entry and early gene expression could be directly affected by the proteasome inhibitor. Our data suggests thatfunctional ubiquitin-proteasome machinery is required during ASFV infection. As in other viral models, core-associated and/or viral proteins involved in DNA replication may be targets for the ubiquitin-proteasome pathway that could possibly assist to the virus in either core uncoating or DNA replication.

18:15-18:30h (CO 44)

CORRELATIVE LIGHT AND ELECTRON MICROSCOPY TO STUDY VIRAL MORPHOGENESIS AND EGRESS

L.SANZ-SÁNCHEZ[1], C.RISCO[1]

[1]*Laboratorio de Estructura Celular, Centro Nacional de Biotecnología, CSIC, Madrid, Spain.*

Arbovirus infections represent an important percentage of all emerging infectious diseases detected recently. These viruses are transmitted to humans and animals by arthropod vectors, mainly mosquitoes and ticks. Arbovirus outbreaks are showing up in new regions due to the introduction of the arthropod vectors in temperate habitats. The *Bunyaviridae* is a large family of RNA viruses, most of them Arboviruses. This family includes several important pathogens that cause encephalitis or haemorrhagic fevers. The best characterized member of the family is Bunyamwera virus (BUNV). In infected cells BUNV builds a complex factory by recruitment of mitochondria and RER elements around Golgi stacks. A Transmission Electron Microscopy (TEM) study of cultured adherent cells´ revealed two previously unreported structures

induced by BUNV in basal regions of the cell: complex multilamellar structures (MLS) and extracellular filament bundles. We have used live cell microscopy followed by Correlative Light and Electron Microscopy (CLEM) to study the structural changes of cells during the late phase of BUNV infection that is egress and propagation. Serial sections and 3D reconstructions showed that MLS exclusively contacted the plasma membrane; however, these virus-induced structures were not similar to any other plasma membrane specializations, such as podosomes, filipodia or invadopodia. Morphology and dimensions of MLS were reminiscent of those reported for the nanostructures on gecko fingertips, which are responsible for the extraordinary attachment capacity of these lizards. As infected cells with MLS were more resistant to detachment than control cells, we propose an adhesive function for these structures, which would compensate for the loss of adherence during release of new virus progeny. The filament bundles visualized in the basal regions of infected cells had numerous viruses attached and often contacted with non-infected cells. These filaments contain actin as confirmed by both confocal microscopy and immunoelectron microscopy. Using a potent inhibitor of actin polymerization, cytochalasin D, the filament bundles were no longer seen and viruses remained attached to the cell surface. We propose that viruses can be transported between cells on these actin-based railways. We are currently using live cell microscopy and CLEM together with two different eGFP-tagged recombinant viruses to study BUNV morphogenesis and egress. This approach will provide new means for identifying viral-cell interactions and targets for novel antiviral compounds.

18:30-18:45h (CO 45)

CD2v INTERACTS WITH ADAPTOR PROTEIN AP-1 DURING AFRICAN SWINE FEVER INFECTION

D. PÉREZ-NÚÑEZ[1], E. GARCÍA-URDIALES[1], M. MARTÍNEZ-BONET[2], M. L. NOGAL[1], S. BARROSO[1], R. MADRID[1] AND Y. REVILLA[1]

[1] *Virology Department, Centro Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain*

[2] *Hospital Gregorio Marañón, Madrid, Spain.*

African swine fever (ASF) is a highly lethal and economically important disease of domestic pigs for which there is no control strategy other than animal quarantine and slaughter. Classified as a notifiable disease by the World Organization for Animal Health (OIE), ASF causes major economic losses to the pig industry in affected countries. Despite the high risk represented by the recent outbreak in the Caucasus in 2007, its subsequent propagation throughout Russia and potential dissemination to neighboring countries, to date, no specific protection or vaccine against ASF is available.

African swine fever virus (ASFV) CD2v protein seems to be involved in virulence enhancement, viral hemadsorption, and pathogenesis, although the molecular mechanisms of the function of this viral protein are still not fully understood. CD2v resembles the T-lymphocyte surface adhesion receptor CD2, and it contains an extracellular N-terminal (Nt) domain composed of two immunoglobulin-like domains, while the cytosolic C-terminal

domain of CD2v (CD2v-Ct) shares no obvious amino acid sequence with the cellular CD2 cytoplasmic domain. During infection, CD2v is assumed to be cleaved into a Nt glycosylated and a Ct non-10 glycosylated form, but both coexist in the infected cell with the full length protein.

Here we describe that CD2v localized around viral factories during ASFV infection, suggesting a role in the generation and/or dynamics of these viral structures and hence in disturbing cellular traffic. We show that CD2v targeted the regulatory trans-Golgi network (TGN) protein complex AP-1, a key element in cellular traffic. This interaction was disrupted by brefeldin A even though the location of CD2v around the viral factory remained unchanged. CD2v-AP-1 binding was independent of CD2v glycosylation and occurred on the carboxy-terminal part of CD2v, where a canonical di-Leu motif previously reported to mediate AP-1 binding in eukaryotic cells, was identified. This motif was shown to be functionally interchangeable with the di-Leu motif present in HIV-Nef protein in an AP-1 binding assay. However, we demonstrated that it was not involved either in CD2v cellular distribution or in CD2v-AP-1 binding. Taken together, these findings shed light on CD2v function during ASFV infection by identifying AP-1 as a cellular factor targeted by CD2v and hence elucidate the cellular pathways used by the virus to enhance infectivity.

18:45-19:00h (CO 46)

THE MAMMALIAN CELL CYCLE REGULATES PARVOVIRUS NUCLEARCAPSID ASSEMBLY

JON GIL-RANEDO[a,], EVA HERNANDO[b], LAURA RIOLOBOS[a,], CARLOS DOMÍNGUEZ[a], MICHAEL KANN[b], AND JOSÉ M. ALMENDRAL[a]

[a]Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), 28049 Cantoblanco, Madrid, Spain. [b]University of Bordeaux, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France.

Cellular and viral life cycles are connected through multiple though poorly understood mechanisms. It is unknown whether the mammalian cell cycle could impact the assembly of viruses maturing in the nucleus. We addressed this fundamental question using MVM, a reference member of the icosahedral ssDNA nuclear parvoviruses, which require cell proliferation to infect by mechanisms partly understood. Constitutively expressed MVM capsid subunits (VPs) accumulated in the cytoplasm of mouse and human fibroblasts synchronized at G0, G1, and G1/S transition. Upon arrest release, VPs translocated to the nucleus as cells entered S phase, at efficiencies relying on cell origin and arrest method, and immediately assembled into capsids. In synchronously infected cells, the consecutive virus life cycle steps (gene expression, proteins nuclear translocation, capsid assembly, genome replication and encapsidation) proceeded tightly coupled to cell cycle progression from G0/G1 through S into G2 phase. However, a DNA synthesis stress caused by thymidine irreversibly disrupted virus life cycle, as VPs

became increasingly retained in the cytoplasm hours post-stress, forming empty, mislocalized capsids in mouse fibroblasts, thereby impairing encapsidation of the nuclear viral DNA. Synchronously infected cells subjected to density-arrest signals while traversing early S phase also blocked VPs transport, resulting in a similar misplaced cytoplasmic capsid assembly in mouse fibroblasts. In contrast, the above-mentioned stressing signals deregulating virus assembly neither perturbed nuclear translocation of the NS1 protein nor viral genome replication occurring under S/G2 cycle arrest. The exquisite cell cycle-dependence of parvovirus nuclear capsid assembly conforms a novel paradigm of time and functional coupling between cellular and virus life cycles. This junction may determine the characteristic parvovirus tropism for proliferative and cancer cells, and its disturbance could critically contribute to persistence in host tissues. These findings may contribute to understand cellular regulations on the assembly of other nuclear eukaryotic viruses, and to develop cell cycle-based avenues for antiviral therapy.

19:00-19:15h (CO 47)

POLYANIONIC CARBOSILANEDENDRIMERS PREVENT THE VAGINAL/RECTAL HSV-2 ENTRY *IN VIVO.*

P. GARCIA-BRONCANO[1*], R. CEÑA-DIEZ[1*], R. GÓMEZ[1], F. J. DE LA MATA[2], MA. MUÑOZ-FERNANDEZ[2].

[1]. Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain.Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.Spanish HIV HGM BioBank, Madrid; Spanish HIV HGM BioBank, Madrid, Spain.

[2]. Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, Alcalá de Henares, Madrid, Spain. CIBER-BBN, Madrid, Spain

Despite a significant worldwide need for effective microbicides to reduce sexually transmitted diseases (STD) and genital herpes simplex virus 2 (HSV-2) transmissions, these microbicides are not currently available. Topical microbicides are applied directly to the genital tract or rectum prior to intercourse to protect against STD. Vaginal and rectal microbicides can reach high local drug concentrations to prevent HSV-2 transmission without toxicity due to the many potential benefits associated with the drug delivery route. We have previously demonstrated that polyanionic carbosilane dendrimers, G1-S4 and 2G-S16 exert their inhibitory effect impeding the viral entry by different mechanisms of action; G1-S4 could bind directly on viral proteins on the surface of HSV-2 particles and inactivate HSV-2, whereas 2G-S16 carry out its inhibitory effect by binding to cellular surface molecules of host cell.G1-

S4 and 2G-S16, showed a good safety profile and did not cause histopathological alterations to the vaginal epithelium at concentrations of 8 mM and 12 mM, respectively. Our data clearly demonstrate that topical vaginal administration of 3% G1-S4 or 3% 2G-S16 formulated as 2% hydroxyethylcellulose (HEC; NIH-ARRRP) gel prevent HSV-2 transmission in BALB/c mice in 100%. This research represents the first demonstration that transmission of HSV-2 can be efficiently blocked by vaginally applied of G1-S4 or 2G-S16. In addition, promising results were also obtained in the case of rectal infection, although without reaching the inhibition values obtained in the vaginal challenge assay. Therefore, further studies should be performed in order to increase their rectal protection efficiency. Our dendrimers have shown a synergistic profile in *in-vitro* when combined with other anti herpetic drugs already described, such as Tenofovir, against HSV-2 as well as against HIV; hence it would be worthwhile to analyze such a combination therapy with those antivirals *in-vivo*.

These results obtained in BALB/c mice provide a strong step forward in the development of G1-S4 or 2G-S16-based vaginal and rectal microbicides to prevent vaginal HSV-2 transmission in humans.

19:15-19:30h (CO 48)

RECRUITMENT OF HOST FACTORS BY THE CRE ELEMENT OF THE HEPATITIS C VIRUS

P. RÍOS-MARCO, C. ROMERO-LÓPEZ Y A. BERZAL-HERRANZ

Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, PTS Granada, Avda. del Conocimiento s/n, Granada, Spain

The hepatitis C virus (HCV) has a positive single stranded RNA genome, with an approximate length of 9.6 Kb. This genome contains a single ORF, flanked by conserved 5' and 3' untraslated regions (UTRs). The 5'UTR contains the most part of the IRES (Internal Ribosome Entry Site), which allows the Cap-independent translation of a viral polyprotein, which is proteolized into ten different viral proteins. The 3'UTR is involved in the viral replication and translation, and is composed of three well-defined elements: the hypervariable region, a poly U/UC domain and the so-called 3'X-tail domain that is formed by three well conserved stem-loop subdomains. Another element of structural and functional importance in the viral genome is the cis-acting replicating element (CRE), located at the 3' end of the region coding for the HCV viral polymerase (NS5B). CRE plays a role at replication by recruiting NS5B and also exerts negative control of the IRES-dependent translation. In fact this region, of approximately 200 nucleotides, is able to establish internal contacts to specific domains belonging to 3'UTR and 5'UTR, modulating structural changes in the genome and regulating essential processes for viral cycle progression. However, the interrelationship of the CRE element with

cellular factors has been little studied so far. This work summarizes the progress of our group in the identification of host proteins and translational machinery with capacity to interact with the CRE element. For example, by proteomic approach, we report a wide group of CRE-binding proteins such as hnRNPs, Ras GTP-activating protein-binding proteins, RNA-helicases or splicing factors. Moreover we provide data indicating that CRE interacts with ribosomal particles and map in detail the nucleotides involved in such interaction. Our data indicates that the CRE stem-loop 5BSL3.2 plays an important role both in recruiting host proteins and in binding of the 40S ribosomal subunit. The knowledge of associations between cellular factors and the virus genome will help to understand the interaction dynamic of RNA-RNA and RNA-protein that allows the progression of HCV viral cycle.

Parallel Session VI: ROLE OF VIRUS IN PEDIATRIC DISEASES (SEV-SEIP)

[Joint Session SEV-SEIP]

Chairpersons:

Mª ISABEL GONZÁLEZ-TOMÉ AND MARISA NAVARRO

Monday June 8, 2015

AUDIOVISUAL ROOM

*Invited paper

17:30-17:45h (CO 49)

IMPLICATIONS OF CONTROL OF VIRAL LOAD DURING PREGNANCY AND RISK OF HIV-1 MOTHER-TO-CHILD TRANSMISSION

L. PRIETO[1]

[1] *Paediatrics Department.Hospital Universitario de Getafe, Getafe, Spain.*

Approximately 3.2 million of children are living with HIV infection worldwide. An estimated 240,000 children were newly infected with HIV in 2014. More than 90 percent of HIV infections in children result from mother-to-child-transmission (MTCT) when the virus passed from a HIV-infected mother to her baby during pregnancy, childbirth, or breastfeeding.

Rates of HIV-1 MTCT in untreated non-breastfeeding populations in developed countries range from 15 to 40%. Nowadays, MTCT is almost entirely preventable infection when interventions including antenatal HIV screening, antiretroviral therapy (ART), appropriate mode of delivery, neonatal antiretroviral prophylaxis and avoidance of breastfeeding are carried out. These interventions have resulted in MTCT rates less than 2% in many developed countries.

However, in the context of low MTCT rates, questions around optimal management remain inconclusive. Maternal HIV-1 viral load is considered the best predictor of the risk of MTCT. Moreover, in the era of highly active antiretroviral treatment (HAART), MTCT rates are directly associated with HIV-1 RNA levels at delivery. Non suppressive maternal viral load at delivery regardless of HAART is associated commonly with lack of prenatal care of HIV-infected women but also with acute HIV infection during pregnancy. Rates of MTCT in these cases remain high.

Strategies for the control of viral load replication during pregnancy and further considerations in the management of HIV-infected women and their exposed infants at high risk of MTCT situations are the scope of this review.

*Invited paper

17:45-18:00h (CO 50)

RSV BRONCHIOLITIS: CHALLENGES IN 2015

R. RODRÍGUEZ-FERNÁNDEZ

Hospital Infantil Gregorio Marañón. Sección de Lactantes. Madrid. Spain

Bronchiolitis by respiratory syncytial virus (RSV) is the most frequent cause of hospitalization in the first year of life in developed societies. It also represents the leading cause of infant mortality after malaria in the first 12 months of life. Despite this impact on global child health, still do not have vaccines or antiviral treatments in daily clinical use. One reason why we have not yet effective therapeutic or preventive interventions is our incomplete understanding of the immune response against RSV, and the lack of development of protective immunity after the first infection. Most infants admitted to hospitals during each epidemic bronchiolitis caused by RSV, are previously healthy infants without underlying diseases. Currently it is impossible to predict the evolution of these patients. At the time of admission is impossible to know which of these RSV-infected infants will be discharged within 24 or 48 hours and which of them are going to get worse and they will need ventilatory support in the following days after admission. Numerous factors have been described that can contribute to the severity of the disease, such as age, viral load, RSV subtype A or B or the infant immune response. Now believed to be a combination of host factors and virus that likely contribute to determine the severity of the disease.

In addition, numerous studies in children hospitalized for bronchiolitis due to RSV, have shown that approximately between 40 and 50% of them develop in the first year of life recurrent wheezing, which in most cases disappear within 3-4 years of life. The high frequency with which this occurs, suggests that there is a relationship between the two events, though still today, it is unclear what the causal mechanism. Most hypotheses suggest that RSV is directly responsible of these persistent or recurrent wheezing, while other authors propose that the virus is simply a marker that identifies children predisposed to persistent wheezing in early childhood.

A better understanding of virus and the infant immune response will allow us to

establish predictive markers of severity and evolution of infants with bronchiolitis.

*Invited paper

18:00-18:15h (CO 51)

NEUROLOGICAL AND SYSTEMIC PARECHOVIRUS INFECTIONS IN CHILDREN

C CALVO[1], M CABRERIZO[2].

[1] *Pediatrics Department.Severo Ochoa Hospital.Leganés. Madrid. Spain* [2]*Enterovirus Unit. National Microbiology Center (ISCIII),Majadahonda, Madrid. Spain.*

Human parechoviruses (HPeV) are RNA viruses belonging to the family of *Picornaviridae*. Formerly described as echovirus 22 and 23 in the *Enterovirus* genus, HPeV were reclassified into their own genus, *Parechovirus*, in 90's, and were renamed as HPeV-1 and HPeV-2, respectively. Additional types of HPeV have been reported and a total of 16 different types have been recognised to date (HPeV-1 to 16). The most common genotype detected worldwide is HPeV-1 followed by HPeV-3. Other types such as HPeV-2 and HPeV-4 are less common.

Infections with HPeV are prevalent in young children and have been associated with mild diseases of the respiratory and gastrointestinal tract, mainly associated to HPeV-1 and HPeV-2, but also with meningitis, encephalitis and sepsis in infants. HPeV-3 might be one of the main agents causing severe neonatal neurological infections in Europe, although its real incidence is unknown since HPeV detection is not routinely performed. Epidemiological and clinical data in our country are scarce.

We are conducting a prospective study in hospitalized young children with fever without source, meningitis, encephalitis and clinical sepsis in 10 hospitals in Spain (PI12-00904) with the objective to know the prevalence of HPeV infections in this group of patients and their clinical characteristics. A comparison with enterovirus (EV) is also performing.

HPeV was tested in those specimens (sera, cerebrospinal fluid, pharyngeal frotis) negative for EV at the CNM, using a real-time RT-PCR designed in the 5-NCR of the genome. Molecular typing of detected EV and HPeV was carried out by amplification of 3'-VP1 or VP3/VP1 regions, respectively, and sequencing.

Although our study is ongoing, we can say that HPeV circulating in our country are relatively common in children <3 years. In infants under 3 months HPeV accounting for 12% of the studied infections. HPeV-3 is the most common. The clinical picture of our patients is characterized by fever without source or suspected sepsis, without leukocytosis and pleocytosis, even though the virus is detected in CSF. Generally, they have a good prognosis.

For pediatricians, we consider that is of interest to suspect these viruses as etiological agents of infections in young children with the mentioned symptomatology and to include them in the routine virological diagnosis in order to avoid unnecessary antibiotherapy and prolonged hospitalizations.

*Invited paper

18:00-18:15h (CO 52)

CONGENITAL CYTOMEGALOVIRUS (cCMV) INFECTION: HOW, WHEN, WHERE.

DANIEL BLÁZQUEZ-GAMERO

Hospital Universitario 12 de Octubre. Sección de Enfermedades Infecciosas e Inmunodeficiencias. Madrid. Spain

Human cytomegalovirus (HCMV) is the leading cause of congenital infections worldwide, the most frequent cause of non genetic hearing loss in children, and a significant cause of permanent neurologic sequelae. In many countries, especially in developing regions with high prevalence of seropositive women, cCMV is a neglected disease, and no screening during pregnancy nor in the newborn period is routinely recommended. In most cases (90%) newborns with cCMV show no symptoms at birth, but up to 15% of these asymptomatic children will develop late onset sequelae. In symptomatic newborns, the rate of long term sequelae is even higher (40-50%). Despite the global impact of this infection, there are still important gaps in the knowledge of disease mechanisms regarding virus transmission, role of viral genotypes, immunological responses and individual host factors. Today there is no effective antiviral treatment available during pregnancy, and other treatments, such as anti-CMV hiperimmune globulin, have shown limited benefits. Antiviral treatment with ganciclovir or valganciclovir during neonatal period in symptomatic children has shown encouraging results. It is of paramount importance to increase the knowledge about mechanisms of transmission and individual factors regarding cCMV infection in order to improve the treatment options and outcome of these children.

Parallel Session VII:
VIRAL ENTRY MECHANISMS

Chairpersons: JOSÉ A. MELERO AND JOSÉ MARÍA ALMENDRAL

Tuesday June 9, 2015

AUDITORIUM REAL CASA DE LA MONEDA

15:00-15:15h (CO 53)

LIPID COMPONENTS IN AFRICAN SWINE FEVER VIRUS ENTRY AND REPLICATION

M. A. CUESTA-GEIJO[1], I. GALINDO[1,] R. MUÑOZ-MORENO[1,2], L. BARRADO-GIL[1] AND C. ALONSO[1].

[1] *Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain*

[2] *Present address: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA*

Since its introduction in Europe through the Caucasus in 2007, African swine fever (ASF) has been spreading westwards from Russia to EU countries threatening porcine industry. Due to the lack of an effective vaccine, ASF control relies on early diagnosis and massive stamping out of animals.

We searched for targets at early stages of infection aiming to discover how African swine fever virus (ASFV) surpasses host cell defenses and reorganizes cellular structures to initiate replication. The virus enters the cell by endocytosis and within few minutes after infection, viral

decapsidation occurs at the acid pH of late endosomes. We report here that the virus exit from the endosome to start replication requires intact cholesterol endosomal efflux. In fact, cholesterol is required at several steps of the virus life cycle starting from virus entry. ASF virus reorganizes cholesterol landscape of the cell to the perinuclear replication site where the viral factory is built. These results add to a growing body of evidence pointing out cholesterol efflux and the endosomal membrane as crucial players for the start of viral replication in several virus models.

15:15-15:30h (CO 54)

POLYANIONIC CARBOSILANE DENDRIMERS AS PROMISING MICROBICIDE CANDIDATES AGAINST HSV-2 INFECTION: BROAD-SPECTRUM ACTIVITY AND ACTION MECHANISM

R. CEÑA-DIEZ[1], E. VACAS CORDOBA[1] P. GARCÍA BRONCANO[1], R. GÓMEZ[2], F. J. DE LA MATA[2],MA. MUÑOZ-FERNANDEZ[1].

[1]. Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain.Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.Spanish HIV HGM BioBank, Madrid, Spain.

[2]. Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, Alcalá de Henares, Madrid, Spain. CIBER-BBN, Madrid, Spain

Although enormous efforts have been made to prevent the sexual transmission of genital herpes simplex virus 2 (HSV-2), there is still neither protective vaccine nor cure against one of the most common sexually transmitted infections in the world. The development of safe topical microbicides, compounds that applied vaginally or rectally, to protect the user from sexually transmitted infections, is of crucial importance; especially when HSV-2 infection is associated with a 3-fold to 4-fold increased probability of HIV acquisition. Nanotechnology offers novel suitable tools to develop new microbicidal compounds, such as dendrimers. Dendrimers are a class of nanoparticles that have shown their potential as therapeutic agents and as carriers of different molecules. The safety and antiviral activity of eight polyanionic carbosilane dendrimers were evaluated to select a proper candidate for the development of a topical microbicide against HSV-2. All dendrimers were non-toxic at the majority of the studied concentrations. The plaque reduction assay on Vero cells showed that 2G-S16, G1-S4 and G3-S16 present the highest inhibitory effect on the HSV-2 infection, reaching values of 100%, 77% and 68%, respectively. Moreover, changes in pH did not alter the inhibitory profile of these dendrimers. Interestingly, we demonstrated that our dendrimers inhibit the viral infection at the first steps of HSV-2 life-cycle, impeding the binding of HSV-2 particles to target cell surface and thus, the viral entry. G1-S4 and G3-S16 sulfate-ended dendrimers bound directly on viral proteins on the surface of HSV-2 particles, inactivating HSV-2; we hypothesize that these dendrimers could bind to HSV-2 glycoprotein B (gB), which has an important role in HSV-2 entry by binding to heparan sulfate on the cell surface. However, 2G-S16 achieves its inhibitory effect by binding to cellular surface

molecules of host cell. Molecular Modeling of the interactions of G1-S4 and 2G-S16 with HSV-2 surface protein gB showed that G1-S4 binds better in general to selected binding sites on gB surface than 2G-S16 and moreover it is better suited to reach worse accessible binding sites. Significantly better binding properties of G1-S4 were found in a position important for affecting transition of gB trimer from its pre-fusion to final post-fusion state and in several positions where it could interfere with gB/gH-gL interaction.Finally, we demonstrated that these nanocompounds have synergistic activity with Tenofovir and acyclovir, two anti-herpetic drugs. Our data indicate that 2G-S16, G1-S4 and G3-S16 are promising candidates to be developed as vaginal microbicides.

15:30-15:45h (CO 55)

ROLE OF CLATHRIN AND CLATHRIN ADAPTOR PROTEIN-1 IN HEPATITIS C VIRUS EGRESS

I. BENEDICTO[1,2], V. GONDAR[1,2], F. MOLINA-JIMÉNEZ[1], L. GARCÍA-BUEY[2,3], M. LÓPEZ-CABRERA[4], P. GASTAMINZA[5], P. L. MAJANO[1,2].

1. *Molecular Biology Unit, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain*
2. *CIBERehd, Instituto de Salud Carlos III, Madrid*
3. *Liver Unit, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain*
4. *Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain*
5. *Centro Nacional de Biotecnología-CSIC, Madrid*

Althoughit is well established that hepatitis C virus (HCV) entry into hepatocytes depends on clathrin-mediated endocytosis,the possible roles of clathrin in other steps of the viral cycle remain unexplored. Thus, we studied whether cell culture-derived HCV (HCVcc) exocytosis was altered after clathrin interference. Knockdown of clathrin or the clathrin adaptor AP-1 in HCVcc-infected human hepatoma cell cultures impaired viral secretion without altering intracellular HCVcc levels or apolipoprotein B (apoB) and apoE exocytosis. Similar reduction in HCVcc secretion was observed after treatment with specific clathrin and dynamin inhibitors. Furthermore, detergent-free immunoprecipitation assays, neutralization experiments and immunofluorescence analyses suggested that whereas apoE associated with infectious intracellular HCV precursors in endoplasmic reticulum (ER)-related structures, AP-1 participated in HCVcc egress in a post-ER compartment. Finally, we observed that clathrin and AP-1 knockdown altered the endosomal distribution of HCV core, reducing and increasing its co-localization with early endosome and lysosome markers, respectively. Our data support a model in which nascent HCV particles associate with apoE in the ER and exit cells following a clathrin-dependent transendosomal secretory route.

This is a significant finding, since to date it has been proposed that HCV and very low-density lipoproteins (VLDL) follow similar exocytic routes. Given that lipid metabolism has recently emerged as a potential target against HCV infection, our data could help to design new strategies to interfere specifically with HCV exocytosis without perturbing cellular lipid

homeostasis, with the aim of achieving more efficient, selective and safe antivirals.

15:45-16:00h (CO 56)

THE pH STABILITY OF FOOT-AND-MOUTH DISEASE VIRUS PARTICLES IS MODULATED BY RESIDUES LOCATED AT THE PENTAMERIC INTERFACE AND IN THE N TERMINUS OF VP1

F. CARIDI, A. VÁZQUEZ-CALVO, F. SOBRINO, M.A. MARTÍN-ACEBES

Departamento de Virología y Microbiología, Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain

The picornavirus foot-and-mouth disease virus (FMDV) is the etiological agent of a highly contagious disease that affects important livestock species. FMDV capsid is highly acid labile and viral particles lose infectivity due to their disassembly at pH values slightly below neutrality. This acid sensitivity is related to the mechanism of viral uncoating and genome penetration from endosomes. In this work, we have analyzed the molecular basis of FMDV acid-induced disassembly by isolating and characterizing a panel of novel FMDV mutants differing in acid sensitivity. Amino acid replacements altering virion stability were preferentially distributed in two different regions of the capsid: the N terminus of VP1 and the pentameric interface. Even more, the acid labile phenotype induced by a mutation located at the pentameric interface in VP3 could be compensated by introduction of an amino acid substitution in the N terminus of VP1.These results indicate that the acid sensitivity of FMDV can be considered as a multifactorial trait and that virion stability is the fine-tuned product of the interaction between residues from different capsid proteins, in particular those located within the N terminus of VP1 or close to the pentameric interface.

16:00-16:15h (CO 57)

ANALYSIS OF THE ORIGIN AND INFECTIVITY OF INFECTIOUS MICROVESICLES DERIVED FROM SEMLIKI FOREST VIRUS DEVOID OF CAPSID

M. RUIZ-GUILLEN[1,2], E. GABEV[1,2], J.I. QUETGLAS[1,2], E. CASALES[1,2],J. POUTOU[1,2], M.C. BALLESTEROS[1,2], A. ARANDA[1,2], J. BEZUNARTEA[1,2], M. ONDIVIELA[3], R. HERNANDEZ-ALCOCEBA[1,2], N. ABRESCIA[3,4], C. SMERDOU[1,2]

[1]*Division of Gene Therapy, CIMA, University of Navarra, Pamplona, Spain.*

[2]*IdiSNA, Navarra Institute for Health Research, Pamplona, Spain*

[3]*Structural Biology Unit. CIC bioGUNE, CIBEREHD .Bizkaia Technology Park, Derio, Spain*

[4]*IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain*

Alphaviruses are enveloped viruses that contain a positive-strand RNA genome packaged into a nucleocapsid of icosahedral symmetry. We have previously observed that alphaviruses derived from Semliki Forest virus (SFV) and Sindbis virus are able to propagate in the complete absence of capsid sequences. This propagation seems to be mediated by the release of infectious microvesicles (iMVs) that contain viral RNA inside and viral envelope proteins on their surface. In the case of SFV, these iMVs are pleomorphic and have a larger size and density than wt virus. In the present work we have

analyzed in detail the cellular origin of iMVs, their mechanism of infection, and their infectivity in mice. These studies showed that while iMVs lack exosomal and endosomal/lysosomal markers, they are highly enriched in components of the plasma membrane, indicating that they are most likely derived from this compartment. In addition, they lack most of the viral replicase components, suggesting that they are not derived from viral replication complexes, which have been described to localize at the plasma membrane at early times post-infection. Infectivity studies showed that iMVs enter the cells through the endosomal pathway and can co-localize with markers of this cellular compartment at early times postinfection. Furthermore, iMVs were not pathogenic to mice when injected intravenously but were able to efficiently infect different organs like lungs and heart. However, in contrast to wtSFV, they were not able to cross the blood-brain barrier. Finally, we have evaluated the possibility of using iMVs as gene transfer vectors. These experiments showed that iMVs are able to transfer and mediate propagation of heterologous genes like GFP and interleukin-12, making them interesting for gene therapy or vaccination applications.

16:15-16:30h (CO 58)

TYPE I INTERFERON RESPONSE IS DELAYED IN HUMAN ASTROVIRUS INFECTED CELLS

S. GUIX[1,2], A. PÉREZ-BOSQUE[2,3], LL. MIRÓ[2,3], M. MORETÓ[2,3], A. BOSCH[1,2], R. M. PINTÓ[1,2]

[1] *Enteric Virus Group, Department of Microbiology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain*

[2] *Nutrition and Food Safety Research Institute (INSA-UB), University of Barcelona, Av. Prat de la Riba 171, 08921 Santa Coloma de Gramanet, Spain*

[3] *Digestive Physiology and Nutritional Adaptations Group, Department of Physiology, University of Barcelona, Av. Joan XXIII S/N, 08028 Barcelona, Spain*

Type I interferon (IFN) activation and its subsequent effects are important in the response to viral infections. Human astroviruses (HAstV) are recognized as common viral pathogens causing gastroenteritis in infants and young children, with very few reports of disease in normal healthy adults, and some reports of severe disease after dissemination to extra-intestinal tissues in immunocompromised patients.They are non-enveloped positive-strand RNA viruses containing a 6.8 kb polyadenylated genome linked to a VPg protein on the 5'end.

Our study shows that HAstVsinduce a mild and delayed IFN response upon infecting CaCo-2 cells. Although IFN-β mRNA is detected within infected cells and supernatant from infected cells show antiviral activity against the replication of other well-known IFN-sensitive viruses, these responses occur at late stages of infection once genome replication has taken place. Interestingly, synthesis of type

III IFN-λ mRNA, which plays an important role in controlling other gastrointestinal viral infections,also takes place within HAstV-infected CaCo-2 cells.

On the other hand, HAstV replication can be partially reduced by the addition of exogenous type I IFN, and inhibition of IFN activation by BX795 enhances viral replication, indicating that HAstVs are IFN-sensitive viruses. Finally, different levels of IFN response were observed in cells infected with different HAstV mutants with changes in the hypervariable region of nsP1a/4, suggesting that nsP1a/4 genotype may potentially have clinical implications due to its correlation with the viral replication phenotype and the antiviral responses induced within infected cells.

16:30-16:45 (CO 59)

LA PROTEÍNA VP5 JUEGA UN PAPEL ESENCIAL EN LA DISEMINACIÓN DEL VIRUS DE LA BURSITIS INFECCIOSA

F. MÉNDEZ, L.L. CUBAS, D. RODRÍGUEZ, J.F. RODRÍGUEZ

Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC, Madrid, Spain

El virus de la bursitis infecciosa (IBDV) es el agente etiológico de una grave enfermedad inmunosupresora que afecta a pollos domésticos responsable de graves pérdidas a la industria avícola mundial. Los viriones de IBDV son icosaedros no envueltos con un genoma formado por dos segmentos de dsRNA.

Estudios recientes llevados a cabo en nuestros laboratorios demuestran que VP5, una proteína asociada a virulencia y prescindible para la replicación del virus *in vitro*, regula el tamaño de placa de lisis. Los mutantes de IBDV carentes VP5 presentan reducciones en el tamaño de placa superiores al 90% con respecto al virus parental. La proteína VP5 se asocia a la cara citoplásmica de diferentes compartimentos membranales, incluyendo la membrana plasmática, a través de su interacción con fosfoinosítidos. Esta interacción está mediada por un dominio electropositivo localizado en la región C-terminal de la proteína. Mutaciones que afectan a la funcionalidad de este dominio alteran la distribución subcelular de la proteína y provocan reducciones significativas en el tamaño de placa.

Los resultados obtenidos sugieren que la eliminación selectiva de la proteína VP5 no afecta significativamente a la replicación del genoma, a la expresión de otras proteínas virales o al ensamblaje del virus. Sin embargo, se observa un efecto dramático sobre la cinética de liberación de partículas infectivas al medio extracelular. Nuestros datos sugieren que, al igual que lo observado recientemente en otros virus carentes de envuelta lipídica, IBDV podría emplear un mecanismo de liberación de virus no asociado a lisis celular cuyo funcionamiento sería dependiente de la proteína VP5. La eliminación de este mecanismo podría explicar la supresión de virulencia documentada en mutantes de IBDV carentes de la proteína VP5.

16:45-17:00h (CO 60)

A MODEL FOR HEPATITIS A VIRUS TRANSCYTOSIS IN HEPATOCYTES

M. DE CASTELLARNAU, S. GUIX, F.J. PÉREZ-RODRÍGUEZ, L. D'ANDREA, A. BOSCH, R.M. PINTÓ

Enteric Virus Laboratory, Department of Microbiology, University of Barcelona, Barcelona, Spain

Hepatitis A is the most common infection of the liver worldwide and is fecal–orally transmitted. Its incidence tends to decrease thanks to improvements in hygienic conditions and vaccination campaigns, but at the same time, its severity increases due to a higher infection rate in adults.

Hepatitis A virus is a unique picornavirus with very special molecular features. Recently, an unexpected exit pathway through its envelopment into exosomes has been described. In the present work, we have studied the exit process in two different cellular models: FRhK-4 cells, which represent a traditional cell culture system utilized to grow HAV *in vitro*, and Huh7-A1 cells, which are more permissive for HAV infection than the parental Huh7 cells from which they derive and which represent the natural hepatocyte model. Additionally, we took advantage of two HAV mutants bearing a VP2 replacement, which potentially affects its binding to ALIX and thus its invagination into multivesicular bodies and the subsequent generation of the "quasienveloped" particles. Huh7-A1 cells were shown to be less efficient in supporting HAV replication, although the mutants gave higher titers in single round replication events. Overall, extracellular release of enveloped particles was higher in Huh7-A1 than in FRhK-4 cells for all viruses, but particularly with mutant viruses. In polarized Huh7-A1 cells, their preferential exit route was through the apical membrane and the viruses present in the canalicular side were enveloped particles. In contrast, those few viruses released through the basolateral membrane were mostly free particles. A new model of HAV entry and exit from human hepatocytes will be presented.

Parallel Session VIII: SPECIFIC IMMUNITY

Chairpersons: MARGARITA DEL VAL AND YOLANDA PACHECO

Tuesday June 9, 2015

WHITE ROOM

15:00-15:15h (CO 61)

THE STRUCTURALLY RELATED FUSION PROTEINS OF HUMAN RESPIRATORY SYNCYTIAL VIRUS AND METAPNEUMOVIRUS ARE ANTIGENICALLY AND IMMUNOGENICALLY DISSIMILAR

L. RODRIGUEZ[1], E. OLMEDILLAS[1], V. MAS[1], C. PALOMO[1], A. TRENTO[1], M. VÁZQUEZ[1], O. CANO[1], B.S. GRAHAM[2], B. VAN DEN HOOGEN[3], J.S. MCLELLAN[4] AND J.A. MELERO[1]

[1]Centro Nacional de Microbiología and CIBERES, ISCIII, Madrid, Spain; [2]Vaccine Research Center, NIH, Bethesda USA; [3]Erasmus Medical Center, Rotterdam, The Netherlands, [4]Geisel School of Medicine, Dartmouth, USA.

The fusion (F) proteins of human respiratory syncytial virus (hRSV) and the highly related human metapneumovirus (hMPV) enable fusion of the viral and cell

membranes at the initial stages of their respective infectious cycles and are the main target of neutralizing antibodies.

Both hRSV_F and hMPV_F experience remarkable structural changes during transit from the metastable prefusion conformation to a highly stable postfusion form. Significant structural information has recently been gained about the refolding process of hRSV_F by solving the structures of soluble prefusion and postfusion forms. Although much less is known about hMPV_F, partial information indicates that it shares structural characteristics with hRSV_F.

Despite the noted structural resemblance and partial amino acid identity, both hRSV_F and hMPV_F have limited antigenic identity reflected in few cross-neutralizing monoclonal antibodies (MAbs). Furthermore, only very limited cross-reactivity is seen in polyclonal responses against various forms of prefusion or postfusion F proteins. More importantly, while depletion of human sera with postfusion hRSV_F removes only a small fraction of neutralizing antibodies, the same procedure depletes most of the neutralizing antibodies directed against hMPV_F, indicating substantially different conformational requirements. All these data provide new insights into hRSV_F and hMPV_F based on structural studies that should contribute to the development of efficient vaccines against these important human pathogens.

15:15-15:30h (CO 62)

VIH-1 INDUCE A DEREGULATION IN B-CELL POPULTIONS THROUGH A PARTIAL REGULATORY B-CELL PHENOTYPE *IN VITRO*

S MARTIN DELGADO[1], J LOPEZ-ABENTE[2], M.A MUÑOZ-FERNANDEZ[1], R CORREA-ROCHA[2] AND **M PION**[1]

[1]Laboratory of Molecular ImmunoBiology, Hospital General Universitario Gregorio Marañón; Instituto de Investigación Sanitaria del Gregorio Marañón. C/ Dr. Esquerdo 46, 28007 Madrid, Spain; [2]ImmunoRegulation Laboratory, Hospital General Universitario Gregorio Marañón; Instituto de Investigación Sanitaria del Gregorio Marañón. C/ Maiquez, 9, 28009 Madrid, Spain

HIV-1 patients usually show general immune system deregulation and hyper-activation. At the humoral immunity level, HIV infection induces hyper-gammaglobulinemia and loss of memory B cells. In our group, we have previously reported that HIV-1 particles caused a direct marked effect on the activation, proliferation and phenotype of B cells. This deregulation could explain why efforts to develop an efficient vaccine against HIV have been unsuccessful. Therefore, it is essential to determine the B-cell deregulation mechanism so as to improve the design of an anti-HIV vaccine and obtain an efficient response of the immune system.

In the present study, we considered the phenotype and functions of HIV-treated B-cell. B cells were extracted from buffy coat and treated with HIV-1 or different stimuli. Using quantitative PCR, we analyzed the expression of different cytokines in B-cell and, by flow cytometry; we analyzed the phenotype of these cells. Moreover, the

function of B cells on CD4+ or CD8+ T cells were determined after co-culture experiments *in vitro*.

Surprisingly, B cells exposed to HIV showed a higher level of mRNA for IL-10, IL-6, EBI3 or IL-12(p35) *in vitro*, presenting a potential immunosuppressive profile. In addition, HIV-treated B cells exposed to lymphocytes were able to reduce the proliferative capacity of CD4+ and CD8+ T cells, confirming the immunosuppressive profile of these B cells. However, HIV-treated B cells did not show a specific ability to reduce the production of TNFα from CD4+ or CD8+ T cells as results of co-culture experiments and the phenotype of the B cells studied by flow cytometry were not conclusive.

We have already established that HIV-treated B cells show a deregulated phenotype and function. But looking deeply at this deregulation, we showed that HIV-treated B cells displayed a partial immunosuppressive phenotype and function. These results may explain the general deregulation of the immune system and the high concentration of IL-10 in plasma observed in HIV patients. These preliminary outcomes are highly promising as a means of understanding the hyper-activation of the immunity in HIV patients and further experiments *ex vivo* are needed to confirm these results.

15:30-15:45h (CO 63)

ENGINEERED THERMOSTABLE EMPTY CAPSIDS OF FMDV FOR IMPROVED VACCINES

S. LÓPEZ-ARGÜELLO[1], V. RINCÓN[1], A. RODRÍGUEZ-HUETE[1], E. MARTÍNEZ-SALAS[1], G. BELSHAM[2], M.G. MATEU[1]

[1]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain, and [2]Technical University Denmark.

Foot-and-mouth disease virus (FMDV) is the causative agent of one of the economically most important animal diseases worldwide. Novel vaccines based on recombinant FMDV empty capsids are being investigated to avoid the risks of virus escape or deficient inactivation during vaccine production, while still preserving the full immunogenicity and antigenic spectrum of current, virion-based vaccines. FMDV empty capsids can be more acid-resistant than virions, but they appear to be even less termostable. Thus, there is a clear need to increase empty capsid thermostability for the development of capsid-based, infection risk-free FMD vaccines.

We have engineered and produced mutant recombinant FMDV empty capsids carrying different mutations that were chosen based on the FMDV atomic structure and our previous work. The empty capsids were produced in eukaryotic cells using an expression system based on recombinant vaccinia virus. The thermal and acid resistance of these mutant capsids against dissociation into pentameric subunits was compared to that of the non-mutated (natural) capsid. Four tested mutants showed substantially increased thermal

resistance although only some of them showed increased acid-resistance. The results are helping to elucidate the structural basis of the thermal sensitivity of FMDV, and the effects of compensatory mutations that were found to restore the viability of FMDV lethal mutants. In addition, the empty capsids of increased thermostability we have engineered could provide a basis for the development of improved empty-capsid-based FMD vaccines.

15:45-16:00h (CO 64)

ROLE OF INTERFERON STIMULATED GENES IN INFLUENZA VIRUS PRODUCTION AND ANTIVIRAL SIGNALLING

M. L. DEDIEGO, L. MARTINEZ-SOBRIDO, D. J. TOPHAM

Department of Microbiology and Immunology, and David H. Smith Center for Vaccine Biology and Immunology. University of Rochester, Rochester, NY, USA

Influenza A (IAV) and B (IBV) viruses are considered one of the most common causes of respiratory virus infections in humans causing annual epidemics and occasional pandemics of considerable public health and economic impact. The innate immune system, leading to the expression of IFN-stimulated genes (ISGs), is the first line of defense against virus infections. To study the IFN-stimulated genes (ISGs) induced during influenza virus infection and their roles in virus production, cellular gene expression in epithelial cells infected with influenza A and B viruses was analyzed. Many well-known ISGs were induced during influenza virus infection, such as RIG-I, MDA5, IRF-7, TLR-3, STAT1, ISG15, ISG20, IFIT1, IFIT2, IFIT3, MX, and OAS. However, we found other genes such as GBP5, GBP6, IFI6, IFI27, IFI35, IFI44, TRIM22, and TRIM34, which were transcriptionally induced after influenza virus infection, but their role during the virus cycle is unknown. Using siRNAs to specifically knockdown the expression of the proteins, and plasmids to overexpress these proteins, we have identified that genes IFI6, IFI27, IFI35, and IFI44, increase viral replication of influenza virus, and also of other viruses such as lymphocytic choriomeningitis virus (LCMV). In addition, we have shown that these genes act as negative feedback regulators of cellular IFN antiviral responses induced by different viruses, by the analog of dsRNA polyinosinic polycytidylic acid, and by IFN itself, most probably accounting for the differences in the virus titers observed. As far as we know, the function of these genes during influenza virus infection has been described for the first time. In addition, the role of IFI6, IFI27, and IFI44 in regulating the cellular IFN responses is novel for these proteins.

16:00-16:15h (CO 65)

EPIDEMIOLOGÍA MOLECULAR DE GRIPE A Y B EN ENFERMEDAD RESPIRATORIA GRAVE Y ESTADO VACUNAL

J PUIG-BARBERÀ (1, 2), KARINA SALVATIERRA (3), SILVIA SANCHO-TELLO (3, 4), JAVIER DÍEZ-DOMINGO (1), F XAVIER LÓPEZ-LABRADOR (3, 5, 6)

(1) Area de Vacunas, Centro Superior de Investigación en Salud Pública (FISABIO-Salud Pública), Conselleria de Sanitat, Valencia, España..

(2) Centro de Salud Pública de Castellón, Conselleria de Sanitat, Castellón, España.

(3) Laboratorio de Virología, Area de Genómica y Salud, Centro Superior de Investigación en Salud Pública (FISABIO-Salud Pública), Conselleria de Sanitat, Valencia, España.

(4) Servicio de Microbiología, Hospital Clínico Universitario de València, España.

(5) Unidades Mixtas Infección-Salud Pública y Genómica-Salud (FISABIO-Salud Pública/Universtat de València)

(6) CIBER-ESP (Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública), Instituto de Salud Carlos III, España.

Introducción: El papel de la vacuna de la gripe en la prevención de ingresos por enfermedad respiratoria es objeto de controversia. Dentro de un estudio prospectivo basado en la vigilancia de hospitalizaciones en las tres provincias de la Comunitat Valenciana (cubriendo 1,266,899 habitantes, 27% de la población), intentamos relacionar las tasas de hospitalización debido a enfermedad respiratoria con la prevalencia de virus respiratorios durante la temporada 2012-13, según el estado de vacunación para gripe (vacuna administrada mínimo 14 días antes del ingreso) y la cepa del virus.

Métodos: El cribado se realizó en todas las admisiones por urgencias de la semana epidemiológica 46/2012 (11/2012) a la 16/2013 (4/2013), en cinco hospitales de referencia. Se obtuvieron frotis nasofaríngeos y nasales (< 14 años) o faríngeos (>=14 años). Se llevó a cabo la detección de 14 virus respiratorios (RT-PCR). De forma sistemática se secuenció el gen completo de la hemaglutinina en asilados de gripe A y gripe B, para compararlo con el de las cepas vacunales.

Resultados: De 1.034 pacientes incluidos en el estudio, en 510 se detectó como mínimo algún virus por RT-PCR, de los que 242 fueron positivos para gripe. Observamos dos olas, la primera de gripe B donde casi todos los asilados pertenecían al linaje B-Yamagata (n=151; 30%; B-Yamagata n=145) y la segunda de gripe A, donde casi todos los aislados fueron A(H1N1)pmd09 (n=85; 35%) y sólo 5 A(H3N2). Los aislados de gripe B Yamagata (54 secuenciados) se distribuyeron en dos clados: (i) clado 2, B/Brisbane/3/2007-like, cercano a B/Massachusetts/02/2012; y clado 3, B/Wisconsin/1/10 (cepa vacunal)-like, cercano a B/England/709/2012. La mayoría de los virus A(H1N1)pdm09 (37 secuenciados) pertenecían al clado 6 (A/St.Petersburg/27/2011-like), y sólo cuatro aislados pertenecían al clado 7 (A/St.Petersburg/100/2011-like). Un total de 18 (34%) vs. 34 (65%) aislados de gripe B, ó 12 (32%) vs. 25 (68%) de gripe A, provenían de pacientes vacunados o no vacunados, respectivamente. No se encontró un agrupamiento filogenético diferencial de los aislados según el estado de vacunación, ni para gripe A, ni para gripe B.

Conclusiones: No se pudo relacionar ningún grupo filogenético de gripe B-Yamagata o A(H1N1pdm09) con fallo

vacunal en ingresos por enfermedad respiratoria grave durante la temporada 2012-13. Otros factores, distintos al clado del virus, pueden contribuir a la poca efectividad de la vacuna en una porción sustancial de casos.

16:15-16:30h (CO 66)

MODIFICATION OF PROMOTER SPACER LENGTH IN VACCINIA VIRUS AS ASTRATEGY TO CONTROL THE ANTIGEN EXPRESSION

M. DI PILATO[1], L. SÁNCHEZ-SAMPEDRO[1], E. MEJÍAS-PÉREZ[1], C. O. S. SORZANO[2] AND M. ESTEBAN[1]

[1]. Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología, Madrid,Spain

[2]. Biocomputing Unit, Centro Nacional de Biotecnología, Madrid, Spain

Vaccinia viruses (VACV) with distinct early promoters have been developed to enhance antigen expression and improve antigen-specific CD8 T cell responses. It has not been demonstrated how the length of the spacer between a gene and its early promoter motif influences antigen expression, and whether the timing of gene expression can modify the antigen-specific CD4 T cell response. We generated several recombinant VACV based on the attenuated modified vaccinia Ankara (MVA) strain, which express GFP or the *Leishmania* LACK antigen under the control of an optimized promoter, using different spacer lengths. Longer spacer length increased GFP and LACK early expression, which correlated with an enhanced LACK-specific memory CD4 and CD8 T cell response. These results show the importance of promoter spacer length for early antigen expression by VACV and provide alternative strategies for the design of poxvirus-based vaccines.

16:30-16:45h (CO 67)

HIGHLY EFFICIENT INSECT CELL-BASED PLATFORM FOR VIRUS-LIKE PARTICLE VACCINES PRODUCTION USING AN IMPROVED BACULOVIRUS VECTOR

J. LÓPEZ-VIDAL[1]*, S. GÓMEZ-SEBASTIÁN[1]*, J. BÁRCENA[2], M. C. NUÑEZ[1], D. MARTÍNEZ-ALONSO[3], B. DUDOGNON[1], E. GUIJARRO[4] AND J. M. ESCRIBANO[4]

[1]Alternative Gene Expression S.L., Pozuelo de Alarcón, Madrid, Spain

[2]CISA-INIA, Valdeolmos, Madrid, Spain

[3]CBMSO, Canto Blanco, Madrid, Spain

[4]Departamento Biotecnología, INIA, Madrid, Spain

** Contributed equally*

Vaccines based on virus-like particles (VLP) have proved their success in human and animal health. Insect cells platform, based on the use of recombinant baculoviruses, is one of the most used technologies to generate this kind of highly immunogenic vaccines. Because production cost is a very relevant constraint to extend the use of these vaccines to human and animal populations, any improvement in productivity may be a relevant factor to reduce the vaccine costs. Here we describe the use of a novel baculovirus expression cassette, denominated TopBac®, to model the production in insect cells of two well defined VLPs. Capsid proteins, from porcine circovirus type 2 (Cap) and from the calicivirus producing the rabbit hemorrhagic disease (VP60), were expressed in insect cells using baculoviruses genetically engineered with

the TopBac® expression cassette. Productivities were compared to that obtained by their conventional counterpart vectors expressing the proteins under the control of *polyhedrin* promoter. Results demonstrated that production yields obtained for these vaccine proteins under the control of the TopBac® cassette were increased by more than 300%. In both cases the recombinant protein was fully functional, forming identical VLPs in size and shape than those produced by the conventional baculovirus, as determined by electron microscopy analysis. The use of the TopBac® expression cassette represents a simply modification of the baculovirus vectors that significantly improves the cost efficiency of VLP-based vaccines production, facilitating the broad application of these vaccines in human and animal health.

16:45-17:00h (CO 68)

VIRAL PROTEINS TARGET COMPLEXES IN THE RIG-I LIKE RECEPTOR

M.T. SANCHEZ-APARICIO[1,2], J. AYLLON[1,2], A. GARCIA-SASTRE[1,2,3].

[1] Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, United States of America. [2] Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, New York, United States of America. [3] Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, New York, United States of America.

The innate immune response relies on a set of Pathogen Recognition Receptors (PRRs) that sensor pathogen patterns (PAMPs). RIG-I is a cytosolic PRR that detects 5'-triphosphate double-stranded RNAs produced during infection. Once activated, the pathways leads to the induction of type I IFN and proinflammatory cytokines, leading to a cellular antiviral state. In a non-infected cell, RIG-I is in an inactive form where a helicase intermediate domain is interacting with the CARDs domains. Upon recognition of the RNA by the RD, RIG-I hydrolyzes ATP and changes its conformation to an active state. The CARD domains are then exposed and become K63-linked polyubiquitinated by E3-ligases, such as TRIM25.

The activation of this pathway is complex and well characterized, but most of the spatio-temporal events, and the subcellular localization where the essential proteins interact, are still under interrogation. Through different techniques, we analyzed how these proteins form complexes that are distributed and reorganized spatially within the cell in order to create an efficient antiviral state.

RIG-I is the main sensor for recognition of many ssRNA viruses such as Paramyxoviruses, Flaviviruses, Rhabdoviruses and Orthomyxoviruses. Many of them have developed numerous and different strategies to overcome the activation of the RLR pathway.

We will discuss and show new insights on how, where, and when, viral proteins can counteract the activation of the RLR pathway. NS1 of Influenza A virus, NS34A of Hepatitis C Virus and NSs of Severe Fever with Thrombocytopenia Syndrome Virus, are IFN antagonistic viral proteins that interact with specific complexes in very well defined areas in the host cell in

order to inhibit the antiviral state in an infected cell.

Parallel Session IX:

MICROBIOME AND HEALTH

[Joint Session SEV-SEM]

Chairpersons: ALBERT BOSCH AND ROSA DEL CAMPO

Tuesday June 9, 2015

AUDIOVISUAL ROOM

*Invited paper

15:00-15:30h (CO 69)

SCIENTIFIC EVIDENCES OF GUT MICROBIOTA IMPLICATIONS IN HUMAN DISEASES

R. DEL CAMPO

Microbiology Department, University Hospital Ramón y Cajal, Madrid, SPAIN

In the recent years, numerous scientific works have pointed to the human gut microbiota as a significant metabolic organ with relevant repercussions in the global human health. Our gastrointestinal tract harbours a considerable microbes ecosystem, constituted by bacteria, parasites, viruses, and archaeas, which interact with the human eukaryotic cells, and the host immune system.

The recent technological advances in molecular tools such as metagenomics and next-generation sequencing have allowed increasing the known of the complete diversity in the gut microbiota ecosystem, including the non-cultivable microorganism, which are the majority.

Some international alliances, as META-HIT in Europe and The Human Microbiome Project in USA, have been created with the aim of to define a standardized "normal gut microbiota composition". These platforms have reported the quantitative and qualitative differences in the microbiota through the different races and age stages, as well as the existence of a universal "core" conserved in almost all humans.

A healthy gut microbiota plays many crucial digestive and metabolic functions in the host, whereas alterations in their composition or reductions in the microbial diversity are highlighted in several pathologic status and diseases. Those modifications in the intestinal microbiota composition and function have been linked to diseases or pathological status, including functional gastrointestinal disorders, obesity and diabetes. Nevertheless, in all cases it is important to define if the alterations observed in the microbiota are the cause or the consequence of the disease.

The most recent studies revealed the existence of a brain-gut axis in which the metabolic molecules produced by the bacteria are structurally similar to neurotransmitter, and due to that might have neuroactive functions. On the other hand, the brain has a connection with the gut microbiota through the enteric nervous system, when released in the intestinal lumen and consequently signal brain function and behaviour.

Dietary supplementation with probiotics and prebiotics are the most widely used dietary adjuncts to modulate the gut microbiota. In some particular diseases, the faecal transplantation might be

indicated for a complete gut microbiota restoration.

*Invited paper

15:30-16:00h (CO 70)

INITIAL BACTERIAL COLONIZATION: IMPACT ON HUMAN HEALTH

E. JIMÉNEZ QUINTANA

ProbiSearch, SL, Tres Cantos, Spain; Departamento de Nutrición, Bromatología y Tecnología de los Alimentos. Universidad Complutense de Madrid. Spain.

The microbial colonization of the infant gastrointestinal tract is an essential process in the human lifecycle since interactions established between the microbiota and the host have important consequences for human health and disease. Traditionally, it has been considered that the intestinal tract was sterile at birth, being rapidly colonized with microorganisms from the mother and the surrounding environment. However, culture dependent analyses have detected microorganisms in amniotic fluid, fetal membranes, umbilical cord and placenta, even in cases where no rupture of membranes has occurred and in elective C-sections. Other studies suggest that, actually, the meconium, the newborn's first intestinal discharge, composed of material that has been ingested or secreted in the gut during fetal life from healthy hosts is not sterile suggesting that gut colonization may start before birth. Different factors, such as mode of delivery, antibiotherapy, diet or environment, affect infant gut colonization although their actual contribution to shape the infant microbiota remains unclear. It has been shown that gestational age and weight at birth also exert a strong influence on this process. Numerous studies monitoring the bacterial communities in preterm infants indicated that the fecal microbiota of premature infants is different compared with that of term infants. In fact, the gut colonization pattern of preterm infants has been described as delayed and aberrant. Abnormal intestinal colonization during the first weeks of life may alter the barrier, nutritional and immunological functions of the host microbiota and, as a consequence, increases susceptibility to disease. Necrotizing enterocolitis, inflammatory bowel disease, obesity, asthma, atopy and even autism are diseases that have been related with the microbial gut composition in infants. The use of probiotics and/or prebiotics for potential modulation of the microbiota early in life may have a long lasting impact on health and could be of great importance for disease prevention.

*Invited paper

16:00-16:30h (CO 71)

METAGENOMIC ANALYSIS OF VIRUSES IN THE HUMAN ORAL CAVITY

M. PARRAS MOLTÓ[1], P. SUÁREZ RODRÍGUEZ[1], A. RODRÍGUEZ GALET[1], A. SIMÓN-SORO[2], A. EGUIA[3], JM. AGUIRRE URIZAR[3], A. MIRA[2], A. LÓPEZ BUENO[1].

[1] *Centro de Biología Molecular Severo Ochoa (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), Madrid, Spain.*

[2] *FISABIO Foundation, Center for Advanced Research in Public Health, Valencia, Spain.*

[3] *Dpto. de Estomatología II, UFI 11/25 Universidad del País Vasco/EHU, Leioa, Spain.*

The oral cavity is not only the main route for bacteria and virus entry but also harbours several microbial ecosystems whose composition and function remained poorly understood. A healthy oral commensal flora protects against pathogenic microorganisms, and some authors propose that changes in stress level, diet or hygiene habits might alter their ecological equilibrium and trigger pathological processes. Although bacteriophages are key players in the regulation of microbial communities, their contribution to oral dysbiosis has not been investigated yet. The development of new techniques of high-throughput sequencing and their applications to the study of natural microbial communities (metagenomics) is providing valuable information about human microbiomes. However few studies have focused on indigenous viruses from our microbiota.

Here we report a metagenomic survey of viruses in the mouth of 35 unrelated human subjects. Viral DNA purified from supragingival plaque (nine patients with active caries and eight healthy subjects) or oral mucosa swabs (seven patients with recurrent aphthous stomatitis and eleven healthy subjects) were sequenced to generate >59 million paired-end reads (35 Gbp). In average, 78% of the reads were assembled in viral contigs based on Blast similarities to comprehensive databases. Oral viromes were dominated by tailed bacteriophages mainly from the *Siphoviridae* and *Podoviridae* families. Due to the unprecedented sequencing depth we have assembled 150 different nearly full-length genomes with an average length of 37 Kbp and average coverage of 357x. Importantly, most of them correspond to novel phages poor related to those deposited in databases. In addition, we have detected high prevalent eukaryotic viruses including human herpevirus 4 and 7, and the complete circular genome of seven human papillomavirus (four novel types), five Circovirus-like viruses and 14 anelloviriridae including two new human viral species (*Parras-Moltó et al.* 2014).

The comparison of the viromes reveals that in spite of the high degree of interpersonal variation, there are few abundant bacteriophages widely distributed along dental plaque and oral mucosa samples. Their closest relatives in databases are phages that infect hosts normally found in the mouth and some of them involved in the development of oral diseases. Finally, although we have not detected statistically significant differences among viromes on the basis of oral health status (healthy versus caries or oral ulcers), some phages are mainly associated with sickness or healthy status.

This study represents the largest metagenomic study of virus in the human mouth and enhances our understanding about this complex biological system.

*Invited paper

16:30-17:00h (CO 72)

VACCINE DRIVEN EVOLUTION OF PORCINE CIRCOVIRUSES

T. KEKARAINEN

Centre de Recerca en Sanitat Animal (CReSA) - Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Bellaterra, Spain

Porcine circovirus 2 (PCV2, family *Circoviridae*) is one of the smallest known viruses with single-stranded circular DNA genome of 1.7kb long. It is one of the most important pig infecting viruses causing great economical losses to pig industry. PCV2 is linked to several diseases named as porcine circovirus diseases (PCVD). The most known PCVD is PCV2-systemic disease (PCV2-SD), also known as postweaning multisystemic waisting syndrome (PMWS). PCV2 targets the lymphoid tissues, which leads to lymphoid depletion and immunosuppression in pigs. The virus modulates the function of immune cells, upregulate IL-10 and proinflamatory cytokines. First vaccine against PCV2 was marketed 2004 and nowadays several pharmaceutical companies offer PCV2 vaccine. It is estimated that up to 90% of Spanish pigs are vaccinated against PCV2. All marketed vaccines are based on the viral capsid protein and they provide high levels of efficacy and excellent return on investment. However, despite of vaccination, pigs still get infected with the virus. Indeed, this is not exceptional since most commercial vaccines should be considered as "imperfect" from a virological point of view, since they do not provide full protection from pathogen infection but protect individuals and populations from disease expression. Such "imperfect" vaccines, however, may affect viral evolution and selection of viral variants *in vivo*. Our research group is interested on vaccine driven evolution of animal infecting viruses. By in depth molecular analysis using next generation sequencing and subsequent bioinformatics analysis, we have shown that PCV2 variant populations are circulating in commercial farms and that this variability differs between vaccinating and non-vaccinating farms.

Parallel Session X: TEACHING AND DISSEMINATION OF THE VIROLOGY

Chairpersons: ESPERANZA GÓMEZ-LUCÍA AND JOSE A. LÓPEZ-GUERRERO

Tuesday June 9, 2015

AUDITORIUM REAL CASA DE LA MONEDA

*Invited paper

17:33-17:41h (CO 73)

TEACHING VIROLOGY AT A PREUNIVERSITARY LEVEL

F.J. MEDINA DOMÍNGUEZ

Departamento de Ciencias Naturales, IES ALPAJÉS (Consejería de Educación, Comunidad de Madrid), Aranjuez, Madrid, Spain

Teaching microbiology and virology into secondary courses present some main difficulties concerning, primary, the dimensional problem: understanding what

to be small means in terms of scaling correctly the different microscopic structures and living beings and to understand life concepts as span life, metabolism rate…

In addition, viruses represent the boundary between molecular and cellular level, an aspect that connect directly with the very definition of a living being. Understanding how some molecular association may present complexes activities usually causes difficulties to our students.

At a third level, viruses and bacteria are conceptually linked to our students as infectious agents. Separate both groups requires a special attention in order not to commit major errors like consider, for instance, antibiotics as a therapeutic tool against viral infections.

Finally, the presence of laboratory activities is reduced in our high school due to the fact that these practices aren't considered too important by our educative authorities, despite the evidence that a lack of a minimum of a laboratory experience may compromise the scientific knowledge and the setting of good bases to follow further studies.

To face these pedagogic challenges we are developed a didactic programme witch combine laboratory activities, aiming to create a trajectory in this field in ours students, and micro-research projects, looking for setting a solid basement, which allow adding new knowledge avoiding misconceptions.

The result of these experiences are collected in different educational platforms: a magazine of science named "Argos" (http://www.educa2.madrid.org/web/argos/inicio) , a blog intending to keep our students in touch with the actuality on health (http://sos-alpajes.blogspot.com.es/) and a wiki (http://sosalpajes.wikispaces.com/) , build by students of 3º ESO (14 years old), replacing the role of the traditional book in our classes.

reducing the formation of defective products, various methods have been suggested and solidphase organic syntheses (SPOS) is one of the popular approaches.

Several SPOS approaches have been reported for the preparation of PAMAM dendrons, but similar by-products are observed while the original protocol developed remains used. Consequently, a new approach is need in the preparation of PAMAM dendrons. Herein, a new SPOS approach to the production of inverse poly (amidoamine) dendrons was developed. A newly designed AB2 building block provided the focal point of the dendrons and provided a means to reduce the formation of side products. A comparison with the classical approach revealed that fewer reaction steps were needed and a smaller amount of the building blocks was required to build dendrons of similar size. This also leads to the more efficient approach. For example, construction of a G5 dendron can be done in 5 days and filtration is the only necessary purification procedure. This approach was also applied to the preparation of other dendrons. This protocol was accomplished on a solid-phase synthesizer that can reduce the costs and time associated with preparing dendrons, especially for high generation of dendrons.

*Invited paper

17:41-17:49h (CO 74)

THE RADIO AS A VIROLOGY INFORMATION DIFFUSOR

M. SEARA VALERO

Director del Programa "A hombros de gigantes". Radio Nacional de España. Prado del Rey. 28223 Madrid. Spain.

Viral diseases news appear occasionally in the media; diseases like AIDS, bird flu, human papillomavirus, measles or, more recently, Ebola, have had a notorius position in newspaper covers. Some, such as African swine fever, have caused significant economic losses in the livestock sector.

Moreover, in latest years, groups against children vaccination have appeared. This entails a serious health threat for children and, eventually, the whole population.

The media have a fundamental role in reporting these diseases to avoid unnecessary alarmism Putting in the right perspective the scope and the consequences is also required. Fighting pseudoscientific movements or inaccurate theories is essential to avoid this kind of threats to our society.

That's at least what we have tried with the "A hombros de gigantes" broadcast program:

(http://www.rtve.es/alacarta/audios/a-hombros-de-gigantes/) that I run in RNE since September 7, 2007.

*Invited paper

17:49-17:57h (CO 75)

VIROLOGY FOR ALL IN FREE AND ONLINE DIVULGATION JOURNALS

A. DOMÉNECH GÓMEZ [1], A. IRURZUN IPIÉNS [2]

[1] *Dpto. Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain*

[2] *Editorial Hélice, Madrid, Spain. www.editorialhelice.com*

Nowadays there is a general growing interest in virus and diseases they cause. In this sense, scientific societies can play an important role as they can give that information in a clear and trustable way using confirmed scientific data, and avoiding unjustified alarms. There are different ways to transmit that information, as courses and congresses/conferences or scientific publications (usually for their members and other researchers). However, these technical forms may be difficult to understand for people not specialist in the topic. For that reason, publications directed to general public, with different levels of education, are more needed and important, and especially those free available in internet and social media (as blogs, twitter or facebook) that may be easily looked upon.

With the aim of approaching virology to society, in 2010 the Spanish Society for Virology (SEV) created "Virología", a free online divulgation journal hosted on the SEV web site (http://sevirologia.es). The journal intends to reach anyone, from expert virologists to general public, which wants to read or learn more about virus, diseases they cause, new treatments,

diagnostic methods or vaccination developed to fight against them. Readers may find in the journal, among other issues, reviews about present topics in virology, written in an easily understandable way, interviews with outstanding virologists, curiosities and stories in virology history, or discover the presence of virus in poetry, painting, philosophy, museums or even in a football world championship!!. In conclusion, the main goal of "Virología" is to disseminate the scientific knowledge of the amazing viral world in an accessible and enjoyable way.

*Invited paper

17:57-18:05h (CO 76)

YOUNG VIROLOGISTS TO RECEIVE THE BATON

RAFAEL N. AÑEZ.

Former student of the Master's Degree in Virology, Universidad Complutense de Madrid, Madrid, Spain; English teacher, Native Professional Teachers, Madrid, Spain; English teacher, Galea Consulting, Madrid, Spain; Secondary teacher in training, IES Alpajés, Aranjuez, Spain

Nowadays, the new generation of scientists is finding itself at a crossroads when it comes to find a new employment. Under the promise of green pastures and plenty of work it lies the ugly truth of free-from-salary jobs and unpaid scholarships.

This generation finds itself between the rock that the old-school scientists represent, not giving enough consideration to any activities beyond the universitarian curriculum, and the proverbial hard place of the shortage of investment that the economical crisis has brought upon us.

In this brief time I will speak about finding ways to live science in a society that does not want any more scientists, and in a scientific community marked with competitiviness and a unthoughtful meritocratic system. Also, I will provide insight on how this generation is developing towards science, with the new opportunities that it has spawned and the new job positions taken over by this group of people in the fields of advertisement, markenting, knowledge transfer or even teaching at various levels.

*Invited paper

18:05-18:13h (CO 77)

DISSEMINATION OF VIROLOGY THROUGH BLOGS AND OTHER SOCIAL MEDIA

MIGUEL ÁNGEL JIMÉNEZ-CLAVERO

INIA-CISA, Valdeolmos, Spain.

Science is an important part of our culture, and as such its dissemination must be considered among the top priorities in the agenda of R&D activities in any advanced society. For that, the involvement of institutions and civil society in general is badly needed, but a special effort from researchers is particularly required, because nobody like them has a deep knowledge on the scientific disciplines they cultivate and its latest developments. New technologies, especially the Internet and social media (blogs, twitter, facebook and other social networking platforms) make available to scientists new tools that facilitate the dissemination of knowledge from the laboratory directly to the citizens. These new tools should be considered as complementary to the more traditional

popular science media (books, magazines, specialized sections in the press and mass media, documentaries, lectures, etc) and all should occupy their own place in the knowledge society, constituting an ecosystem in which contributing active scientists coexist and interact with journalists and communication experts with specialized knowledge in the different scientific disciplines.

In the case of virology, dissemination of knowledge based on scientific evidence deserves special significance due to the wide public impact of epidemics caused by viruses, notably those producing high mortality, such as the recent Ebola virus disease epidemic. Very often, news on biological alerts concerning virus epidemics raise fear and insecurity among the population in a most irrational way. This pitfall clearly indicates that special efforts are certainly needed to give reliable, scientifically based information to provide the citizens with the knowledge necessary to "metabolize" the flood of information that occur from time to time associated with virus emergencies. Though, an important problem arising with social media is that not all the information published through these networks is reliable, so in order to make these new communication tools useful, it is increasingly necessary to establish quality systems that allow the public to identify trustworthy information through the Internet.

With the aim to contribute to disseminate relevant topics in the virology area, targeting a broad Spanish-speaking public, the blog "Emerging Viruses and Global Change" hosted on the web "madri+d" (http://www.madrimasd.org/blogs/virusemergentes/) reports since 2012 on current issues around emerging viruses and the diseases they produce, in a context of global change. With 70 posts published up to now and around 100,000 visits received, it has been awarded in the last two editions of the madri+d Awards for Science Communication.

*Invited paper

18:13-18:21h (CO 78)

GAMES AS TOOLS FOR TEACHING VIROLOGY

E. GÓMEZ-LUCÍA

Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense (UCM), Madrid, Spain

Traditionally, teaching virology (as well as other subjects) has orbited almost exclusively around lectures. These are usually followed by the student studying his notes or checking books, and the professor evaluating the acquisition of the information. This system, although it is good for reaching a large number of students, fails to really motivate the student, who, as a result, will not store a long term memory of the information received. New approaches are being continually tested, in order to promote what is called “life-long-learning”, or in other words, stimulate the pleasure obtained from learning. Since playing is an emotional need, and young animals use it for their learning, currently, in many schools (especially up to high school, but also at the university level), games are being implemented for teaching. The game is a way of using the mind, or even an attitude about how to use the mind.

Games may seem irrelevant at first sight, but they help the student to fix concepts in a subliminal way. Some of the games used to teach microbiology, virology or infectious diseases represent real-world scenarios of disease outbreaks, in which the student has to carry out virtual research on the virus and the host defence mechanisms. Other games test on aspects of these subjects, being informative and with a self-assessment component. In general, the advantages of using games are numerous: they are motivating, relaxing for the student, distancing him from his normal activity, and improve self-esteem, they generate pleasure, mobilize the subject, develop creativity, curiosity and imagination, activate the divergent thinking, promote communication, integration and group cohesion. The presentation will discuss two games for learning and self-assessment of Virology: Viropolis (http://www.interbionet.com/viropolis/juego/) and VirTUal epidemic (epidemia.sevirologia.es).

Parallel Session XI: ANTIVIRAL DRUGS

Chairpersons: JULIÁ BLANCO AND RAFII MOHAMED

Tuesday June 9, 2015

WHITE ROOM

17:30-17:45h (CO 79)

CHD1 CHROMATIN REMODELER IS A POSITIVE MODULATOR OF INFLUENZA VIRUS REPLICATION THAT PARALLELS RNAP II DEGRADATION IN THE INFECTED CELLS

LAURA MARCOS-VILLAR [1, 2], ALEJANDRA PAZO [1, 2] AND AMELIA NIETO[1, 2]

[1]*Centro Nacional de Biotecnología-CSIC, Madrid, Spain.*

[2]*Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (Ciberes), Spain.*

Influenza A virus polymerase associates with chromatin components of the infected cell, such as the CHD6 chromatin remodeler. Here we show that CHD1, a member of the same family, also interacts with the viral polymerase complex and positively modulates viral replication. Silencing of CHD1 causes reduction on viral polymerase activity, viral RNA transcription and production of infectious particles. Similar results are obtained during infection with H1N1 and H3N2 influenza virus subtypes, but not with Vesicular stomatitis virus or Adenovirus 5, indicating that CHD1 is an important protein for influenza virus replication and that chromatin plays a significant role on influenza virus life cycle.

Influenza virus transcription requires a functional coupling with cellular transcription for the cap-snatching

process. Despite that, the RNAP II is degraded during the infection in a process triggered by the viral polymerase, once viral transcription is finished and on-going cellular transcription is not required. Since CHD1 specifically modulates influenza virus RNA transcription, and associates with Mediator, a transcriptional coactivator complex of RNAP II–mediated transcription, its possible degradation during influenza virus infection was evaluated. Reassortant viruses from strains that induce or not RNAP II degradation have allowed the identification of PA and PB2 subunits as responsible for the degradation process, the involvement of specific residues within these subunits and the correlation between absence of RNAP II degradation and attenuation of pathogenicity in mice. Here we show that CHD1 associates with RNAP II and strictly parallels its degradation pattern during influenza virus infection, suggesting that degradation of both host factors is involved in viral pathogenicity.

17:45-18:00h (CO 80)

LOCAL RNA FLEXIBILITY PERTURBATION OF THE IRES ELEMENT INDUCED BY A NOVEL LIGAND INHIBITS VIRAL RNA TRANSLATION

G. LOZANO[1], J. RAMAJO[1], A. TRAPOTE[2], J. ROBLES[2], E. PEDROSO[2] AND E. MARTÍNEZ-SALAS[1]

[1] *Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Cantoblanco 28049 Madrid, Spain*

[2] *Departament de Química Orgànica and IBUB, Facultat de Química, Universitat de Barcelona, 08028 Barcelona, Spain*

Several RNA viruses initiate translation using a cap-independent mechanism mediated by the internal ribosome entry site (IRES) that is located at their 5´ untranslated genomic region. Picornavirus IRES activity is highly dependent on both its structural organization and its interaction with host factors. Small molecules able to interfere with RNA function are valuable candidates as antiviral agents. Here we show that IRAB, a small molecule based on a benzimidazole compound, inhibited foot-and-mouth disease virus (FMDV) IRES-dependent protein synthesis in RNA-transfected cells leading to a decrease of the virus titer. Interestingly, IRAB preferentially inhibited IRES-dependent translation in cell free systems in a dose-dependent manner. RNA structural analysis by Selective 2´-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) demonstrated an increased local flexibility of the IRES structure upon incubation with IRAB, which affected four stem-loops (SL) located on the apical region of domain 3. Fluorescence binding assays conducted with individual aminopurine-labeled oligoribonucleotides indicated that the SL3A binds IRAB (EC_{50} 18 µM). Additionally, the results derived from fluorescence binding assays suggested that the target site of IRAB within the FMDV IRES might be a folded RNA structure that involves the entire apical region of domain 3. Our data suggest that the conformational changes induced by this compound on a specific region of the IRES structure which is essential for its activity is, at least in

part, responsible for the reduced IRES efficiency observed in cell free lysates and, particularly, in RNA-transfected cells.

18:00-18:15h (CO 81)

ANTIVIRAL ACTIVITY OF POLYANIONIC CARBOSILANE DENDRIMERS AGAINST HEPATITIS C VIRUS IN CELL CULTURE

DANIEL SEPÚLVEDA-CRESPO[1,2†], PEDRO L. MAJANO[3,4†], RAFAEL GÓMEZ[5], FRANCISCO JAVIER DE LA MATA[5], JOSÉ LUIS JIMÉNEZ[2]*, Mª ÁNGELES MUÑOZ-FERNÁNDEZ[1,2]*, PABLO GASTAMINZA*[6]

*† These two authors contributed equally to this work. * Corresponding authors*

[1] *Laboratorio InmunoBiología Molecular, IISGM, BioBank VIH HGM, CIBER-BBN, Madrid, Spain*

[2] *Plataforma de Laboratorio, Hospital Gregorio Marañón, IISGM, BioBank VIH HGM, CIBER-BBN, Madrid, Spain*

[3] *Molecular Biology Unit, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain*

[4] *CIBERehd, Instituto de Salud Carlos III, Madrid*

[5] *Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, Alcalá de Henares, CIBER-BBN, Madrid, Spain*

[6] *Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (CNB-CSIC), Campus Cantoblanco, 28049 Madrid, Spain*

Hepatitis C virus (HCV) infection is a major worldwide biomedical problem. Although new direct antiviral agents have been successfully developed for the treatment of chronic HCV infection, the potential threat of resistance of this genetically diverse family of viruses and the difficulties to make treatment available to all infected patients worldwide should fuel the study of novel antiviral agents that may contribute to eradicate this important human pathogen.

Polyanionic carbosilane dendrimers (PCDs) have demonstrated potent and broad-spectrum anti-HIV-1/HSV-2 activity *in vitro* and *in vivo.* However, the potential anti-HCV effect of PCDs and their mode of antiviral action remain to be determined. In this study, we used an unbiased, cell-based system to screen a battery of PCDs aiming at identifying non-toxic antiviral compounds targeting different steps of the HCV lifecycle. We selected the PCDs displaying the best 5 selectivity indexes to characterize them by determining the genotype spectrum and step targeted in the HCV lifecycle using HCV-pseudotyped retroviral particles (HCV_{pp}) and trans-complemented, spread-defective HCV virions (HCV_{tcp}).

Our results show that PCDs inhibit infection by genotype 2a HCVtcp and HCVpp of the major genotypes (1, 2, 3, and 4) at nanomolar concentrations with no associated cellular toxicity. Given the fact that virocidal activity against other viruses has previously been ascribed to members of this class of molecules, we investigated their impact on HCV virion stability. While exposure to most compounds did not alter virion infectivity, one of the PCDs irreversibly destabilized infectious virions, making infectivity undetectable and strongly reducing viral RNA integrity, even after PCD removal.

In summary, PCDs are identified as novel anti-HCV agents that target either the virion itself or early aspects of HCV infection and constitute a step forward in the development of PCDs as nanotools to prevent HCV transmission in humans.

18:15-18:30h (CO 82)

APTAMERS DESIGN AS ANTIVIRAL AGENTS AGAINST INFLUENZA VIRUS

P. RODRÍGUEZ-RODRÍGUEZ[1,2], V.M. GONZÁLEZ[3], M.E. MARTÍN[3] AND A. NIETO[1,2].

1 Centro Nacional de Biotecnología-CSIC, Madrid, Spain.

2 Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (Ciberes), Spain.

3 IRYCIS-Hospital Ramón y Cajal, Madrid, Spain.

Influenza A virus (IAV) causes respiratory disease and continue to be one of the largest global threats to human health. The virus possesses a negative-oriented segmented RNA genome that encodes for its own RNA-dependent RNA polymerase, which is error-prone. In addition, its segmented genome allows for exchange of RNA segments between genotypically different influenza viruses. These features confer a high genetic diversity and lead to generation of novel strains or/and subtypes and thus contribute to the permanent exposure of the human population to newly emerging and re-emerging influenza viruses.

Viruses have developed different strategies to allow selective translation of their mRNAs using cellular translation factors as targets. Influenza virus mRNAs are formally equivalent to the cellular mRNAs and a sophisticated strategy has been selected by the virus to enhance specifically the translation of viral mRNAs. Previous work has demonstrated that NS1 viral protein interacts directly and specifically with eIF4GI translation initiation factor and with the polyA binding protein 1 (PABPI). Consequently, translation of cellular mRNAs is strongly inhibited in influenza virus-infected cells while viral mRNAs are actively translated. Thus, the inhibition of NS1-PABPI interaction or its destabilization can be potentially used as an antiviral strategy.

Recently, nucleic acid aptamers have been put forward for use as therapeutic agents against many human diseases due to their inhibitory ability and target specificity. In the present study, we have selected ssDNA aptamers specific to the human PABPI, as possible inhibitors of IAV mRNA translation and as potential antivirals for influenza virus replication.

Two aptamers (ApPABP#7 and ApPABP#11), which bind PABPI with high affinity have been selected and characterized. ApPABP#11 inhibits the polyA-PABPI binding and moreover, the translation of CAP and IRES-dependent cellular mRNAs in in vitro assays. Both aptamers inhibit cellular and viral translation in in vivo experiments but, under specific experimental conditions, viral translation is specifically inhibited while cellular one is preserved. Accordingly, under these experimental conditions, both aptamers reduce two logarithms influenza viral replication in multistep curves, independently of the H1N1 or H3N2 subtype and reduced viral protein accumulation in single infection curves.

These results provide support for a potential use of aptamers targeting viral-cellular interactions as novel antivirals against influenza virus replication.

18:30-18:45h (CO 83)

SIMVASTATIN AND METFORMIN INHIBIT CELL PROLIFERATION AND HEPATITIS C REPLICATION IN VITRO, BY DOWNREGULATING TCTP AND INCREASING PTEN

M. GARCÍA-VALDECASAS[1], A. GIL-GÓMEZ[1], A. ROJAS[1], J. AMPUERO[1], R. GALLEGO-DURÁN[1], B. FOMBUENA RUBIO[1], J. MUNTANÉ[2], FJ. PADILLO[2], M. ROMERO-GÓMEZ[1], JA. DEL CAMPO[1]

[1] *Digestive Disease Departament and CIBER-ehd.Valme University Hospital. Seville.*

[2]*Oncology Surgery Department, Cell Therapy and Transplant Organs Departament.IBiS. Seville*

Introduction

Chronic hepatitis C infection (HCV) induces fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Statins and metformin have been shown to delay the development and improve the prognosis of HCC. MTOR pathway is frequently deregulated in cancer, and represents a suitable therapeutic target for HCC.

AIM: To evaluate the effect of simvastatin and metformin on mTOR pathway, using an *in vitro* model and primary hepatocytes.

Methods

Huh7.5 cells were grown in supplemented DMEM at 37ºC, 5%CO2. Human hepatocytes were prepared from liver biopsies obtained from patient submitted to a tumor resection. Hepatocytes isolation was based on the two-step collagenase procedure. Huh7.5 cells were infected with JFH1 (1 particle/cell) and treated with metformin (2-10 mM) and simvastatin (2-4 µM) 3 hours after cell seeding. Cell quantification was performed using a Neubauer chamber. Total RNA and protein were extracted after 72 hours. Gene and protein expression were analyzed by qRT-PCR (Quantace, Bioline) and Western-blot using standard protocols.

Results

Simvastatin (4µM) and metformin (10mM) treatments inhibited Huh7.5 cells proliferation 58±8.6% and 38±2.2% respectively, in a dose-dependent manner after 72h. In cells treated with metformin, *TCTP, PTEN1*, and *MAPLC3B* gene expression was increased whereas MTOR and TCTP protein expression was down-regulated (2.08±0.28 and 1.89±0.02 fold respectively). Simvastatin treatment inhibited mTOR protein (2.11±0.73), and could induce PTEN and TCTP proteins (1.48±0.05 and 1.96±0.03 fold, respectively).

In Huh7.5 cells infected with JFH1, *TCTP, PTEN1* and *MAPLC3B* gene expression was increased. JFH1 infection inhibited PTEN1 protein expression (1.75±0.04) and increased TCTP protein level (2.12±0.36). PTEN1, TCTP and MTOR proteins were down-regulated in infected cells treated with metformin (2.32±0.03, 2.00±0.18 and 3.04±0.61, respectively). On the other hand, simvastatin treatment increased TCTP (2.76±0.38) and decreased MTOR (3.5±0.42).

A significant effect on viral replication was observed: metformin and simvastatin treatment could inhibit JFH1-RNA levels (52.4%±6.39) and Core protein expression (60.0±10.0 fold).

When primary hepatocytes were treated with metformin (2mM) mTOR and TCTP protein levels were found reduced (1.6 and 1.5 fold, respectively). By contrary, Caspase 3 was found induced (2.02 fold).

CONCLUSIONS

Simvastatin and metformin inhibited cell proliferation and HCV replication *in vitro*, decreasing TCTP levels (oncogene) and increasing PTEN1 (tumor suppressor). *MAPLC3B* gene expression, a marker for autophagy, is found increased after metformin treatment. Simvastatin and metformin could contribute to the prevention and therapy for HCV-related HCC, although *in vivo* experiments are needed to assess this role.

18:45-19:00h (CO 84)

HEPATITIS C VIRUS REPLICATION FACTORY STUDIED BY CRYO SOFT X-RAY TOMOGRAPHY: PLATFORM FOR PHARMACEUTICAL TRIALS OF NEW ANTIVIRAL DRUGS AT CELLULAR LEVEL

AJ PEREZ-BERNA[1], MJ RODRÍGUEZ[2], FJ CHICHON[2], M FRIESLAND[2], A SORRENTINO[1], JL CARRASCOSA[2], P GASTAMINZA[2] AND E PEREIRO[1]

[1]*ALBA Synchrotron Light Source, MISTRAL Beamline – Experiments Division, Cerdanyola del Vallès, Barcelona, Spain*

[2]*Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (CNB-CSIC), Campus Cantoblanco, Madrid, Spain*

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million people infected worldwide. Low yields, poor stability and inefficient infection systems have severely limited the analysis of the HCV life cycle and the development of effective antivirals and vaccines. HCV is a positive strand RNA that replicates its genome in intracellular membranes forming a complex membranous web. Nevertheless, the three-dimensional structure of this membranous web in whole infected cells is still unknown.

In this study we have performed full-field cryo soft X-ray tomography (cryo-SXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by the HCV replicon in conditions close to the living physiological state. We have obtained the first complete cartography of the dramatic cellular modification caused by the stable subgenomic HCV replicon transfected in cell culture. Moreover, in order to identify the viral proteins allocation in the different subcellular compartments, we have correlated the three-dimensional structure obtained with X-rays with electron microscopy immunelabeling and confocal immunofluorescence. The morphology of the membranous HCV factory web is a cytoplasmic accumulation of large and small heterogeneous vesicles, mitochondria and lipid drops.

Using this system as a platform we test the consequences of the treatment of infected culture cells with different drugs against HCV. The reversion of pathological ultrastructural alterations at cellular level has never been carried out. In conclusion, cryo-SXT provides a powerful new tool for the analysis of host-virus interactions and facilitates the trial of new antiviral drugs and vaccines at cellular level.

19:00-19:15h (CO 85)

DETECTION OF A SEXUALLY TRANSMITTED HEPATITIS C VIRUS PROTEASE INHIBITOR-RESISTANCE VARIANT IN A HUMAN IMMUNODEFICIENCY VIRUS-INFECTED HOMOSEXUAL MAN

S. FRANCO[1],M. NEVOT[1],C. TURAL[2-5],J. MOLTÓ[2-5],J. K. ROCKSTROH[6],B. CLOTET[1-5]AND MA. MARTINEZ[1]

[1]. Fundació irsiCaixa, Badalona, Spain.

[2].Department of Internal Medicine, Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

[3].Universitat Autònoma de Barcelona (UAB), Spain.

[4].Universitat de Vic (UVic) Barcelona, Spain.

[5].Fundació Lluita contra la SIDA, Badalona, Spain.

[6].Department of Medicine I, Bonn University Hospital, Bonn, Germany.

In the last decade, an increase in the number of cases of acute infections (AHC) for hepatitis C virus (HCV) in men who have sex with men (MSM) co-infected with virus human immunodeficiency type 1 (HIV-1) have been detected. Previous studies have demonstrated the existence of epidemiological networks of transmission among this group of patients. Recently, new direct acting antiviral agents (DAA) have been approved to increase the response to HCV treatment. In this study, we describe the first documented case of transmission of a HCV DAA resistant variant from a patient co-infected with HIV who was treated with telaprevir to his sexual partner and its relation to the transmission of HCV as described previously between MSM and co-infected HIV-1.

We analysed the baseline and post-treatment plasma samples of two patients of our clinical unit infected with HCV genotype 1a and co-infected with HIV-1. Patient A was treated with Telaprevir + IFN-PEG + Ribavirina, experienced a viral breakthrough, and therefore stop treatment. Patient B, his sexual partner, was cured for treatment with Daclatasvir+IFN-PEG+Ribavirina, but at week 48 after therapy this patient experienced an AHC. We amplified three regions of the HCV (NS3, NS5B and E2) by RT-PCR. The sequences obtained were analysed with sequences described in a previous work of our group. The analysis of the NS3 protease quasispecies revealed a resistant variant to telaprevir (V36M) in patient A detected after stopping treatment. Phylogenetic analysis of the NS3 showed that patient A, who developed resistance, transmitted the virus to his sexual partner (B). Analyses of other regions of the HCV (E2 and NS5B) confirmed that the resistant virus belonged to an epidemiological network of transmission.

These results confirm the sexual transmission of resistant variant to telaprevir and establish that this resistant virus belongs to an international network of HCV transmission among MSM. Since there is an international epidemic of HCV infection among HIV-infected MSM with a high rate of re-infections, attention should be paid to the transmission of HCV DAA-resistant variants, which may impair future therapeutic interventions. In addition, this case history strongly underlines that successful treatment of HCV does not preclude HCV re-infection and therefore emphasizes the need for behavioural interventions in patients at increased risk for HCV re-infection in order to preserve

cost-effectiveness of HCV treatment strategies.

19:15-19:30h (CO 86)

TRANSIENT INCREASES IN THE ERROR RATE CAN OPEN NEW ADAPTIVE PATHWAYS IN AN RNA VIRUS

E. LÁZARO[1], L. CABANILLAS[1], M. ARRIBAS[1]
Centro de Astrobiología (INTA-CSIC), Madrid (Spain)

The replication error rate is one of the main factors influencing the extension of genetic diversity contained in a population. RNA viruses together with viroids are the biological entities with the highest error rates found in nature, which is associated to a wide exploration of the genotype space and a great ability to adapt to new selective pressures, including the immune response and antiviral treatments. However, since most mutations are deleterious, there must be an upper limit for the error rate, and additional increases above this value can compromise both survival and adaptability of populations. These considerations have inspired a new antiviral therapy, named lethal mutagenesis that consists in the artificial increase of the error rate through the use of nucleoside mutagenic analogues. One of the potential problems of lethal mutagenesis is whether transient increases in the error rate, which can occur when a mutagenic treatment fails to extinguish virus infectivity, could improve virus adaptability to new selective pressures. To get a deeper insight into this point, we propagated an RNA virus, the bacteriophage Qβ, under different transmission regimes that included transient increases in the error rate through the presence of a mutagenic nucleoside analogue (5-azacytidine or AZC). Then, these populations were exposed to a new selective pressure that consisted in an increase in the replication temperature. After a number of transfers under the new conditions (42º C), we determined the degree of adaptation reached and the mutations fixed in the consensus sequences.

The results obtained show that populations previously exposed to an increase in the error rate rapidly fixed several mutations that confer advantages when replication takes place at 42º C. These mutations were not detected in the populations that always had replicated at standard error rate, suggesting that transient increases in this parameter can open new adaptive pathways. We are currently investigating whether the expansion of the mutant spectrum that takes place as a consequence of the increase in the error rate also offers advantages for adaptation to other selective pressures that are different from temperature changes.

Parallel Session XII:
REPLICATION MECHANISMS

Chairpersons: AMELIA NIETO AND LUIS MENÉNDEZ

Tuesday June 9, 2015

AUDIOVISUAL ROOM

17:30-17:45h (CO 87)

MODULATION OF P85β ACTIVITY BY SUMO

C.F. DE LA CRUZ-HERRERA[1], M. BAZ-MARTÍNEZ[2], V. LANG[3], A. EL MOTIAM[2], J. BARBAZÁN[4], R. COUCEIRO[4], M. ABAL[4], A. VIDAL[2], M. ESTEBAN[1], C. MUÑOZ-FONTELA[5], A. NIETO[1], M. S RODRÍGUEZ[3], M. COLLADO[4], C. RIVAS[1,2].

[1] *Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC, Madrid, Spain*

[2] *Centro de Investigación en Medicina Molecular (CIMUS), Universidade de Santiago de Compostela, Instituto de Investigaciones Sanitarias (IDIS), Santiago de Compostela, Spain.*

[3] *Ubiquitylation and Cancer Molecular Biology laboratory, Inbiomed, San Sebastián-Donostia, Gipuzkoa, Spain*

[4] *Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), SERGAS, Santiago de Compostela, Spain*

[5] *Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany*

Virus infection activates host cellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3K/AKT) signaling, which regulates many cellular processes such as protein synthesis, metabolism, cell survival and proliferation. The activation of this pathway by influenza A depends on the interaction of the viral non-structural NS1 protein with the regulatory subunit of the PI3K, p85β. The mechanism by which this interaction leads to PI3K activation is not fully understood. Here we show that NS1 inhibits p85β SUMOylation, increases p85βinteraction with Src tyrosine kinases, and promotes the tyrosine phosphorylation of the regulatory subunit. These findings highlight the relevance of SUMO modification in the regulation of cellular signalling pathways, such as the one controlled by PI3K, and provide an example of virus-host interaction in which influenza A takes advantage of the host SUMOylation machinery

17:45-18:00h (CO 88)

EXPRESSION OF PSEUDORABIES VIRUS IE180 PROTEIN UNDER THE CONTROL OF HUMAN TUMOR-SPECIFIC PROMOTERS (hTERT AND CEA): I.- APPLICATION TO OBTAIN CITOLYTIC VECTORS IN TUMOR CELLS

L. LERMA, B. MARTÍN, B. SAINZ JR., E. TABARÉS.

Department of Preventive Medicine, Public Health and Microbiology.School of Medicine.Autónoma University of Madrid. Spain.

Pseudorabies virus (PRV), which belongs to the viral subfamily *Alphaherpesvirinae*, has been proposed for use as a vector in cancer virotherapy since it shares the same advantages described for other anti-cancer viral vectors such as herpes simplex type 1 (HSV-1), but at the same time has additional inherent advantages including the absence of virulence, recombination and seroprevalence in the human population. Furthermore, PRV has a single

immediate early gene, encoding the IE180 protein, which controls viral temporal replication and thus represents a more simplified system for the development of anti-cancer viral vectors.

This study focused on enhancing the tumor selectivity and cytolytic efficacy of recombinant PRV for tumor cells by controlling IE180 gene transcription using cellular promoters preferentially active in tumor tissues: human telomerase reverse transcriptase (hTERT) and carcinoembryonic antigen (CEA).

PRV-TER and PRV-CEA viruses were obtained by homologous recombination with PBAC90. They have a single copy of the IE180 gene under the control of a tumor-specific promoter in their genome. PRV-T180TER and PRV-T180CEA viruses were obtained by homologous recombination with PBAC80. They have two copies of the IE180 gene, one of them under the control of a tumor-specific promoter and the other under the control of a tetracycline-inducible (Ptet) promoter. Genomes of recombinant viruses were characterized by PCR and IE180 mRNA expression was assessed by RT-PCR. Virus growth was studied in the human osteosarcoma (U2OS), cervical cancer (HeLa) and colon cancer (HT29) cell lines, using lung fibroblasts (FP7) as a normal cells line reference. In addition, this study was also carried out in primary pancreatic ductal adenocarcinoma cultures (185, 215 and 354) with human pancreatic duct epithelial cells (HPDE) as a non-tumor cell control. Recombinant virus growth was compared to the parental virus vBecker2 and to N1aHTK (which expresses the HSV-1 TK protein) and its parental PRV-NIA3 virus. Results show a titer reduction between 3 and 4 logs for the recombinant virus relative to the parental virus vBecker2 in control FP7 cells and only a 1 to 3 log reduction in U2OS, HeLa, HT29, 215 and 354 cells. Interestingly, recombinant virus growth was undetectable in HPDE and 185 cells. These data indicatethat recombinant PRV viruses may represent potential candidates for the design of anti-cancer specific oncolytic viruses.

18:00-18:15h (CO 89)

THE EXONUCLEASE XRN1P IS SPECIFICALLY REQUIRED FOR THE TRANSLATION OF BROME MOSAIC VIRUS

B. BLASCO-MORENO[1], J. JUNGFLEISCH[1], S. LEIDEL[2], J. DÍEZ[1]

[1]. Virology Unit, CEXS, Universitat Pompeu Fabra (UPF), Barcelona, Spain

[2]. RNA Biology Group, Max Planck Institute for Molecular Biomedicine, Münster, Germany.

The exonuclease Xrn1p is a crucial factor in controlling the degradation of most messenger RNAs (mRNAs). Given that positive-strand RNA viruses mimic cellular mRNAs, Xrn1p is expected to be a restriction factor for this group of viruses. Such role has already been proven for hepatitis C virus. However, other viruses such as Dengue use Xrn1p to precisely degrade the genomic RNA and produce a pathogenic subgenomic RNA. Here, we report a new function of Xrn1p in the translation of the [(+)-RNA] brome mosaic virus (BMV) in yeast.

We demonstrate that translation of BMV RNA is highly and specifically inhibited when Xrn1p is deleted, in spite of the over-accumulation of viral RNA. By sequential

deletion analysis, we identify the 5'UTR and a stem-loop structure in the ORF as the main determinants in the Xrn1p-dependence for translation. Moreover, polysome profiling analyses indicate that Xrn1p is needed for efficient BMV RNA translation initiation.

Three main conclusions arise from studying mutants targeting key Xrn1p features. First, both the Xrn1p exonuclease activity and its capability to bind 5' uncapped mRNAs are required for efficient BMV RNA translation. Second, expression of the nuclear exonuclease in the cytoplasm (Rat1ΔNLS) does not rescue BMV RNA translation in *xrn1Δ.* Importantly, Rat1ΔNLS can recover normal growth and steady-state BMV RNA levels. Third, an Xrn1p mutant unable to be imported to the nucleus is still capable of promoting viral RNA translation. This indicates that the role of Xrn1p in translation is independent from its recently described function in transcription. Together, our data suggest a novel function of Xrn1p in the specific regulation of BMV RNA translation.

18:15-18:30h (CO 90)

IFN-α TREATMENT CAUSES A MASSIVE APOPTOSIS IN IBDV INFECTED CELLS

L.L. CUBAS, M. CISCAR, J.F. RODRÍGUEZ, D. RODRÍGUEZ.

Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC. Madrid, Spain.

The infectious bursal disease virus (IBDV) is the best characterized member of the *Birnaviridae*family, that groups naked icosahedral viruses with bi-segmented double-stranded RNA (dsRNA) genomes. IBDV infects different bird species and causes an acute immunosuppressive disease, known as IBD or Gumboro disease, that affects domestic chickens (*Gallus gallus*), and is responsible for major economic losses to the poultry industry worldwide. The most obvious pathogenic sign is the atrophy of the bursa resulting from the infection and destruction of pre-B lymphocytes. Although the molecular bases for IBDV pathogenesis are still poorly understood, it has been suggested that an exacerbated innate immune response that leads to a massive production of proimflamatory cytokines is related with IBDV-induced pathogenicity.

A crucial component of the host innate immune response is the IFN system. IFNs have been extensively studied in the context of host defense against viral infection. However, type I (IFNα/β) and type II (IFNγ) may have dual biological roles: elicit an antiviral state in uninfected cells through the transcriptional activation of anti-viral proteins such as PKR, OAS and Mx, while selectively inducing apoptosis in virus-infected cells, thus limiting viral replication and spreading of the infection.

We focus our study on the interaction between IBDV and the host innate immune response. In this respect, we have observed a generalized apoptosis in cultures infected with IBDV and treated with IFN at different times post-infection. As observed by different assays, the apoptotic effect is milder when IFN is added at later times post-infection. Significantly, in cells that do not express PKR (siRNA) the IFN treatment after IBDV infection does not cause extensive apoptosis. To further analyze the cellular

response, we studied the expression of different ISGs and pro/-anti-apoptotic genes by qRT-PCR. In these assays, we determined that TNF-α is upregulated in cultures infected with IBDV and treated with IFN at earlier times post-infection, but this effect is not observed in PKR-silenced cells. These results suggest that IFN secreted by infected cells may contribute to trigger TNF-α mediated apoptosis which, in turn, could explain the destruction of the bursa of Fabriciusin IBDV-infected chickens, which leads to immunosuppression.

18:30-18:45h (CO 91)

BIOGENESIS AND DYNAMICS OF TOROVIRUS REPLICATIVE STRUCTURES

G. ÁVILA [1], M.T. REJAS[2], F.J. CHICHÓN[3], M. GUERRA[2], J.L. CARRASCOSA[3], J.J. FERNÁNDEZ[3], D. RODRÍGUEZ [1].

[1]*Department of Molecular and Cellular Biology and* [3]*Department of Macromolecular structures, Centro Nacional de Biotecnología (CNB-CSIC), Madrid.*

[2]*Centro de Biología Molecular Severo Ochoa (CBM-CSIC). Madrid, Spain.*

Plus-stranded RNA viruses replicate in the cytosol of infected cells, in membrane-bound replication complexes containing the replicase proteins, the viral RNA and host proteins. The formation of the replication complexes through the rearrangement of cellular membranes is currently being actively studied for viruses belonging to different viral families. We previously identified double membrane vesicles (DMVs) in the cytoplasm of cells infected with the equine torovirus Berne virus (BEV), the prototype member of the *Torovirus* genus (*Coronaviridae* Family, *Nidovirales* Order). The presence of DMV clusters, considered to be involved in the replication process, is a common feature in Nidovirus infected cells. Other structures that have also been related with the replication factories, such as convoluted membranes, are present only in some of the examined coronaviruses. In addition, in cells infected with the infectious bronchitis virus (IBV), a member of the gammacoronavirus, new structures known as spherules, which strongly resemble the replication sites of other positive-strandedRNA viruses, have been recently described. Our purpose was to perform an in-depth ultrastructural analysis of cells infected with BEV to characterize the architecture of torovirus replication factories, and to learn about their biogenesis and dynamics during the infection. Previous analysis by conventional transmission electron microscopy suggested that the DMVs form a reticulovesicular network (RVN) resembling those described for the related severe acute respiratory syndrome (SARS) coronavirus and the equine arteritis virus (EAV). Here, we used serial sectioning and electron tomography of cells infected with BEV and fixed at different post-infection times to obtain three-dimensional images of the replication factories. We confirmed the formation of a RVN in BEV infected cells where the DMVs outer membranes are interconnected with each other and with the ER. Like in EAV, convoluted membranes were not observed in the RVNs. However, we observed paired or zippered ER membranes lacking luminal space, which in some cases are connected with the DMVs, and likely represent early structures that will evolve to give rise to

DMVs. Interestingly, curled membranes resembling the spherules described in IBV were observed at late time post-infection in BEV-infected cells. After careful examination of the tomograms we hypothesize that these structures probably represent remnants of paired membranes unused for the formation of DMVs, which accumulate at late times post-infection. Hence, BEV shows important similarities, but also some differences, in the architecture of the replication factories with other related viruses in the *Nidovirales* order.

18:45-19:00h (CO 92)

STRUCTURAL BASIS OF INFLUENZA VIRUS RNP ACTIVITY

R.COLOMA[1], R. ARRANZ[2], J. ORTIN[1] AND J. MARTIN-BENITO[2].

[1].*Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología, Madrid, España.*

[2].*Departamento de Estructura de Macromoléculas, Centro Nacional de Biotecnología, Madrid, España.*

The influenza A virus genome is formed by a set of 8 ribonucleoprotein particles in which each RNA molecule is associated to the polymerase complex and many monomers of the nucleoprotein (NP). These complex molecular machines are central in crucial viral processes. RNPs are transcribed and replicated inside the nucleus of the infected cell, from which they are exported to cytoplasm where the morphogenesis of virions takes place. As shown by multiple studies, most of them with NP mutants, the mRNA synthesis depends strongly on the correct arrangement of the whole complex, showing that the structure of the RNP is a key factor for its functionality. Using electron microscopy and image processing we have determined the structure of native influenza RNPs derived from virions. The basic arrangement shows a double-helical conformation in which two NP/RNA strands are associated each other in an antiparallel way; both strands are connected by a loop at one end of the particle and associated to the polymerase at the other end.

Our group is currently conducting an other structural study of native influenza RNPs derived from virions much more detailed thanks to the use of a high-end microscope equipped with a direct detector. This study is revealing a more complex structure in which we have found a broad conformational variability that could be key for the movement of the polymerase along the RNP during the transcription and replication processes.

19:00-19:15h (CO 93)

THE DEAD-BOX HELICASE DHH1 PROMOTES TRANSLATION OF HIGHLY STRUCTURED mRNAS

J JUNGFLEISCH[1], D NEDIALKOVA[2], I DOTU[3], E RAINERI[4], S LEIDEL[2], J DÍEZ[1]

[1]. *Laboratorio Virologia Molecular, Universitat Pompeu Fabra, Barcelona, Spain*

[2]. *RNA Biology Laboratory, Max Planck Institute for Molecular Biomedicine, Münster, Germany*

[3]. *Hospital del Mar Medical Research Institute, Barcelona, Spain*

[4]. *Statistical Genomics, Centro Nacional de Analisis Genomica, Barcelona, Spain*

Translation control and mRNA decay are central to maintain proper gene expression allowing to respond rapidly to

perturbations. The group of Dhh1/DDX6 DEAD-box helicases plays a key role in these processes since its members act at the interface of mRNA translation and decay They promote translation repression of cytoplasmic mRNAs that are then fed into decay or stored. Intriguingly, we have previously shown that Dhh1/DDX6 activated translation of positive-strand RNA viral genomes. However, the mechanism involved and whether this role is extended to cellular mRNAs is unknown. By using a model system that allows the replication of the Brome mosaic virus in yeast here we show that the ATPase activity of Dhh1 was required for its positive role in translation. Moreover, polysome profile analyses indicated that Dhh1 promotes translation initiation. This role was linked to the concurrent presence of the 5´ and 3´UTRs, two highly structured sequences known tocontrol translation, and of a newly determined stem-loop in the ORF region. Consistent with a direct role of Dhh1 in translation, Dhh1 co-immunoprecipitated with the viral RNA without affecting its stability. Excitingly, genome-wide ribosome profiling analyses in yeast demonstrated that Dhh1 also promotes translation of a specific subset of cellular mRNAs that are enriched in previously described Dhh1-bound mRNAs. These mRNAs present higher base pair probablilities at their ORFs than those translationally-repressed or not translationally affected by Dhh1 and are enriched in mRNAs related to ribogenesis processes. As a consequence modulation of Dhh1 activity will lead to their fast coregulation, as needed for example under stress conditions. In sum, our results uncover a novel role of Dhh1 in the cell that has been hijacked by viruses to control their gene expression and points out at this DEAD-box helicase as a key cross-talk mediator between translation, translational repression and decay.

19:15-19:30h (CO 94)

INCREASED PATHOGENESIS OF INFLUENZA A H1N1 VIRUS LED BY A PA RESIDUE DETECTED IN A FATAL CASE

J. VASILIJEVIC[1,2], A. NIETO[1,2] AND A. FALCON[1,2].

[1]*Centro Nacional de Biotecnología-CSIC, Madrid, Spain.*

[2]*Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (Ciberes), Spain.*

Pandemic 2009 H1N1 (pH1N1) influenza viruses caused mild symptoms in most infected patients. However, a greater rate of severe disease was observed in healthy young adults and children without comorbid conditions, suggesting that viruses with different pathogenicity could cocirculate.

Our previous data indicated that a strain of pH1N1 virus isolated from a fatal case presented enhanced pathogenicity compared to a virus isolated from a mild case, which circulated during the 2009 pandemic. PB2 A221T, PA D529N, HA S127L changes appeared as particularly interesting and suggested that one or combination of these changes could play an important role in increased pathogenicity. Biological properties of recombinant viruses (pH1N1 California/04/) carrying each of these residues or combination of them have been analyzed both in vitro (human lung alveolar epithelial cells) and in vivo (murine

model). Wild-type recombinant virus and viruses carrying PA529N, PB221T or both changes replicated at similar rate, but HA recombinant virus had a slightly higher replication rate at 9 and 12 hpi in cell culture. In vivo analysis showed a significantly decreased LD50 of 50 and 10 fold for PA and PA/PB2 recombinant viruses, respectively, compared to that of the control virus. Viral titer in lungs of PA recombinant virus infected mice was higher up to 7 dpi., moreover a high proportion of mice presenting infectious virus in the heart, was found in these infected animals whose replication was detected by the presence of NEP (Nuclear Export Protein) mRNA. Analysis of CD45+ cells in lungs of infected mice showed higher percentage of neutrophils and dendritic cells by 1 and 2 dpi, as well as rapid loss of alveolar macrophages by the 2 dpi in PA and PA/PB2 recombinant viruses infected mice compared with the control virus infected mice.

These results indicate that PA529N residue leads to increased pathogenicity of influenza A H1N1 virus mediated by several biological processes.

FLASH PRESENTATIONS

Chairpersons: SUSANA ALVAREZ AND JOSÉ LUIS JIMÉNEZ

Wednesday June 9, 2015

AUDITORIUM REAL CASA DE LA MONEDA

10:20-11:30 h

*Flash presentations

(P01)

THE COAT PROTEIN OF THE POTYVIRUS PLUM POX VIRUS CAN BE PHOSPHORYLATED *IN VIVO* AND THIS MODIFICATION ESTABLISHES A CROSS-TALK WITH ITS PREVIOUSLY DESCRIBED *O*-GlcNAcylation

S. MARTÍNEZ-TURIÑO[1], J. J. PÉREZ[1], R. NAVAJAS[1], S. CIORDIA[1], J. A. GARCÍA[1]

[1]. Centro Nacional de Biotecnología CNB-CSIC, Madrid, Spain.

Plum pox virus (PPV), a member of the genus *Potyvirus* (family *Potyviridae*), causes sharka, one of the most damaging diseases of stone fruit trees. PPV genome is a positive-sense single-stranded RNA encapsidated by a single type of capsid protein (CP) in flexuous rod particles. It is translated into a large polyprotein, and a frameshift product, that are proteolytically processed in at least 11 final products.

O-GlcNAcylation is a post-translational modification (PTM) that adds single *O*-linked N-acetylglucosamine residues to nuclear and cytoplasmic proteins. Contrary to animals, this PTMhas been barely studied in plants. The PPV CP is the best-characterized target of *O*-GlcNAcylation produced in plants. It is modified by secret agent (SEC), one of the two *O*-linked N-acetylglucosamine transferases (OGT) identified in *Arabidopsis thaliana*, in up to seven specific threonines located towards the N-terminal region of PPV CP.*O*-GlcNAcylation of CP has a positive role in the infection process, probably intervening in virion assembly and/or stability.

A "Yin-Yang" mechanism has been proposed to regulate reciprocal phosphorylation and *O*-GlcNAcylation of different mammalian proteins. Some previous evidence suggested that PPV CP could be phosphorylated. In this study, we made use of proteomics analyses to demonstrate that PPV CP is phosphorylated *in vivo* at its N-terminal region. In contrast with the classical "Yin-Yang" mechanism, phosphorylation affects residues different from the *O*-GlcNAcylated ones (serines Ser25, Ser81, Ser101 and Ser118). However, quantification by a differential proteomics strategy based on iTRAQ (Isobaric Tags for Relative and Absolute Quantitation) of peptides of CP from virions purified from wild type and SEC-deficient plants led to uncover the existence of some cross-talk between *O*-GlcNAcylation and phosphorylation in PPV-CP. Accordingly we speculate that some sort of regulation could direct reciprocal and dynamic changes between this two PTM affecting neighbouring Thr/Ser residues that are susceptible to be modified.

*Flash presentations

(P02)

NEW THERAPEUTIC ALTERNATIVES FOR THE TREATMENT OF ADENOVIRUS INFECTIONS IN IMMUNOSUPPRESSED PATIENTS: DESIGN, SYNTHESIS AND EVALUATION OF THE ANTI-ADENOVIRUS ACTIVITY OF PIPERAZINE DERIVATIVES

P. MARTÍNEZ-AGUADO[1], M. VEGA HOLM, A. SERNA GALLEGO[1], J. I. CANDELA, J.A. MARRUGAL LORENZO[1], I. GÓMEZ-MARÍN[1], F. IGLESIAS GUERRA, J. M. VEGA PÉREZ AND J. SÁNCHEZ-CÉSPEDES[1]

[1]*Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocío/CSIC/University of Seville, Clinical Unit of Infectious Diseases, Microbiology and Preventive Medicine, Seville, Spain*

[2] *Department of Organic and Pharmaceutical Chemistry, School of Pharmacy, University of Seville, Seville, Spain*

Adenoviruses (HAdV) are the cause of many different acute infections mostly in the respiratory and gastrointestinal tracts, as well as conjunctivitis. HAdV disease in immunocompetent individuals is mostly self-limiting, however, in immunocompromised individuals, especially in pediatric units, HAdV infections are cause of high morbidity and mortality. Unfortunately, despite the significant clinical impact, there are no antiviral agents that are approved for the treatment of HAdV. Sub-optimal therapeutic options to treat HAdV infections in immunosuppressed patients include the use of broadly acting antivirals such as ganciclovir, acyclovir, vidarabine, ribavirin and cidofovir, with highly variable results. To address this situation, we used high-throughput screening (HTS) of synthetic small molecule libraries to identify compounds that restrict HAdV infection. Since different combinatorial piperazinone libraries have previously been described and identified as potent antiviral compounds we choose this ring as the central core for the design and evaluation of a new small library. Based on the structure of a previously reported anti-HAdV piperazinone (15D8) that targeted the HAdV replication process, we designed, synthesized and evaluated a library of new piperazine derivatives with potential anti-HAdV activity. We substituted the piperazin-2-one ring from compound 15D8 by one of piperazine, moving the carbonyl group at N1 position. Starting with an initial first generation of compounds two more generations were designed based on the structure-activity relationship (SAR) of the active compounds obtained in each previous generation. We found five phenylpiperazine compounds that significantly inhibited HAdV infection. These compounds showed substantial anti-HAdV activity at low micromolar concentration targeting the HAdV DNA replication process. Moreover, we found that the presence of a phenylpiperazine ring and a urea group at N1 carrying electron-withdrawing groups conferred little or no cytotoxicity to these molecules. The selected phenylpiperazines potentially represent strong hit compounds for the development of a new class of antiviral compounds to treat HAdV infections in immunossupressed patients and could represent a useful tool to better understand the complex events involved in HAdV DNA replication.

*Flash presentations

(P03)

IN VITRO IDENTIFICATION OF A STRUCTURAL MOTIF IN THE 3' UTR REGION OF THE IFNA5 MRNA FAVOURED BY miR-122 WHICH ENABLES THE RECOGNITION OF THE 40S SUBUNIT OF THE RIBOSOME IN THIS REGION

R. DÍAZ-TOLEDANO [1,2], N. CALERO-MUÑOZ[1], A. ARIZA-MATEOS[1,2]AND J. GÓMEZ[1,2]

(1) Laboratory of RNA Archeology, Instituto de Parasitología y Biomedicina 'López-Neyra', CSIC, Armilla, 18100 Granada, Spain. (2) Ciberehd.

*In silico*predictions have allowed the detection of a microRNA binding site within the non-coding 3' region of the alpha interferon subtype 5 that is specifically expressed in the liver. We have analysed the RNA structure in this region using RNases that specifically recognise single and double chain RNA. We observed that the presence of miR-122 modifies the digestion pattern of these RNases in the region predicted for their annealing. The modifications affect the RNA structure in the "stop" codon region, and suggests the possible appearance of a pseudoknot-type structure. The resulting conformational change post-hybridisation with miR-122 generates an RNA mimetic structure which can be recognised *in vitro* by human RNase P and the ribozyme of the cyanobacterium *Synechocystis* sp. In addition, the presence of miR-122 promotes, in a mild yet specific way, the interaction of the 40S subunit with IFNA5 mRNA in the absence of other protein factors. We have seen that the 3' region of mRNA is responsible for this binding. It is not yet known if this interaction plays a role in the functional recruitment of the 40S subunit to the mRNA.

*Flash presentations

(P04)

QUERCETIN MODIFIES LIPID DROPLET MORPHOLOGY AND IMPAIRS HEPATITIS C VIRAL LIFE-CYCLE STEPS FROM ASSEMBLY TO REPLICATION

Á.ROJAS*[1], S.CLEMENT[2], J.A. DEL CAMPO[1], M.LEMASSON[3], M.GARCÍA-VALDECASAS[1], L. ROJAS[1], A.GIL-GÓMEZ[1], I. RANCHAL[1], J.BAUTISTA[4], A.R. ROSENBERG[3], F.NEGRO[5], M.ROMERO-GÓMEZ[1].

[1]. Unit for Clinical Management of Digestive Diseases and CIBERehd, VALME UNIVERSITY HOSPITAL, Seville, Spain

[2]. Division of Clinical Pathology , University Hospital, Geneva, Switzerland

[3]. Hepatitis C virology, University Paris Descartes, Paris, France

[4] . Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Seville, Seville, Spain

[5]. Division of Clinical Pathology and Gastroenterology and Hepatology, University Hospital, Geneva, Switzerland

Background and Aims:

Hepatitis C virus (HCV) life cycle can be divided into several steps: (i) entry of viral particles, (ii)

translation of the viral proteins, (iii) replication of the viral genome, a step which requires the activity of HCV non structural proteins including the protease NS3, and (iv) assembly of new viral particles, a step which requires the localization of HCV core and NS5A protein to lipid droplets mediated by the host diacylglycerol acyltransferase type 1

(DGAT1).

Quercetin, a bioflavonoid, seems to prevent the localization of HCV core protein to lipid droplets and to inhibit HCV replication (Rojas et al., AASLD 2013). Here we aimed at evaluating the potential of quercetin as antiviral drug and further defining its mechanism(s) of action on the different steps of HCV life cycle.

Methods: To reproduce the complete HCV life cycle, Huh7.5 cells and primary hepatocyte were infected with JFH1and subsequently treated with doses of quercetin. i) Replication of HCV genome was assessed by measuring the intracelular levels of negative-strand HCV RNA by qRT-PCR. ii) Production of infectious virus was assessed by measuring the infectivity titers in filtered culture supernatants with focus-formation assay and by COBAS® TaqMan® HCV Test v2.0. iii) NS3 protease activity in vitro was measured using a comercial Kit SensoLyte® 520 HCV Protease Assay. (iv) DGAT activity was analyzed using the protocol previously described by McFie and coll. (2011) in Huh7.5 cells infected by JFH1.

Results: Infectivity assay in Huh7.5.1 and primary hepatocytes were IC50: 37.83 and 23.63 µM respectively. At 50µM HCV-RNA levels decreased (Huh7.5.1: 39% and PHH: 24%). The amount of HCV-RNA (evaluated by the quantity of viral RNA produced in the supernatant) was decreased as well by 60%±26.7 ($p<0.05$) compared to the supernatant from Huh7.5 infected by JFH.1 (1MOI). In vitro NS3 activity was inhibited by quercetin by 45.40%±1.15 RFU ($p<0.001$) compared to the vehicle, DMSO (no inhibition). DGAT enzyme activity in infected cells was increased relative to noninfected cells (fold induction 2.29±0.23 $p<0.01$). However, this increase was significantly inhibited by treatment with quercetin [63.5±2.9% ($p<0.01$)].

Conclusions: In the current study, quercetin was observed to inhibit DGAT activity, to decrease NS3 activity, as such, resulting in impairment of viral infectivity and replication. Thus, the antiviral activity of this flavonoid is promising and mediated through several viral and host mechanisms.

*Flash presentations

(P05)

DELAYED LIVER FIBROSIS IN HTLV-2-INFECTED PATIENTS CO-INFECTED WITH HIV-1 AND HCV WITH SUPPRESSIVE ANTIRETROVIRAL TREATMENT

ABAD-FERNÁNDEZ M, MORENO A, DRONDA F, DEL CAMPO S, QUEREDA C,CASADO JL, PÉREZ-ELÍAS MJ, MORENO S, **VALLEJO A**

Department of Infectious Diseases, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Universitario Ramón y Cajal, Madrid, Spain.

Objectives: HIV-1 and HTLV-2 co-infection is found with relatively high frequency among injection drug users in North America and Western Europe since 80´s. There is still no clear evidence that HTLV-2 causes any human disease. Nevertheless, several studies analyzing the effects of HTLV-2 on HIV-1 pathogenesis in dually infected HTLV-2-HIV-1 individuals revealed delayed progression of HIV-1 to AIDS. On the other hand, among individuals coinfected with HIV-1 and HCV, the influence of HTLV-2 on HCV pathogenesis has been poorly studied.

Design: Retrospective study to clarify the influence of HTLV-2 in HCV pathogenesis and hepatic fibrosis among patients co-infected with HIV-1.

Methods: This was comparative cohort study including 39 HTLV-2-HIV-1-HCV-coinfected patients and 42 HIV-1-HCV-coinfected patients. They were evaluated for transaminase levels, hepatic fibrosis stage, IL-28B genotype, Th1/Th2/Th17 cytokine levels, immune activation, inflammation, and microbial translocation.

Results: HTLV-2-HIV-1-HCV-coinfected patients had lower alanine aminotransferase levels (p=0.023) and hepatic fibrosis (p=0.012) compared to HIV-1-HCV-coinfected patients. Moreover, Kaplan-Meier survival analysis showed a delay in hepatic fibrosis development for up to five years (p=0.032). HTLV-2-HIV-1-HCV-coinfected patients also had higher Th1/Th2 ratio (IFNγ/IL4 ratio, p=0.045, TNFα/IL4 ratio, p=0.011) and Th17 response (p=0.047), while lower CD8 T cell activation (p=0.013) and LPS level (p=0.002).

Conclusions: Findings strongly support that HTLV-2 co-infection might delay fibrosis developmentin HCV-HIV-1 co-infected patients.

*Flash presentations

(P06)

RNA-seq PROFILES FROM RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) HEAD KIDNEY AFTER INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS INFECTION.

N. A. BALLESTEROS[1], H. ARTAZA[1], G. PADILLA[1], L. ALONSO[1], S. RODRÍGUEZ SAINT-JEAN[1], S. PEREZ-PRIETO[1].

[1.] Centro de Investigaciones Biológicas, (CSIC), Dpto. Microbiología Molecular y Biología de la infección, 28040, Madrid, Spain.

Infectious hematopoietic necrosis virus (IHNV) is a single-stranded, negative-sense RNA virus and a member of the *Rhabdoviridae* family. IHNV is endemic within both wild and cultured host populations in North-West of USA. The virus establishes acute, lethal infection in juvenile Pacific salmonids and results in a significant loss to hatchery programs and aquaculture industries every year.

In Europe, the rainbow trout is the most affected species. The aim of this work was to contribute to a better understanding of how fish respond to the viral infection. This information will be essential for the knowledge of immunity and to afford new perspectives in the design of oral vaccines. We have focused in the transcriptome response (physiological and pathological state) of rainbow trout at 3 and 7 days after IHNV challenge, which correspond to asymptomatic and symptomatic (respectively) viral infection.

The massive RNAseq technique was used for this study and the head kidney (main hematopoietic organ) was selected for sampling. The transcriptome of rainbow

trout from the National Animal Genome Research Program was used as reference, and the information was divided in two different groups (Ohnologous and No ohnologous genes) as reference. These RNA-seq libraries were sequenced with a read length of 75 nucleotides, single-end reads in two different lanes of an Illumina GAiix Format. The number of reads in each group was well balanced with 16.122.108 reads in the infected fish at 3 days post infection (dpi) group, 16.378.765 reads in the infected fish group sampled at 7 dpi and 16.192.950 reads in the non-infected fish group (control group). The results rendered 6875 differentially expressed transcripts (DETs) at 3 dpi in the IHNV infected group and 5857 DETs at 7 dpi in comparison with the control group. In addition, gene pathway analysis of the differentially expressed gene set highlighted several putative genes involved in the immune response activity.

The expression patterns of 7 differentially expressed genes involved in immune response were validated by quantitative real-time RT-PCR. Our results provide valuable information on gene functions associated with IHNV infection.

This work was supported by projects AGL2010-18454 (Spanish Ministry of Economy and Competitiveness,MINECO) and 2010-20E084 (CSIC). N. Ballesteros wants to thank the MINECO for their PhD student fellowship.

*Flash presentations

(P07)

"VIR(TU)AL EPIDEMIC": A GAME ABOUT VIRUSES FOR SMARTPHONES

E. GÓMEZ-LUCÍA[1], L. BENÍTEZ[2], M.M. BLANCO[1], M.T. CUTULI[1], A. DOMÉNECH[1], R. FLORES[3], J. QUER[4], J. ROMERO[5], R. AÑEZ[1]

[1]Dpto de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense Madrid

[2]Dpto de Microbiología III, Facultad de Ciencias Biológicas, U.Complutense Madrid

[3]Instituto de Biología Molecular y Celular de Plantas (Universidad Politécnica-CSIC), Valencia

[4] Liver Unit. Hospital Universitari Vall d'Hebrón, Barcelona

[5]Dpto de Protección Vegetal, INIA, Madrid

A team of teachers of the Complutense University of Madrid, virologists of the SEV and computer science designers (Sr. Brightside) has developed a free online game (http://epidemia.sevirologia.es) for Smartphone, which also may be played with tablets and conventional computers. It is aimed both for the self-evaluation of Virology and to learn more about various aspects of this science in a subliminal way, enjoying the knowledge about viruses. At the moment, the game is offered in Spanish and in English. More than 200 multiple choice questions inquire about what are viruses and their differences with bacteria and eukaryotic organisms, the diseases that they produce in man, animals and plants, their treatment, diagnosis and prevention, as well as about bacteriophages and the possible applications of viruses. As soon as the question is answered, the correct answer is available, along with additional information on the treated aspects.

The name "Vir(tu)al epidemic" alludes to its design, representing a challenge to save the World from a lethal viral epidemic. At the beginning of the game, the program asks for the name of the player, who begins with 0 points and five lives. Whenever the player fails a question, he/she loses one life, which can be recovered when responding correctly to three questions. Each question has a value that is added to the score of the player. Also, following every few correct answers, an encouraging message is received. The game ends when the player has responded to 20 questions, being his/her name and score inscribed in a public ranking. As it is competitive due to this ranking, it is very attractive and contributes to approach Virology to the general public and to students, who generally consider viruses as distant biological entities, possibly due to their small size and the many different aspects involving their study.

The game has been presented in High Schools of the Community of Madrid, and in a course of the 8th edition of "Teachers and Science" sponsored by the Fundació Catalunya La Pedrera, Barcelona. A satisfaction survey has been prepared to present it in the reference institutes, and to evaluate the acceptance of the project.

Funded by FECYT, SEV, UCM y Erasmus+

*Flash presentations

(P08)

INNOVIROLOGY: THE NETWORK OF EUROPEAN TEACHERS/TRAINERS OF VIROLOGY

E. GÓMEZ-LUCÍA[1], A. DOLEI[2], R. LAVIGNE[3], S. LEPODER[4], C. LOGUE[5], D. RADIN[6], M. SZYNDEL[7], B. WÖLK[8,9]

[1]Dpto de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense Madrid (Spain). [2]Department of Biomedical Sciences, Università degli Studi di Sassari (Italy).[3]Katholieke Universiteit Leuven (Belgium).4Université Paris-Est. Ecole Nationale Vétérinaire d'Alfort. UMR 1161 Virologie INRA-ENVA-ANSES (France). [5]Public Health England, Leeds, (England). [6]Faculty of agriculture, Belgrade (Serbia).[7]Szkola Glowna Gospodarstwa Wiejskiego (Poland).[8]LADR GmbH, MVZ Kramer and Colleagues, Geesthacht (Germany), [9]Medizinische Hochschule Hannover (Germany)

The European Union has funded an initiative for innovation in teaching and training of Virology as well as for improving its dissemination through its programme Erasmus+ (Innovirology, project number 2014-1-ES01-KA203-004962) under the orchestration of eight institutions from different European countries. The idea is to create a network for teachers and trainers of Virology to connect to each other, share and develop teaching materials and contribute to the general spread of knowledge in the Virology field. The specific aims include:

- To compile protocols for teaching laboratory Virology which different teachers choose to share, so that any member of the network can freely access it and select laboratory techniques best suited to the characteristics of their group of students.

- To share and make available to other Virology teachers specific educational tools developed by the network, such as (but not exclusively) information and communication technologies (computer programs, games, tools for self-assessment or evaluation, etc.).
- To develop online courses for life-long learning and bring Virology to those who are interested in learning more about it. Online courses will be "What are viruses?", "Basic and applied Virology", "Clinical Virology", "Veterinary Virology", "Plant viruses", "Viral molecular diagnostics", "Emerging viral diseases" and "Food Virology". These courses will be designed so that they can be followed by anyone with a general interest in virology. The possibility of including, in addition to an online exam, a graded face-to-face test so the course grants a degree of official recognition, is being discussed. Also, the consortium aims to write a book on Virology, online and open access. It is intended to have plenty illustrations, attractive to young high school students and to the person of the street.
- To create and maintain social networks (Facebook, twitter, etc) about news on all fields of Virology.

Any teacher of Virology in Europe is welcome to join the network. This invitation includes all Virology teachers interested independent of the type of their institution, their major field or their interest in different subspecialties of Virology. At the end of the project, a questionnaire will be prepared to evaluate the usefulness of the different aspects of the project, which we foresee will help students and general public to get more acquainted with the different aspects of Virology.

*Flash presentations

(P09)

APPLICATION OF A RT-*q*PCR TECHNIQUE IN THE CONFIRMATION OF CASES AND DEATHS BY YELLOW FEVER VIRUS

M. ROSSI S.[1,2], J. MÉNDEZ R.[3], A HERNÁNDEZ[2], F. LASALA[1], J.M. LUQUE[1], F. MOLERO[1], G. CÉSPEDES[2], A. TENORIO[1], M.P. SÁNCHEZ-SECO[1], A. NEGREDO[1]

[1]*Laboratorio de Arbovirus y Enfermedades Víricas Importadas, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, España.*

[2]*Sección de Investigaciones en Patología Ultraestructural y Biología Molecular, Instituto Anatomopatológico José A. O´Daly, Facultad de Medicina, Caracas, Venezuela.*

[3]*Instituto Nacional de Salud, Bogotá, Colombia.*

Yellow fever (YF) is an arbovirosis caused by the Yellow Fever Virus (YFV), characterized by the presentation of fever, jaundice and haemorrhages of less than 10 days and a high lethality. In South America, between 1985 and 2004, more than 90% of cases and outbreaks of YF were described in Peru, Bolivia, Brazil, Colombia, Ecuador and Venezuela, with a total of 3,559 cases of sylvatic and 2,068 deaths (58% of lethality). In Venezuela the YF-cases occurs as sporadic and self-limiting outbreaks in the Central west region, dedicated to forestry, crop and livestock, and characterized by poor socio-sanitary conditions with difficulties to access to the national health system. In most of the cases the aetiological of the cases must be done post-mortem at

immunohistochemical level due the difficulties of geographic access. The aim of this work was to evaluate the applicability of a RT-*q*PCR technique for the confirmation of deaths caused by YFV in human primates (HPr) from Venezuela and Colombia and non-human primates (NHPr) from Venezuela, using samples of formalin-fixed and paraffin-embedded tissues (FFPET) and freeze tissues (FT). Sections of tissues were dewaxed according histologic techniques, dried at ambient temperature, digested with Proteinase K and aliquots of the lysate were added to vials containing AVL previous to the extraction of the RNA. The amplifications of the cDNA were done in a LightCycler 2.0 (Roche) and detected using TaqMan probes. Of a total of 11 HPr-deaths and 5 NHPr-deaths with immunohistochemical diagnosis of YFV, respectively 9 (81.82%) and 5 (100%) were positive in the RT-*q*PCR with an average *Ct* of 32.95 for the FFPET of the HPr and 27.88 for the NHPr samples. Of all the samples of FT (80%; 8/10) were positive with an average *Ct* of 25.97. The *Ct*-values of the FFPET samples from NHPr suggest that its viral load is higher than the viral load described for HPr-samples. The differences in the *Ct*-values between the samples of FT and FFPET should be associated with the deleterious activity of formaldehyde on nucleic acids such as fragmentation, methylol bridges formation, etc. Results are discussed in the context of the importance of RT-*q*PCR as a sensible and specific tool in anatomic pathology as well as for the detection of different lineages of YFV and its use for the confirmation of deaths caused by this arbovirus in Venezuela.

*Flash presentations

(PO 10)

CONSERVATION OF G PROTEIN EPITOPES IN RESPIRATORY SYNCYTIAL VIRUS (GROUP A) DESPITE BROAD GENETIC DIVERSITY: IS ANTIBODY SELECTION INVOLVED IN VIRUS EVOLUTION?

A. TRENTO[1, 2], L. ÁBREGO[3], R. RODRIGUEZ-FERNANDEZ[4], M. I. GONZÁLEZ-SÁNCHEZ[4], F. GONZÁLEZ-MARTÍNEZ[4], A. DELFRARO[5], J. M. PASCALE[3], J. ARBIZA[5] AND J. A. MELERO[1, 2].

[1] *.Unidad de Biología Viral, Centro Nacional de Microbiología. Instituto de Salud Carlos III, Majadahonda. Spain*

[2] *.CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Majadahonda, Spain.*

[3] *.Departamento de Investigación en Virología, Instituto Conmemorativo Gorgas de Estudios de la Salud, Panamá.*

[4] *.Hospital General Universitario Gregorio Marañón, Madrid, Spain.*

[5] *.Sección Virología, Facultad de Ciencias, Universidad de la República, Uruguay.*

Human respiratory syncytial virus (hRSV) is recognized as the major cause of severe acute lower respiratory tract infections (ALRI) in infants and young children worldwide. Phylogeny of group A sequences of the G glycoprotein of hRSV revealed diversification in major clades and genotypes over more than fifty years of recorded history. Multiple genotypes co-circulated during prolonged periods of time but recent dominance of the GA2 genotype was noticed in several studies and it is highlighted here with sequences from viruses circulating recently in Spain and Panama. Reactivity of group A viruses with MAbs that recognize strain-variable epitopes of the G glycoprotein failed to

correlate genotype diversification with antibody reactivity. Additionally, no clear correlation was found between changes in strain-variable epitopes and predicted sites of positive selection, despite both traits being associated to the C-terminal third of the G glycoprotein. Hence, our data do not lend support to the proposed antibody-driven selection of variants as major determinant of hRSV evolution. Other alternative mechanisms are considered to account for the high degree of hRSV_G variability.

*Flash presentations

(PO 11)

AN ATYPIC ISOLATE OF PEACH LATENT MOSAIC VIROID WITH IMPORTANT SEQUENCE CHANGES THAT PRESERVE THE RNA CONFORMATION AND INCREASE THE BIOLOGICAL FITNESS

P. SERRA[1], E. BERTOLINI[2,3], M.C. MARTÍNEZ[2], M. CAMBRA[2], R. FLORES[1]

[1]*Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia, Spain*

[2]*Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain*

[3]*Departamento de Fitossanidade, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

Viroids, despite their minimal genomes (they are non-protein-coding circular RNAs of about 250-400 nt), can infect and frequently incite diseases in plants. Like other members of the family *Avsunviroidae,* peach latent mosaic viroid (PLMVd) replicates in plastids by a rolling-circle mechanism involving cleavage of the resulting multimeric intermediates of both polarities by cis-acting hammerhead ribozymes. To parasitize the transcription and processing machinery of its host, PLMVd depends on sequence and structural motifs. *In silico, in vitro* and*in vivo* approaches support that the most abundant PLMVd strand (arbitrarily assigned the plus polarity) folds into a multibranched conformation stabilized by a kissing-loop interaction. In the course of routine testing by real-time RT-PCR, PLMVd could not be detected in a sample that reacted positively by RNA gel-blot hybridization with a full-length riboprobe. These conflicting results led us to consider that, instead of the low variability presumed for the viroid region used to design the TaqMan probe, this region might be quite different in the novel isolate. Conventional RT-PCR with two pairs of adjacent primers of opposite polarity derived from distinct regions of the molecule, cloning and sequencing confirmed that this was indeed the case. Intriguingly, when compared with PLMVd isolates of known sequence and biological properties, the novel isolate presented extensive covariations preserving the two stems whose capping loops form the kissing-loop interaction, thus upholding the functional relevance of this interaction. Sequence analysis of multiple clones of the novel isolate showed relatively low internal variability, and inoculation of peach seedlings with *in vitro* transcripts from a recombinant plasmid with a head-to-tail dimeric insert of a representative variant revealed a non-symptomatic infection. Moreover, when *in vitro* transcripts of this variant and of a symptomatic variant (gds6) from another isolate were co-

inoculated, the first outcompeted the second as revealed by real-time RT-PCR (with specific TaqMan probes for each variant) and by conventional RT-PCR, cloning and sequencing of the resulting progenies, which did not display detectable recombination. Based on these data, a new "universal" TaqMan probe was designed for the concurrent detection of both variant classes. Altogether these results provide further insights into PLMVd variability (which preserves key structural elements like the hammerhead ribozymes and the kissing-loop interaction), and into the strong interference existing between coinfecting variants (possibly mediated by RNA silencing). From a more applied perspective, they also alert on diagnosis techniques just relying on a fragment of the RNA to be detected.

*Flash presentations

(PO 12)

HUMAN VIRUS IN FECALLY CONTAMINATED WATER, THE VIROME OF URBAN SEWAGE AND CLINICAL GASTROENTERITIS FECAL SAMPLES

X. FERNANDEZ-CASSI[1], M. RUSIÑOL[1], N. TIMONEDA[1], R.BARTOLOMÉ[2], S. BOFILL-MAS[1], J.F. ABRIL[3], R. GIRONES[1].

[1.] *Laboratory of Virus Contaminants of Water and Food, Microbiology Department, Biology faculty, Barcelona University, Catalonia, Spain.*

[2.] *Hospital Universitari Vall d'Hebron, Microbiology Service, Barcelona, Catalonia, Spain.*

[3] *Computational Genomics Lab (Compgen), Genetics Department, Biology faculty, Barcelona University, Catalonia, Spain.*

Many viral infectious diseases are transmitted by consumption or contact with water or food contaminated with the discharge of untreated or even treated sewage. Among the most frequently detected human viruses excreted in urban sewage are the known groups of viruses such us adenoviruses, astroviruses, enteroviruses, rotaviruses, as well as noroviruses.

To improve the knowledge on the excreted virome and the viruses that could represent a risk associated to water or food for the population, NGS techniques have been applied to study viral richness in urban sewage from Barcelona. The metagenomics study using Illumina platform allowed the description of more than 25 different viral families. Among those 9 are related to human viral pathogens. The presence of the recently described virus *Salivirus/klassevirus*, a new genus belonging to *Picornaviridae* family in urban sewage, has been confirmed by conventional RT-PCR.

The study of excreted viruses has continued by analyzing 56 clinical samples from patients presenting gastroenteritis without an identified etiological agent. Using metagenomics on different pools of samples members of the family *caliciviridae*, *astroviridae*, *adenoviridae*, *picornaviridae* and *parvoviridae* have been found. These findings show that conventional clinical tests designed to identify known etiological agents do not identify many viral pathogens related to gastroenteritis. Metagenomics is a very useful technique for the identification of etiological agents in clinical samples presenting negative results for the commonly applied diagnostic tests.

*Flash presentations

(PO 13)

APPROACHES TO DIVA ASSAYS FOR WEST NILE VIRUS

B.REBOLLO[1], S.LECOLLINET[2], A.CAMUÑAS[1], E.SORIA[1], A.J SANZ[1], A.VENTEO[1]

1. INGENASA, Inmunología y Genética Aplicada, Madrid, Spain

2. ANSES, Animal Health Laboratory, EU on equine West Nile disease, France

West Nile virus infection is identified by several diagnostic tools, being the most commonly ones focused on the identification of the agent (RT-PCR), the virus neutralization test or the detection of IgM/IgG against structural proteins. Over the last decade, several outbreaks caused by West Nile virus have been detected in different parts of Europe, which increased the vaccination of horses in many countries. The currently diagnostic methods cannot differentiate infected from vaccinated animals. The main goal of this work is to deal with this problem when both events may occur in a horse population at the same time. Based on the difference of antibody response between infected and vaccinated horses against the structural (E protein) and the Nonstructural (NS1 protein), a DIVA (differentiating infected from vaccinated animals) assay has been designed. A panel of Monoclonal antibodies (Mabs) against E and NS1 proteins was obtained and the best were selected to coat ELISA plates. After that, the inactivated whole culture virus was added and each specific protein was captured by the specific Mab. Horse samples were analyzed in an indirect ELISA DAS format (IDAS). Different groups of animals sera were used for this for study: vaccinated and infected animals from controlled experiments bled at different days and vaccinated and/or infected field animals. To set up the assay, the sera from the experimental infected/vaccinated animals were analyzed. These samples reveals a positive result in the ELISA based on E protein with the same high OD values. Nevertheless, the antibody response against NS1 in vaccinated animals showed decreased OD values comparing with those obtained with infected animals. The ratio OD with E protein/NS1 protein was higher than 4 in vaccinated animals whereas this ratio, in infected animals, was lower than 4.Similar results were obtained using the field samples. Additionally to test the specificity a group of 90 negative sera showed a very low signal in both assays. In conclusion, We have observed a different antibody response to the structural E protein and to the non-structural NS1 protein in infected and vaccinated horses. Based on this difference, using an IDAS-ELISA we could differentiate vaccinated from infected horses. The design of this assay carried out during this study could help to the development of a DIVA assay. Further experiments are needed to adjust the ELISA conditions and test a bigger panel of infected sera in order to check the utility of the assay in field.

*Flash presentations

(PO 14)

PCV2 GENOME CAN NOT ENCODE VIRAL miRNAs IN AN EXPERIMENTAL INFECTION

F. NÚÑEZ-HERNÁNDEZ[1], LJ. PÉREZ[2], G. VERA[3], S. CÓRDOBA[3], J. SEGALÉS[1,4], A. SÁNCHEZ[3,5], JI.NÚÑEZ[1*]

[1]. Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, Bellaterra, Cerdanyola del Vallès, Spain.

[2]. Centro Nacional de Sanidad Agropecuaria (CENSA), La Habana, Cuba.

[3]. Departament de Genètica Animal, Centre de Recerca en AgriGenòmica (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

[4]. Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

[5]. Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain.

Porcine circovirus type 2 (PCV2) is a small single stranded circular DNA virus of 1768-9 nt long. PCV2 is the essential etiological infectious agent of PCV2-systemic disease (PCV2-SD), formerly known as postweaning multisystemic wasting syndrome, (PMWS). MicroRNAs (miRNAs) are 19-24 nt long non- coding single stranded RNAs with post- transcriptional regulation functions. They mediate the silencing of their target mRNAs by binding to complementary sites usually located in the 3' untranslated regions (UTRs) of the mRNA. More than 28600 miRNAs have been described as shows miRBase, the miRNA database. These miRNAs are expressed by all kind of organisms, from mammals to plants and viruses. Since this time, only DNA viruses with a nuclear phase have been described to be able to encode viral miRNAs, while the capacity of the RNA virus to encode viral miRNAs is a matter of controversy. Herpesviruses are the best example of virus that encodes viral miRNAs with the capability to express high amounts of viral miRNAs but it has also been observed in other kind of viruses. In this study, the capability of PCV2 to encode viral miRNAs in a subclinical infection has been tested. For this purpose four pigs were intranasally infected with $7x10^{4.8}$ $TCID_{50}$ of PCV2 isolate Sp-10-7-54-13 and two pigs received PBS by the same route as controls. At 21 days p.i. pigs were euthanized. Small RNAs libraries were created from tonsil and mediastinal lymph node and later sequenced by next-generation sequencing techniques. For viral miRNA discovery, the obtained sequences were blasted to the viral genome considering 100% of alignment but also allowing mismatches in the extremes considering miRNAs variability. One candidate was found with 58 copies which sequence corresponds to the Vmir precursor candidate with the highest score. The posterior analysis revealed that the candidate was an isomir of the cellular miR-29a-5p. After this study we can confirm that PCV2 does not encode viral miRNAs in a subclinical infection. On the other hand, the study of the homology of a viral sequence with a cellular miRNA could shed light on how miRNAs affect viral evolution.

(PO 15)

QUANTIFICATION OF GENOMES ENCODING LARGE AND SHORT FORMS OF HEPATITIS DELTA ANTIGEN TO EXPLORE THE REPLICATION CAPACITY OF HEPATITIS DELTA VIRUS

M. HOMS(1,2), M. BLASI(1,2), A. RUIZ(2), D. TABERNERO(1,2), P. REIMUNDO(2), J. QUER(1,3), R. CASILLAS(2), J. GREGORI(3), M. RIVEIRO-BARCIELA(4), L. NIETO(2), R. ESTEBAN(1,4), M. BUTI(1,4), F. RODRIGUEZ-FRIAS(1,2)

1-CIBEREHD, Barcelona, Spain

2-Liver Pathology Unit, Biochemistry and microbiology Departments, Vall d'Hebron Hospital, Barcelona, Spain

3-Liver Diseases, Research Institute, Vall d'Hebron Hospital, Barcelona, Spain

4- Hepatology Department, Vall d'Hebron Hospital, Barcelona, Spain

Background The genome of hepatitis delta virus (HDV) has a single open reading frame able to encode two antigens. The stop codon at position 196 is edited to Tryptophan and elongates the translation to the codon 214. The genomes with Stop codon in 214 encodes for large delta antigen (LHDAg), which is involved in the assembly and virion formation, and inhibition of HDV replication. The genomes with stop codon in196 are the only ones that can be edited, encode for the short delta antigen (SHDAg), and have replicative capacity.

Aims To quantify the genomes encoding SHDAg and LHDAg (Genomes S/L-Ag) in the quasispecies of VHD and relate with viral replication.

Patients and methods Sixteen baseline samples from 13 patients (1-13) with chronic hepatitis delta were analyzed. 2 samples from 3 patients (1-3) followed up for 6 months without treatment were included. HDV RNA was quantified the by an in-house (range, 3.5-8 log copies / mL) method. The proportions of Genomes S/L-Ag were determined by massive sequencing (nt 339-891).

Results The patients showed a mean 6.3-HDV RNA logs copies / mL (SD, 0.76) and a mean of 65% of genomes encoding for the short delta antigen. HDV RNA did not correlated with the percentage of genomes S/L-Ag.

The 3 followed patients did not present changes in HDV-RNA, but a dynamic in the S/L-Ag genomes.

Four samples (1b, 3a, 6 and 11) presented an enriched population of L-Ag genomes, indicating a population of defective viral replication.

Conclusion The lack of correlation between HDV-RNA quantification and the proportion of genomes encoding the short and large delta antigens, and the dynamics genomes encoding the short and large delta antigens in the absence of treatment suggest that assessment of HDV RNA alone does not reflect the replicative activity HDV.

*Flash presentations

(PO 16)

BEFORE-TO-CART IMMUNOVIROLOGICAL TRAITS OF HIV SUBJECTS WITH LOW CD4 RESTORATION

I. ROSADO SÁNCHEZ[1], M.M. POZO-BALADO[1], R.S. DE PABLO-BERNAL[1], I. JARRÍN VERA[2], A.I. ALVAREZ-RÍOS[3], M. BENHNIA RAFII-EL-IDRISSI[1], E. RUIZ-MATEOS[1], M. LEAL NOVAL[1], Y.M. PACHECO LÓPEZ[1].

[1] *Laboratorio de Inmunovirología del Instituto deBiomedicina de Sevilla (IBiS), UGC Enf. Infecciosas, Microbiología y Med. Preventiva, Hospital Universitario Virgen del Rocío, Sevilla, España*

[2] *Instituto de Salud Carlos III, Madrid, España*

[3] *Departamento de Bioquímica, Hospital Universitario Virgen del Rocío, Sevilla, España*

Background

HIV-infected subjects who persistently maintain low CD4 counts despite having achieved undetectable viremia during combined antiretroviral therapy (cART) are at an increased risk of death, and no therapeutic alternative is available. These patients show several immunovirological traits like X4 viral tropism, increased T-cell activation, senescence and apoptosis, and increased Treg frequency. However, whether these factors are cause or consequence of the failed CD4 restoration is unknown. This is the first study analyzing samples from these patients before the initiation of cART.

Methods

Patients with failed CD4 restoration (FR patients) were patients starting cART with less than 200 CD4/µL and achieving less than 250 CD4/µL 2 years after suppressive cART. In the Spanish CoRIS cohort, we selected 21 male FR patients and 20 male control patients with successful CD4 restoration (SR patients; with CD4 reaching more than 250 CD4 after 2 years of successful cART), who had available baseline sample at the Spanish RIS Biobank. SR patients were baseline age, CD4 and viral load-matched subjects. Plasma levels of monocyte activation (sCD14), endothelial activation (ICAM, VCAM), platelet/lymphocyte activación (sCD40, β2-microglobulina), IFN-γ-Inducible Protein 10 (IP-10), coagulation marker (D-Dimer), proinflammatory markers (hsCRP, IL6) and soluble cytokines (IL10, TGF-β, IFN-γ, IL4, IL7, TNF-α, IL17a) were measured. Genotypic viral tropism, cell subsets (CD3, CD4, CD25, FOXP3) and cellular markers of activation (HLA-DR, CD38), senescence (CD57, CD28), and cycling (ki67) were also determined.

Results

Before cART initiation, FR patients compared with SR patients showed higher levels of IL-6 (7.3[3.6-12.1] vs. 4.9[2.6-6.8] ρg/ml, respectively; p=0.034), a higher proportion of patients with hsCRP>5 mg/L (44.4% vs. 5.6% respectively; p=0.007), a higher frequency of Treg (1.14[0.54-3.60] vs. 0.46[0.22-1.37]; p=0.044) and a higher frequency of CD4+Ki67+ (11.2[7.9-18.8] vs. 7.6[3.8-10.1] respectively; p=0.047). However, no differences were found in the cellular makers of activation and senescence, soluble cytokines or viral tropism.

Conclusion

Increased inflammatory levels (IL6, hsCRP), Treg and cycling CD4 cells preexist in patients with low CD4 restoration before

the cART initiation, and consequently, these factors could be involved in the subjacent mechanisms causing such failure.However, X4 viral tropism, as well as T-cell activation and senescence, usually associated to these patients, are more probably consequence of such failed CD4 restoration.

*Flash presentations

(PO 17)

MONOCYTE PHENOTYPE AND POLYFUNCTIONALITY ARE ASSOCIATED WITH ELEVATED SOLUBLE INFLAMMATORY MARKERS, CYTOMEGALOVIRUS INFECTION AND FUNCTIONAL AND COGNITIVE DECLINE IN THE ELDERLY

RS. DE PABLO[1], MI GALVÁ[2], R. RAMOS[2], J. CAÑIZARES[2],S. FERRANDO[3], M.B. RAFII-EL-IDRISSI[1], YM. PACHECO[1], M. LEAL[1], E. RUIZ-MATEOS[1]

[1]Biomedicine Institute of Seville, Seville, Spain.

[2]Heliopolis nursing home, Seville, Spain.

[3]Vaccine Research Center NIAID NIH, Bethesda, MD, United States.

Monocytes are mediators of the inflammatory response. High levels of soluble inflammatory biomarkers have been associated to aging, CMV infection and adverse health outcomes in the elderly. Monocytes comprise three subsets, the classical (CD14++CD16−), intermediate (CD14++CD16+), and non-classical (CD14dimCD16+). However, little it is known about the phenotypical and functional age-related changes of monocytes and the association with soluble inflammatory biomarkers, CMV infection and functional and mental function decline. We assayed the activation *ex-vivo* and the responsiveness to TLR2 and TLR4 agonists *in-vitro* in the three subset of monocytes, assessing the intracellular production of IL1-alpha(α), IL1-beta (β), IL-6, IL-8, TNF-α and IL-10 of old subjects (median age 83 [67-90] years old; n=20) compared to young controls (median 35 [27-40] years old; n=20). *Ex-vivo*, elderly showed higher percentage of classical monocytes that expressed intracellular IL1-α (p=0.001), IL1-β (p=0.001), IL-6 (p=0.002) and IL-8 (p=0.007). Similar results were seen for intermediate and non-classical subsets. *In-vitro*, higher monocyte responsiveness to TLR2 and TLR4 agonists with aging was observed. Multiple cytokine polyfunctionality was higher in the elderly. Single and multiple intracellular functionality *ex-vivo* were strongly associated to soluble coagulation and inflammatory markers. The activation phenotype was independently associated to anti-CMV IgG levels and functional and cognitive decline in the elderly. These data demonstrate that monocytes are key cells as potential source of the high soluble inflammatory levels. Our findings suggest that CMV infection might be a driving factor of the activation of monocytes which was associated to the functional and cognitive decline in the elderly.

*Flash presentations

(PO 18)

NEAR-ATOMIC RESOLUTION CRYO-EM STRUCTURE OF ROSELLINIA NECATRIX QUADRIVIRUS 1

C.P. MATA[1], J. GÓMEZ-BLANCO[1], D. LUQUE[1,2], N. SUZUKI[3], S. KANEMATSU[4], W. HAVENS[5], S. A. GHABRIAL[5], B.L. TRUS[6], J.L. CARRASCOSA[1], J.R. CASTÓN[1]

[1]Department of Structure of Macromolecules, Centro Nacional de Biotecnología/CSIC, 28049 Madrid, Spain; [1]Centro Nacional de Microbiología/ISCIII, 28220 Majadahonda, Madrid; [3]IPSR, Okayama University, Kurashiki, 710-0046 Japan; [4]Apple Research Station, NARO, Morioka, 020-0123 Japan; [5]University of Kentucky, Lexington, KY 40546-0312 USA; [6]Center for Information Technology/NIH, Bethesda, MD 20892-5624, USA

Most dsRNA viruses have an icosahedral T=1 capsid based on 60 asymmetric coat protein dimers of a single protein (also referred to as a "T=2", 120-subunit capsid). This ubiquitous stoichiometry provides an optimal framework for genome replication and organization. Whereas there are numerous structural studies of the family *Reoviridae*, additional studies are needed of many fungal and protozoal dsRNA viruses. Penicillium chrysogenum virus (PcV), a mycovirus of the family *Chrysoviridae*, has an authentic T=1 capsid formed with 60 copies of one polypeptide; the capsid protein is an almost perfect structural duplication of a single domain, in which many secondary structural elements match very well. This conserved core represents a hallmark fold preserved in the dsRNA virus lineage.

Here we describe the 3D structure of Rosellinia necatrix quadrivirus 1 (RnQV1), the type species of the family *Quadriviridae*. Quadriviruses are fungal viruses with a multipartite genome. Each dsRNA segment is encapsidated separately in a similar particle. The ~45 nm-diameter RnQV1 capsid is built of 60 heterodimers with two 1,356- (P2) and 1,059-residue proteins (P4), as deduced from analytical ultracentrifugation analysis. In RnQV1 strain W1075, P2 and P4 are processed into several peptides without altering its structural stability, whereas in strain W1118, both proteins remain nearly intact. The 3D structure of RnQV1 W1118 was determined by single-particle cryo-EM analysis at 3.73 Å resolution. Data were acquired in a Tecnai Titan Krios electron microscope (Laboratory of Molecular Biology, Cambridge, UK) equipped with a direct electron detector and processed using RELION software. Heterodimers are organized following a quaternary organization similar to reovirus, chrysovirus and totivirus. The full-atom model of the capsid showed the critical contacts among structural subunits that mediate capsid assembly, and indicated that P2 is processed in its C-terminal end that faces the outer surface and lacks 383 residues. Despite the lack of sequence similarity, superimposition of P2 and P4 showed a common core in which many □-helices and □-chains matched very well. There are two preferential "hot spots" into which structural and functional variations can be introduced by insertion of distinct segments. Overlaying PcV and L-A (a totivirus) capsid proteins on either of the P2 and P4, while maintaining the same spatial arrangement in the shell, highlighted the same conserved motif and hot spots for insertions. The RnQV1 capsid

is the first pseudo T=1 (or "P=2") capsid reported to date.

*Flash presentations

(PO 19)

INFLUENCE OF CODON PAIR USAGE IN THE EVOLVABILITY OF HUMAN IMMUNODEFICIENCY TYPE 1 VIRUS (HIV-1)

M. NEVOT, G. MARTRUS, M. PARERA, M. CLOS, M.A. MARTÍNEZ

Irsicaixa, Hospital Germans Trias i Pujol, Badalona, Spain

HIV-1 populations, like other RNA viruses, are described as a closely related mutant spectra or mutant clouds termed viral quasispecies. Mutant cloud composition can impact virus evolvability, fitness and virulence. The extraordinarily large number of possible encodings in natural genes is to some extent restricted by two encoding biases referred to as codon bias and codon pair bias. An unexplored aspect of the genetic architecture of HIV-1 is how codon choice influences population diversity and evolvability. Here we compared the development of HIV-1 resistance to protease inhibitors (PIs) of wild-type (WT) virus and a synthetic virus (MAX) carrying a codon-pair re-engineered protease sequence with 38 (13%) synonymous mutations. A sequence analysis of 200 individual clones, obtained by virus RNA endpoint dilution, demonstrated that after one passage in MT-4 cells MAX protease quasispecies diversity (p-distance) was significantly higher than that of the virus carrying the WT protease (0.0014±0.00003 vs 0.0012±0.00004, p=0.0027, unpaired t-test). However, after 15 passages (45 days) the diversity was lower in MAX protease compared with WT protease (0.0021±0.00003 vs 0.0029±0.00006, p<0.0001, unpaired t-test). These differences were not observed when protease flanking regions were analyzed. These results suggested that WT and MAX proteases might occupy different sequence spaces. To explore the evolvability of the codon pair re-coded protease, WT and MAX viruses were subjected to the selective pressure of PIs [atazanavir (ATV) and darunavir (DRV)]. After the same number of serial passages in MT-4 cells in the presence of PIs, WT and MAX viruses developed phenotypic resistance to PIs (IC50 14.63±5.39 nM and 21.26±8.67 nM, for ATV; and IC50 5.69±1.01µM and 9.35±1.89 for DRV, respectively). Sequence clonal analysis showed the presence, in both viruses, of previously described resistance mutations to ATV and DRV. However, a different resistance variant repertoire appeared in the MAX virus protease when compared to WT. Specifically, the G16E substitution was only observed in the WT protease. In addition, the L10F, L33F, K45I, G48L and L89I substitutions were only detected in the re-coded MAX protease population. The differences in the mutation pattern that emerged after PIs treatment suggested again that WT and MAX virus proteases occupy different sequence spaces although both virus proteases were able to develop PIs resistance. Further studies will required to elucidate whether HIV-1 sequences have evolved to optimize not only the protein coding sequence but also the DNA/RNA sequences.

*Flash presentations

(PO 20)

THE (DIS)ASSEMBLY PATHWAY OF A SIMPLE VIRUS CAPSID INVOLVES A COMPLEX SERIES OF TRANSIENT INTERMEDIATES

M. MEDRANO, M.A. FUERTES, P.J.P. CARRILLO, A. RODRÍGUEZ-HUETE, A. VALBUENA, M.G. MATEU.

Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain.

Capsid assembly is an obligate step of the viral cycle; the reverse process, disassembly, is required for some viruses to release its genome into the host cell during infection. The study of capsid assembly and disassembly is contributing to the development of antiviral drugs, the production and modification of virus-like particles for vaccination and other biomedical or biotechnological applications, and the engineering of self-assembling nanoparticles for technological uses.

In principle, assembly/disassembly of a virus capsid can be considered a reversible reaction, although it can later become irreversible through a maturation process. Thus, the study of either capsid assembly or disassembly under appropriate conditions can provide the same information on this reversible process. Theoretical studies on simplified virus models are delivering important predictions on (dis)assembly pathways. Unfortunately, the experimental study of the (dis)assembly of simple viral capsids has been severely hampered because they have been usually observed as two-state reactions with no significantly populated intermediates.

We have undertaken the experimental *in vitro* study of the (dis)assembly pathway of one of the simplest viral capsids known, that of the minute virus of mice (MVM). In a previous study we used atomic force microscopy (AFM) to study the mechanical disassembly of single MVM particles. In the present study, MVM capsids were treated with limited, controlled amounts of guanidinium chloride in order to stabilize transient intermediates in the (dis)assembly pathway. The disassembly reaction was followed over time, and any intermediates were visualized and quantified using mainly transmission electron microscopy and AFM.

The results revealed that (dis)assembly of the MVM capsid appears to proceed sequentially through a series of relatively stable intermediates, whose abundance and structural organization were estimated over time. We conclude that (dis)assembly of even a very simple virus capsid can be a marginally cooperative or non-cooperative process in which a series of intermediates gradually appear and disappear in succession as a consequence of a gradual, loss (disassembly) or accretion (assembly) of subunits. These results tend to confirm the predictions of theoretical calculations and simulations on assembly/disassembly of the protein homo-oligomers that form the smallest virus capsids.

*Flash presentations

(PO 21)

RELEVANCE OF miRNAs IN SARS-CoV-PATHOGENESIS

L. MORALES[1], J.C. OLIVEROS[2], L. ENJUANES[1], I. SOLA[1]

[1]*Department of Molecular and Cell Biology.National Center of Biotechnology (CNB-CSIC). Madrid. Spain.*

[2]*Computational Genomics Service.National Center of Biotechnology (CNB-CSIC). Madrid. Spain.*

Severe acute respiratory syndrome coronavirus (SARS-CoV) causes a respiratory pathology characterized by an exacerbated inflammatory response. microRNAs (miRNAs) are small noncoding RNAs implicated in the regulation of several cell processes, including the modulation of the inflammatory response, by silencing associated mRNA targets. Recently, a novel role of miRNAs as regulators of the host response to virus infection has been proposed. The relevance of miRNAs as mediators of the host innate immune response against SARS-CoV infection is being analyzed. We have previously shown that the mouse-adapted SARS-CoV lacking the envelope E protein (SARS-CoV-MA15-ΔE) is attenuated and causes a milder lung inflammation and pathogenicity in infected Balb/c mice as compared to wild type SARS-CoV. miRNAs differentially expressed in lung tissues from mice infected with wt SARS-CoV and SARS-CoV-ΔE have been analyzed by next generation sequencing (NGS). These miRNAs may regulate cell pathways related with the differential inflammation induced in infections by both viruses. Bioinformatics analysis revealed that most small RNA sequences were 22 nts in length in agreement with mammalian miRNAs average length. Most of the small RNA sequences differentially expressed during SARS-CoV infection were potential unknown cellular miRNAs, whereas few small RNA sequences corresponded to annotated miRNAs. The discrete changes, around twofold, in miRNAs expression induced by viral infection complicated further validation studies. In addition to cell miRNAs, other small RNA sequences specifically aligning to SARS-CoV genome were discovered in infected tissues. Some of these sequences might comprise viral degradation products, which are equally distributed across the viral genome. In contrast, a significant proportion of reads concentrated in peaks that aligned to specific viral genome regions, representing viral-derived small RNAs (svRNAs). Interestingly, several of these vsRNAs were detected in the serum of infected mice, confirming their abundance in infected tissues. The functional relevance of these svRNAs in the host response to SARS-CoV infection or in the viral cycle will be validated by using inhibitors that interfere with the biological effect of svRNAs. A deeper knowledge of miRNAs and svRNAs involved in SARS-CoV pathogenesis will allow the development of new therapeutic approaches to prevent mortality caused by SARS-CoV.

*Flash presentations

(PO 22)

STRUCTURAL STUDY OF FOOT-AND-MOUTH DISEASE VIRUS REGULATORY ELEMENTS IN LIVING CELLS

R. DÍAZ-TOLEDANO, G. LOZANO, E. MARTÍNEZ-SALAS.

Departamento de Dinámica y Función del Genoma. Centro de Biología Molecular "Severo Ochoa" (CISC/UAM), Madrid, Spain.

The genome of foot-and-mouth disease virus (FMDV) consists of a positive-sense RNA of about 8500 nts. It is organized in a single open reading frame flanked by highly structured 5' and 3' untranslated regions (UTRs). Initiation of translation of the viral RNA is controlled by an internal ribosome entry site (IRES) located within the 5'UTR. In addition, the 3'UTR is essential for viral replication and infectivity. Viruses are obligatory intracellular parasites. Host cell and viral proteins cooperate with viral RNA elements to allow virus multiplication. Currently, knowledge of RNA structure *in vivo* is limited due to the

lack of suitable methodologies.

The 2'-hydroxyl group is a universal chemical feature of RNA. Selective 2´-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) has become the gold standard for monitoring the secondary structure of complex RNAs. SHAPE

reactivity reveals flexible regions or nucleotides constrained in a conformation where the ribose 2´-OH is susceptible to modification. Conversely, nucleotides involved in canonical base pairing or stable U:G, A:A, and A:G pairs are not reactive. In this work, we have used the acylation electrophile, 2-methyl nicotinic

acid imidazolide (NAI) to modify RNA within cells and thus, to report the IRES and 3'UTR RNA structure in the viral RNA context.

Our *in vivo* results show differences compared with previous *in vitro* results. The IRES pattern observed *in vivo* showed an increased reactivity in the pyrimidine tract of domain 5 and a decreased reactivity on residues 142-147, 165-168 and the C-rich loop of domain 3 compared to data observed *in vitro*. Furthermore, a marked decrease of reactivity in the apical loop of domain 2, the GNRA motif in the domain 3, and the pyrimidine tract of domain 5 were apparent in the presence of the 3'UTR.

RNA structure *in vivo* is dynamic; the directionality and velocity of transcription and translation, as well as trans-acting factors such as proteins, small ligands or other RNA molecules are likely to influence the RNA architecture. Knowledge of the viral RNA structure in living cells would shed light on the molecular mechanism governing virus infections.

*Flash presentations

(PO 23)

IN VITRO SELECTION AND CHARACTERIZATION OF RNA AND DNA APTAMERS TARGETING THE RNA-BINDING PROTEIN PCBP-2

M. MORENO[1], M. FERNÁNDEZ-ALGAR[1], J. FERNÁNDEZ-CHAMORRO[2], J. RAMAJO[2], E. MARTÍNEZ-SALAS[2], C. BRIONES[1,3].

[1]. Laboratory of Molecular Evolution. Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid, Spain.

[2]. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain.

[3]. Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain.

Aptamers are single-stranded oligonucleotides (ssRNA or ssDNA) selected from combinatorial libraries by an amplification-selection *in vitro* process termed SELEX. They possess a specific three-dimensional structure depending on their sequence and the physicochemical features of the folding buffer (1). These *in vitro* selected nucleic acids are able to recognize and, eventually, alter the activity of their target molecules by establishing non-covalent aptamer-target molecular interactions. The current technology allows generating aptamers with very high affinity and specificity for a broad range of targets including low molecular weight compounds, proteins, nucleic acids and macromolecular complexes. Therefore, SELEX technology is increasingly been used in a growing number of diagnostic (2) and therapeutic (3) applications over the last decade.

Among the factors involved in post-transcriptional regulation, the poly(rC)-binding proteins (PCBPs) are of interest due to their role in translation initiation of some viral genomic RNAs. In particular, translation initiation of picornavirus genomes requires the assembly of ribonucleoprotein complexes involving host cell factors, including PCBPs, on a highly structured RNA functional elementtermed internal ribosome entry site (IRES), located at the 5' untranslated region (5'UTR) of the viral genome (4).

We have selected and characterized in parallel ssRNA and ssDNA aptamers against PCBP-2. In both cases, molecular cloning of the 76 nt long evolving population after 10 rounds of *in vitro* selection allowed us to identify the fittest aptamers according to quantitative amplification of the bound aptamer, as revealed by the analysis of their affinity constant (Kd) and maximum binding capacity (Bmax). Additionally, gel-shift analyses have confirmed that the assayed aptamers bind to the target PCBP-2 in solution. Herein, we present sequence and structure comparison of the *in vitro* selected nucleic acid molecules, and discuss on the differential behaviour of RNA and DNA aptamers targeting the RNA-binding protein PCBP-2.

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POSTERS SESSION

(PO 24)

MEDILABSECURE: IMPLEMENTING A LABORATORY NETWORK FOR VECTOR-BORNE DISEASES BRINGING TOGETHER ANIMAL VIROLOGY, HUMAN VIROLOGY AND ENTOMOLOGY IN THE MEDITERRANEAN AND BLACK SEA REGIONS THROUGH A ONE HEALTH APPROACH

MIGUEL ÁNGEL JIMÉNEZ-CLAVERO[1], ELISA PÉREZ-RAMÍREZ[1], JEAN-CLAUDE MANUGUERRA[2], CAMILLE ESCADAFAL[2], VINCENT ROBERT[3], MARIE PICARD[3], MARIA GRAZIA DENTE[4], SILVIA DECLICH[4], FLAVIA RICCARDO[4], FANNY CHERBLANC[2], KATHLEEN VICTOIR[2]

[1] *Center for Research in Animal Health INIA-CISA, Valdeolmos (Madrid), Spain*

[2] *Institut Pasteur, Paris, France*

[3] *Institut de Recherche pour le Développement IRD, Montpellier, France*

[4] *Istituto Superiore di Sanitá (ISS), Rome, Italy*

As (re-)emerging viruses are threatening global health, the EU-funded MediLabSecure project (2014-2017) aims at enhancing the preparedness and response to viral threats by establishing an integrated network of animal virology, human virology and entomology laboratories in 19 non-EU countries of the Mediterranean and Black Sea areas in partnership with 4 Institutes in three countries: France (Institut Pasteur, Paris in charge of the coordination of the project and the human virology workpackage, and Institut de la Recherche pour le Developpement, Montpellier, in charge of the entomology workpackage); Spain (Center for Research in Animal Health INIA-CISA, Valdeolmos (Madrid), in charge of the animal virology workpackage); and Italy (Istituto Superiore di Sanitá, Rome, in charge of the epidemiology workpackage). The MediLabSecure project is reinforcing the public health laboratory and epidemiology networks previously established by the EpiSouth Plus project (2010-2013) by additionally involving animal virology and medical entomology laboratories in a fully integrated "one health" approach for surveillance and control of emerging arboviral diseases.

One laboratory per field of study (human virology, animal virology, medical entomology) and per country was selected in 2014. A first meeting involving the heads of laboratories was held in Paris in January 2015 with the aim of defining priorities and adapting upcoming activities of the project to the needs and interests of participating countries. A "Needs assessment" questionnaire was implemented to assess laboratory capacities and needs regarding biosafety, diagnostic methods and integration of laboratory and epidemiological surveillance for emerging vector-borne viruses.

Forty-seven laboratories were selected to actively join the project. The January meeting allowed the project partners and head of laboratories to meet and exchange on the objectives and future steps of the project as well as on their experiences, needs and expectations. Based on these discussions and on the responses to the "Needs assessment" questionnaire, the first tailored training sessions will be organized in June 2015, enabling laboratories to implement harmonized and up-to-date techniques to perform (1) laboratory diagnosis of relevant vector-

borne viral diseases such as West Nile, Chikungunya and Rift Valley Fever in humans and animals and (2) mosquito species determination and entomological field surveys.

By enhancing diagnostic capacities and regional multidisciplinary cooperation, the Medilabsecure network could represent the cornerstone of a corporate preparedness and response to vector-borne viral threats in the Mediterranean and Black Sea regions based on a One Health approach.

(PO 25)

GENOMIC FEATURES OF DENV IMPORTED TO EUROPE

A ROJAS[1,2], F MOLERO[1] , MD FERNÁNDEZ [1,3] , L HERNÁNDEZ[1], A POTENTE[1], ANDREAS NEUMAYR[4], A WILDER SMITH[5], C HATZ[4], ATENORIO[1] ,MP SÁNCHEZ –SECO[1], ANA VÁZQUEZ[1], L FRANCO[1]

1-Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid-Spain

2-Master en Virología, Universidad Complutense de Madrid, Madrid-Spain

3-Pastuer Institute,Dakar-Senegal

4- Swiss Tropical and Public Health Institute, Basel,-Switzerland

5-Umea University,Umea-Sweden

Dengue is caused by 4 different related viruses, (DENV-1 ,2, 3, 4) transmitted to humans through the bites of *Aedes* mosquitoes. The disease is endemic in more than 100 countries from Asia, America, Africa and Oceania. In 2010, dengue re-emerged in the French Riviera and Croatia, with small outbreaks. Two years later, in October 2012, a sustained and explosive epidemic appeared in Madeira archipelago. Both, 2010 and 2012 outbreaks were caused by DENV-1. In Europe, travelers can act as vectors to introduce DENV, to uninfected areas or region, as occurred previously. The present study is aimed to characterize DENV serotypes and genotypes obtained from infected travelers returning from different continents with acute dengue infections. Samples were obtained from from 11 European clinics belonging from Tropnet network and participating in the DengueTools project(www.denguetools.net), from 2011 to 2014. Sequences of the Envelope gene were used for sero and genotyping of 120 viraemic samples. In addition, complete genome sequences were obtained from relevant lineages and new emerging clades, including all DENV-1 introduced in Europe in recent years. A genomic analysis from all studied samples will be presented and discussed.

(PO 26)

NEW TOMOGRAPHIC APPROACHES TO VISUALIZE THE STRUCTURE OF VIRUSES AND THEIR INTERACTION WITH HOSTS

F.J. CHICHÓN[1,2], G.N. CONDEZO[1,2], J.J.CONESA[1,2], C. SAN MARTIN[1,2], E. PEREIRO[3] AND J.L. CARRASCOSA[1,2,4]

[1]Department of Macromolecular Structures, Centro Nacional de Biotecnología (CNB-CSIC), 28049 Madrid, Spain

[2]NanoBiomedicine Initiative, Centro Nacional de Biotecnología (CNB-CSIC), 28049 Madrid, Spain

[3]ALBA Synchrotron Light Source, MISTRAL Beamline – Experiments Division, 08290 Cerdanyola del Vallès, Barcelona, Spain

[4]Instituto Madrileñoo de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), 28049, Madrid, Spain

In recent years, the use of electron and Soft X-ray tomography are providing virologist with very valuable data about the structure of viruses as well as its interaction with the host. Both electron and X-ray tomography are based on the acquisition of projections at different angles. Then the projections are aligned and combined to obtain a three-dimensional map, which must be subsequently analyzed to extract biological information in a process called segmentation. Both electron and soft X-ray tomography data collection can be carried out in cryo-conditions. These require a complex system to maintain the sample at low temperatures along the acquisition process once vitrified, but produce unique structural details in a hydrated environment, avoiding the addition of contrast and fixing agents.

Due the poor penetration of electrons into the biological matter, limited to half a micron, the use of electron microscopy requires the generation of sections to analyze viruses interacting with their hosts. Soft X-ray microscopy offers a new valuable tool to study in three dimensions viruses and cells in their hydrated state, without sectioning or staining the sample. Moreover, this technique presents an intermediate resolution and magnification ranges between electron and confocal microscopy, opening new correlative approaches to link functional and structural information in the same sample.

In summary, these new techniques allow to obtain three-dimensional information on the architecture of viruses or cells in a close-to-native state. It is therefore a great advance, which allows not only to develop descriptive microscopy, but also to generate quantitative models. In this work we show several examples of the use of these techniques applied to the field of Virology.

1. *Electron tomography (ET):*
   a. Immature Vaccinia Virus Structure. (Chichón et al. 2009).
   b. Native Structure of influenza virus nucleoprotein. (Arranz et al. 2012)
   c. Organization of the adenovirus mini-chromosome. (Pérez-Berná et al. 2015)
2. *Soft X-ray Tomography (SXT) (Pereiro and Chichón 2014):*
   a. ET *vs.*SXT (Carrascosa et al. 2009)
   b. SXT of the infected cells (Chichon et al. 2012)

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(PO 27)

FLAVIVIRUS DETECTION IN INSECT FROM THE BALEARIC ISLANDS, SPAIN, 2012.

F. DEL VENTURA – VILLARROEL*[1], S. GUIMARÃES*[1], D. ROIZ[2], L. HERRERO[1], E. GÓMEZ-DIAZ[3], A. POTENTE[1], R. SORIGUER[2], M.P. SÁNCHEZ-SECO[1], J. FIGUEROLA[2], Y A. VÁZQUEZ[1].

1. Arbovirus Laboratory and Imported Viral Diseases , Instituto de Salud Carlos III . Madrid, Spain .

2 .Wetland Ecology Department, Doñana Biological Station, CSIC, Sevilla, Spain

3. Emory University, Atlanta, USA.

The Flavivirus (Flaviviridae) are viruses that cause diseases in animals and humans, such as the yellow fever virus, Dengue, West Nile, Usutu and Bagaza, among others. They are usually transmitted by arthropods such as mosquitoes and ticks. In this study, the molecular identification of Flavivirus was performed in a total of 274 insect pools, belonging to the Psychodidae (*Phelbotomus perniciosus*, *Sergentomyia minuta*) and Culicidae Families (*Culex pipiens, Cx. modestus, Cx. laticintus, Cx. perexigu, Culiseta anulata, Cs. longiareolata, Aedes (Ochlerotatus) caspius, Ae. (Och) mariae and Ae (Och) detritus*) captured in the Balearic Islands in 2012. The studies about arboviruses circulation in our country have been carried out primarily in the Iberian peninsula, therefore the aim of this work was to determine which arboviruses are circulating in vectors from island territories, and then to analyze their importance and develop more direct and specific investigations. The viral RNA was extracted using the kit QIAamp Viral RNA extraction (Qiagen, Izasa, Spain). A RT-Nested-PCR generic for Flavivirus to amplify a fragment of 143 bp was used for detection of the virus. Positive samples were confirmed using a PCR that amplifies a fragment of 1,010 bp of viral polymerase gene, useful to carry out studies of phylogeny. Phylogenetic analysis performed including sequences of different flavivirus demonstrated that the sequences detected in this work in the mosquitoes Oc *caspius* and Oc *mariae*, from the Ibiza and Cabrera islands sequences were grouped in the insect specific flavivirus cluster, related to Ochlerotatus insect flavivirus (OcFV) previously described in Oc. *caspius* mosquitoes in Spain.

(PO 28)

CHARACTERIZATION OF *USUTU VIRUS* DETECTED IN MOSQUITOES IN SPAIN AND ITALY

I. BRIOSO[1], L. HERREROL[1], M. GRISENTI[2], J. MORENO[3], S. RUÍZ[3], J. FIGUEROLA[4], R. SORIGUER[4], A. RIZZOLI[2] M.P. SÁNCHEZ-SECO[1], A. VÁZQUEZ[1].

1. Laboratory of Arboviruses and Viral Imported Diseases, Institute of Health 'Carlos III',

Ctra Pozuelo-Majadahonda, Km 2, 28220 Majadahonda, Madrid, Spain

2. Department of Biodiversity and Molecular Ecology, Research and Innovation Centre,

Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Trento, Italy

3. Mosquito Control Service of Huelva (Andalucı´a), Huelva, Spain.

4. Wetland Ecology Department, Doñana Biological Station, CSIC, Sevilla, Spain

Usutu virus (USUV) is a mosquito-borne virus that belongs to the family *Flaviviridae*, genus *Flavivirus*, Japanese

encephalitis serocomplex. The natural cycle involves birds as the main amplifying hosts and mosquitoes (particularly of the genus Culex) as vectors. It emerged in Europe for the first time in Austria in 2001, causing a high mortality rate in wild birds. The factors that determine the severe symptoms caused by this strain of USUV are still under discussion. Since its emergence in Europe, it has continued to expand, causing cases in humans as well as in birds, in several European countries. In the summer of 2009 it was detected the first human case in Italy, associated with neurological disease, and in 2012 in Croatia. In addition, there have been detected antibodies in blood donors in Germany and Italy. This is the reason why until its expansion in Europe, USUV was not considered as a potential pathogen for human, for the reason that it has never been associated to severe illness.

In Spain it was firstly detected in 2006 and 2009 in *Culex* mosquitoes from Catalonia and Andalusia, not having been reported human cases. As well, during 2012 virus genome has been detected on samples of death birds in Andalusia. The phylogenetic analysis of a partial coding section of the NS5 protein gene region indicated that USUV strains circulating in Europe come from three different clusters, African strains, Spanish strains (from mosquitoes) and central Europe ones (including the strain isolated from Spanish birds). Therefore, different strains are circulating in Europe, with differences on its pathogenicity.

The objective that we set here has been to amplify the complete genome of two virus strains detected on Spanish and Central Europe mosquitoes as well as performing a molecular and phylogenetic characterization of them. For this cause, there have been designed 17 pairs of overlapping primers, representing the complete viral genome. The obtained results of the analysis of these sequences will be discussed in this work.

(PO 29)

CONTRIBUTIONS OF TWO RT-*q*PCR TECHNIQUES IN THE CONFIRMATION OF DEATHS ASSOCIATED WITH THE INFECTION BY DENGUE FEVER VIRUS IN VENEZUELA (2005-2010) USING FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUES FROM AUTOPSIES

M. ROSSI S.[1,2], N. MAYO[1,3], A. HERNÁNDEZ[2], A. POTENTE VILLAFAÑE[1], F. MOLERO[1], G. CÉSPEDES[2], A. NEGREDO[1], A. VÁSQUEZ[1], M. P. SÁNCHEZ-SECO[1], A. TENORIO[1], L. FRANCO[1]

[1]*Laboratorio de Arbovirus y Enfermedades Víricas Importadas, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, España.*

[2]*Sección de Investigaciones en Patología Ultraestructural y Biología Molecular, Instituto Anatomopatológico José A. O´Daly, Facultad de Medicina, Caracas, Venezuela.*

[3]*Maestría en Virología, Universidad Complutense de Madrid, Madrid, España.*

Dengue fever is the arbovirosis more widespread globally and responsible for the outbreaks and epidemics that may affect more than 2.500 million people in urban and rural communities from tropical and subtropical countries. The disease is caused by dengue viruses (DENV-1, 2, 3 y 4). In America the Infection has been estimated in 400 million of cases each year. Between 2001 and 2012, the Venezuelan government agencies 659.728

cases of DENV infection, 47.718 of which were haemorrhagic with a total of 32 deaths from the year 2001 to 2005. Most of the fatal cases occurred in communities from rural, marginal or border areas characterized by poor socio-sanitary conditions and with difficulties to access to the national health system. These conditions not only hinder the early diagnosis and treatment but also conditioned the confirmation of deaths at anatomic pathology level. The aim of this work was to evaluate the applicability of two RT-*q*PCR techniques for the confirmation of deaths caused by DENV, as well as its serotyping, using for these purposes samples of formalin-fixed and paraffin-embedded tissues. Briefly, tissue sections (liver, kidney, spleen and lung) were recovered in vials, dewaxed according histologic techniques, dried at ambient temperature and digested with Proteinase K previous to the extraction of the RNA´s using silica columns. The amplifications of the cDNA´s were done in 2 different real time platforms using TaqMan probes. Of a total of 87 deaths with presumptive pathological diagnosis of dengue, 27 (31%) were positive for DENV with an average *Ct* of 36.36 ($CI_{95\%}$: 35.85, 36.88). Positive samples showed a minimal viral load between 20-200 copies/µl. Eighty one percent (22/27) of the positive samples were from liver and 18.52% (5/27) from kidney. Serotyping was possible in the 96.29% of positive cases, The results demonstrate at molecular level that the majority of deaths were due to DENV-2 (88.89%), one case due to DENV-1 and one mixed infection with DENV-2 and DENV-3. Results are discussed in the context of the importance of both RT-*q*PCR as sensible and specific tools in anatomic pathology, as well as in relation with the casuistry officially communicated and the epidemiologic features of dengue outbreaks.

(PO 30)

ECOLOGICAL CONNECTIVITY SHAPES QUASISPECIES STRUCTURE OF RNA VIRUSES IN AN ANTARCTIC LAKE

A.LÓPEZ-BUENO, A. RASTROJO, R. PEIRÓ, M. ARENAS & A. ALCAMÍ

Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), Madrid, Spain

Metagenomics has revealed the enormous diversity of viruses in nature and facilitates the detection of minority variants. Although RNA and DNA viruses appear to be equally abundant in aquatic environments, our knowledge of viral RNA communities is scarce. RNA viruses exist as complex mixtures of genotypes, known as quasispecies, where the evolution potential resides in the whole community of related genotypes. Quasispecies structure and dynamics have been studied in detail for virus infecting animals and plants but remain unexplored for those infecting microorganisms in environmental samples.

Here we report a metagenomic study of RNA viruses in an Antarctic lake (Lake Limnopolar, Livingston Island). Similar to low latitude aquatic environments, this lake harbours an RNA virome dominated by positive single strand RNA viruses from the order *Picornavirales* likely infecting microorganisms. Antarctic Picorna-like virus 1 (APLV1), an abundant virus in the

lake from 2006 to 2010, does not fix any change in the consensus sequence and shows stable quasispecies with low complexity indexes. By contrast, APLV2-3 are detected in the lake water exclusively in summer samples, and are major constituents of surrounding cyanobacterial mats. Their quasispecies exhibit low complexity in cyanobacterial mat, but their runoff-mediated transfer to the lake results in a remarkable increase of complexity that may reflect the convergence of different viral quasispecies from the catchment area or replication in a more diverse host community. This is the first example of viral quasispecies from natural aquatic ecosystems and points to ecological connectivity as a modulating factor of quasispecies complexity.

(PO 31)

IMPLICATIONS OF THE CELULAR LIPIDIC METABOLISM IN WEST NILE VIRUS INFECTION AND CHARACTERIZATION OF THE VIRAL ENVELOPE LIPIDIC COMPOSITION

T. MERINO-RAMOS[1], A.B. BLÁZQUEZ[1], J. CASAS[2], E. ESCRIBANO-ROMERO[1], F. SOBRINO[3], J.C. SAIZ[1], M.A. MARTÍN-ACEBES[1,3]

[1]*Departamento de Biotecnología. INIA, Madrid.*

[2]*Departamento de Química Biomedicinal, Instituto de Química Avanzada de Cataluña (IQAC-CSIC), Barcelona, Spain*

[3]*Departamento de Virología y Microbiología. Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain.*

West Nile virus (WNV) is an emerging zoonotic mosquito-borne flavivirus responsible for outbreaks of febrile illness and meningoencephalitis, for which no vaccines or antivirals for human use are available. The replication of WNV takes place on virus-modified membranes from the endoplasmic reticulum of the host cell and virions acquire their envelope by budding into this organelle. Consistent with this view, the cellular biology of this pathogen is intimately ligated to modifications of the intracellular membranes, and the requirement of specific lipids, as cholesterol and fatty acids, has been documented. In this study, we evaluated the impact of WNV infection on two important components of cellular membranes, glycerophospholipids and sphingolipids, by mass spectrometry of infected cells. A significant increase in the content of several glycerophospholipids (phosphatidylcholine, plasmalogens and lysophospholipids) and sphingolipids (ceramide, dihidroceramide and sphingomyelin) was noticed in WNV-infected cells, suggesting functional roles of these lipids during WNV infection. Furthermore, the analysis of the lipid envelope of WNV virions and recombinant virus-like particles revealed a unique composition of their envelopes that were enriched in sphingolipids (sphingomyelin) and showed reduced levels of phosphatidylcholine, in a manner similar to that of sphingolipid enriched lipid microdomains. Inhibition of neutral sphingomyelinase (which catalyzes the hydrolysis of sphingomyelin into ceramide), either by pharmacological approaches or siRNA mediated silencing, reduced the release of flavivirus virions as well as virus-like particles, suggesting a role of sphingomyelin to ceramide conversion in flavivirus budding and

confirming the importance of sphingolipids in the biogenesis of WNV. These results has allowed the identification of the sphingolipids metabolism as a new therapeutic target for the development of antivirals against WNV and other related flaviviruses.

(PO 32)

BEGOMOVIRUS QUASISPECIES ADAPT TO HOSTS BYEXPLORING DIFFERENT SEQUENCE SPACE WITHOUTCHANGING THEIR CONSENSUS SEQUENCE

S. SÁNCHEZ-CAMPOS[1,2], G. DOMÍNGUEZ-HUERTA[1,2], D.M. TOMÁS[1], J. NAVAS-CASTILLO[1], E. MORIONES[1], A GRANDE-PÉREZ[2]

1. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM, UMA-CSIC), Estación Experimental "La Mayora", 29750 Algarrobo-Costa, Málaga, Spain.

2. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM, UMA-CSIC), Área de Genética, Campus de Teatinos, 29071 Málaga, Spain.

Geminiviruses possess single-stranded circular DNA genomes that depend oncellular polymerases for replication in host nuclei. In plant hosts, geminiviruspopulations behave as ensembles of mutant and recombinant genomes. Thisfavours the emergence of new geminiviruses able to cause new diseases orovercome the genetic resistance of cultivars. In warm and temperate areasseveral whitefly-transmitted geminiviruses of the genus Begomovirus cause thetomato yellow leaf curl disease (TYLCD) with important economicconsequences. TYLCD is frequently controlled in commercial tomato productionusing the Ty-1 resistance gene. Over a 45 day period we studied theevolutionof infectious clones from three TYLCD-associated begomoviruses: Tomatoyellow leaf curl Sardinia virus, Tomato yellow leaf curl virus and therecombinant Tomato yellow leaf curl Malaga virus. The evolution of virusquasispecies was examined in susceptible tomato (ty1/ty1), resistant tomato(Ty1/ty1), common bean, and the wild reservoir Solanum nigrum. We found thatin addition to affecting viral accumulation kinetics, the host influenced thesequence space explored by the begomovirus quasispecies. In tomato, viraldynamics were not influenced by the presence of the Ty-1 gene. Interestingly,positive adaptation of the coat protein gene observed in the common bean andS. nigrum correlates with these plants having viral quasispecies with the highestdegree of complexity and heterogeneity. Our results underline the importance ofthe mutant spectra of begomovirus infections, especially in wild reservoirs,which have the potential to give rise to large numbers of emergent variants inspite of the invariance of their consensus sequences.

(PO 33)

WEST NILE VIRUS RECOMBINANT SUBVIRAL PARTICLES INDUCED PROTECTION AGAINST HOMOLOGOUS AND HETEROLOGOUS CHALLENGE AND CROSS-REACTIVE ANTIBODIES AGAINST OTHER FLAVIVRUSES

E. ESCRIBANO-ROMERO[1], T. MERINO-RAMOS[1], A.B. BLÁZQUEZ[1], R. CAÑAS-ARRANZ[2], F. SOBRINO[2], J.C. SAIZ[1], M.A. MARTÍN-ACEBES[1,2]

[1]*Departamento de Biotecnología. INIA, Madrid, Spain.*

[2] *Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, Madrid, Spain.*

West Nile virus (WNV) is a neurovirulent mosquito-borne flavivirus that infects multiple vertebrate hosts including birds, horses and humans. Phylogenetic analyses have identified 8 different lineages, being lineage 1 strains considered as the most neurovirulent; however, recent outbreaks have unveiled circulation of highly neurovirulent lineage 2 strains. Since co-expression of flavivirus prM and E glycoproteins drives the assembly of recombinant subviral particles (RSPs) that share antigenic features with virions, a mammalian cell line stably transfected with a plasmid encoding the WNV prM and E glycoproteins of a neurovirulent lineage 1 strain (NY-99) was generated. WNV-RSPs secreted to the culture medium were characterized and their immunogenicity was evaluated. Injection of RSPs induced a potent humoral response against WNV in mice with production of neutralizing antibodies. A single inoculation of RSPs formulated with $Al(OH)_3$ as adjuvant protected animals against a lethal challenge with either homologous (lineage 1, NY-99) or heterologous (lineage 2, SRB-Novi Sad/12) WNV strains. The cross-reactivity of the response elicited by these RSPs against Usutu virus (USUV) –the only other mosquito-borne flavivirus circulating in Europe, which shares multiple ecological and antigenic features with WNV and that has recently caused a considerable avian mortality and a few neurological human cases in Europe- was analyzed. Mice immunization with WNV-RSPs increased specific antibody titers found upon subsequent USUV infection, proving that RSPs prime a humoral response to this related virus. These results expand the ability of RSP-based vaccines to control the two main WNV lineages and to induce cross-reactive humoral responses against an antigenically related flavivirus and, thus, show their potential to the control of neglected flaviviruses, as USUV, which co-circulates with WNV.

(PO 34)

GENETIC VARIABILITY OF HUMAN RESPIRATORY SYNCYTIAL VIRUS A IN SPANISH HOSPITALISED CHILDREN: EMERGENCE OF ON1 GENOTYPE

A. CALDERÓN REÑÓN [1], F. POZO SÁNCHEZ[1], C. CALVO REY [2], ML. GARCÍA GARCÍA[2], M. GONZÁLEZ ESGUEVILLAS[1], M. MOLINERO CALAMITA[1], U. PÉREZ SAUTU[1], I. CASAS FLECHA[1]

[1]*Respiratory Virus and Influenza Unit,National Center for Microbiology, Instituto de Salud Carlos III Majadahonda, 28220 Madrid, Spain.* [2]*Pediatrics Department, Severo-Ochoa Hospital, 28911 Madrid, Spain.*

Human respiratory syncytial virus (HRSV) is the most frequently identified virus in infants hospitalized with acute lower respiratory infections, and it is a significant pathogen among vulnerable adults. Groups A and B are serologically and genetically distinguishable among isolates. Phylogenetic G glycoprotein studies have identified numerous genotypes in both A and B antigenic groups and demonstrated a complex circulation pattern during the same epidemic annual season. The emergence and dissemination worldwide of human respiratory syncytial virus group A, named ON1, with a72-nt insertion in the second hypervariable region of the G gene, allowed us to use it as a natural tag to examine the evolution of HRSV-A. The circulation pattern and the genetic variation in the complete G protein gene of HRSV-A virus were analyzed during four consecutive winter seasons, from 2010 to 2014. Out of 2546 respiratory specimens taken from children 542 (21%) were positive to HRSV, and 288 (54%) grouped as HRSV-A, whichwas predominant during two consecutive epidemics 2011-2012 (74%) and 2012-2013 (95%). Complete G gene sequences were obtained and the phylogenetic analysis was carried out on 122 HRSV-A virus collected among our pediatric population during the study period. Two different HRSV-A genotypes were identified, NA1 and the recently discovered genotype ON1. In 2011-2012, ON1 viruses emerged in Madrid sporadically with 2 positives and become predominant in the 2012-2013, with a total of 38 positives (95%). Clinical outcome of children positives for ON1 virus was established and bronchiolitis was diagnosed in 25 (67.6%) and recurrent wheezing/asthma exacerbations were found in 9 (24.3%). Improving virological and clinical surveillance is required to clarify genetic diversity and transmissibility of the new ON1 genotype.

(PO 35)

MOLECULAR EPIDEMIOLOGY OF MUMPS IN SPAIN 2000-2015. GENOTYPE CIRCULATION AND STRAIN DISCRIMINATION BASED CON SEQUENCING HIPERVARIABLE REGIONS.

A.RUEDA[1], J.E. ECHEVARRÍA[1,2], F. DE ORY[1,2], A. CASTELLANOS[1,2], A. FERNÁNDEZ-GARCÍA[1,2]

1. Centro Nacional de Microbiología. Instituto de Salud Carlos III. Majadahonda. Madrid. Spain.

2. CIBER de Epidemiología y Salud Pública (CIBERESP).Madrid. Spain.

Introduction: Mumps Virus (MuV) is responsible of mumps, a highly contagious disease. In Spain the vaccine was introduced in 1981 with the MMR (measles, mumps and rubella) vaccine. Despite the high rates of vaccination, epidemic waves and outbreaks affecting vaccinated population are still observed. The knowledge of the circulation patterns of MuV genotypes is important for epidemiological surveillance. From 2005 to now the genotype G is being the most prevalent worldwide. Genetic variation within the fragment of the SH gene recommended for genotyping by the WHO is low and there are identical sequences spread all over the world along time, so that it is not easy to establish circulation patterns and transmission chains. The purpose of this work is to describe the molecular epidemiology of MuV in Spain,

including the development of new methods to improve strain discrimination.

Methods: A total of 1679 SH sequences of the MuV were analysed, including 397 from Spain and 1282 from other locations taken from GenBank database. They were aligned (Bioedit v.7.0.5) and their haplotypes determined (DNAsp v.5). Phylogenetic analysis was carried out through maximum likelihood (RaxML v.7 and PhyML v.3). For the RT-PCR we use SimPlot v.3.5.1 to look for variable regions in the MuV genome and PerlPrimer v1.1.21 to assist the primer design.

Results: We found 51 different haplotypes, from genotypes A (4), D (9), G (30), H (5), J, K and N. A change on the dominant genotype from H to G was observed between 2003 and 2005 coincident with the lowest incidence ever reported in our country. All genotype G SH sequences belong to the same phylogenetic cluster. We located three variable intergenic regions (VR) which are candidates for strain discrimination: VR1 1499-2580 nt, NVP (genes N and P/V); VR2 2727-3669 nt, PM (genes P and M); VR3 4180-4760 nt, MF (genes M and F). By the moment we have only tested the VR1 region.

Discussion: After 2005, genotype G is the main circulating genotype all over Europe. It agrees with the circulating genotype in Spain. Our study also confirms the existence of importations from other countries like Japan or the Unites states of America. The main haplotype of the G genotype has been circulating from 2005 to 2015 all over Spain causing numerous outbreaks. The RT-PCR we are optimizing will allow increase the phylogenetic information to distinguish variants or transmission chains inside each haplotype, to improve the surveillance.

(PO 36)

EVALUATION OF ELISA FOR THE DETERMINATION OF CHIKUNGUNYA VIRUS-IgG AND -IgM

F. DE ORY[1,2], T. MINGUITO[1], P. BALFAGÓN[1], L. FRANCO[1,2,3], M. P. SÁNCHEZ-SECO[1,2,3].

[1]Centro Nacional de Microbiología, Instituto de Salud Carlos III, [2]Virored, [3]Red Colaborativa en Investigación de Centros de Enfermedades Tropicales.

In December 2013 Chikungunya virus (CHIKV) was introduced in the Americas, first on the island of Saint Martin, with subsequent spreading to other Antillean islands and continental countries, some of which are important touristic destinations for Spanish travelers. This epidemic implies a real challenge for Spain, since a competent vector (*Aedes albopictus*) is present in the Mediterranean basin. The bite of a viremic patient would allow the indigenous circulation of the virus, as recently happened in neighboring countries (Italy, 2007, France, 2010 and 2014). Bearing in mind the symptoms and epidemiology of CHIKV and dengue virus (DENV) differential diagnosis of both viruses is an important issue. CHIKV viremia is intense, but very short, thus serological methods for diagnosis are required. Indirect immunofluorescence (IIF) techniques are hampered by the difficulties due to the interpretation of results, since nonspecific reactivities caused by autoantibodies are frequent, being required a highly experienced personnel. ELISA techniques avoid these

problems. The aim of the present study was the evaluation of indirect ELISAs using recombinant structural protein as antigen to determine IgM and IgG against CHIKV (EuroImmun, Germany).

A total of 158 samples (from 148 cases), received in our lab in January-September, 2014, for diagnosis of CHIKV infection, were included in the study. Ninety nine cases (108 samples) were classified as caused by CHIKV, since they showed a positive result by PCR (23 cases) and/or CHIKV positive IgM (88 cases, 95 samples). Thirty cases (31 samples) were classified as DENV infections, and 19 cases (19 samples) as negative to both viruses. The cases were classified by the determination of CHIKV IgM and IgG using IIF (Euroimmun) and/or nsP4 and E1 genes amplification. DENV IgM was determined by a capture ELISA and VD IgG by an indirect one (both from Panbio, Korea).

As compared with IIF, ELISA IgM CHIKV assay showed overall agreement, sensitivity and specificity of 94.3%, 95.8% and 92.1%. These figures were improved to 97.2%, 97.8% and 94.1% when only CHIKV infection cases were considered. For IgG assay, the corresponding figures were 89.9%, 82.6% and 98.6%. The low sensitivity of this assay may be caused by the difficulties for detecting this isotype in recent infection cases, in which the sensitivity was 83.1%. This was probably due to the use of structural antigen with more specific reactivity than those detected in the IIF.

ELISA methods are adequate approaches for the serological diagnosis of CHIKV infections.

(PO 37)

MOLECULAR EPIDEMIOLOGY AND CLINICAL ASSOCIATION OF ENTEROVIRUS D68 INFECTIONS IN SPAIN

I. TARAVILLO[1], M. ARANZAMENDI[2], M.P. ROMERO[3], A. MORENO-DOCÓN[4], C. MUÑOZ-ALMAGRO[5], N. RABELLA[6], A. OTERO[1], G. TRALLERO[1], M. CABRERIZO[1]

[1] Enterovirus Unit, CNM, Institut of Health Carlos III, Madrid, Spain.[2]Hospital Cruces, Bilbao, Vizcaya, Spain. [3]Hospital La Paz, Madrid, Spain. [4]Hospital Virgen de la Arrixaca, Murcia, Spain. [5]Hospital San Joan de Deu, Barcelona, Spain.[6]Hospital Santa Creu i Sant Pau, Barcelona, Spain.

Introduction. Enterovirus 68 (EV-D68) is member of *Enterovirus genus* (species D) within the *Picornaviridae* family. EV-D68 was firstly isolated in 1962 from a patient with respiratory illness. It shares features with rhinovirus (RV) and had been detected only sporadically associated with mild respiratory infections. In last years, however, the circulation of this serotype has increased in different parts of the world. Specifically, during 2014, several EV-D68 outbreaks were described in the USA and Canada, causing substantial hospitalization of children with severe respiratory diseases. Fatal cases were also reported. In Spain there are no studies about the incidence and characteristics of EV-D68 infections.

Objectives. To characterize the serotype of EV detected in children with respiratory illnesses and to study the epidemiology and clinical association of EV-D68 infections in Spain.

Patients and methods. Clinical samples collected between October 2014 and February 2015 from 53 hospitalized

children with respiratory symptoms were included in the study. The mean age of the patients (57% male) was 1.6 years. The clinical diagnosis was pneumonia (6 cases), bronchiolitis (5 cases), bronchospasm/wheezing/respiratory distress (11 cases) and upper tract respiratory infection (31 cases). All samples were throat swabs and were positive for EV/RV by RT-PCR previously. Samples were sent to CNM for genotyping by specific RT-PCRs of species EV-A, B, C and D which amplify 3'-VP1 region of the viral genome, sequencing and phylogenetic analysis.

Results. EV were confirmed in 29 (55%) of the total of 53 specimens analyzed and RV in 24 (45%). Of the 29 EV, 22 (78%) were genotyped being EV-D68 the most frequently detected serotype, accounting for 18% (4/22). Other serotypes identified were echovirus (E) -30, coxsackievirus (CV) -B2 and E-6 (3/21, 14%), CV-B4 and CV-A8 (2/21, 9%), and E-13, E-16, E-20, CV-B5, CV-CV-A10 and A5 (1/21, 5%). The mean age of EV-D68-infected children was 2.4 years (range, 2.5 months-4.8 years). Three of them (75%) were male. Clinically, they were diagnosed with pneumonia (1 case), bronchospasm/wheezing (2 cases) and respiratory symptoms with acute liver failure (1 case). This last child and other with bronchospasm required admission to PICU, but none had further complications. Phylogenetic analysis showed Spanish sequences belonged to the same clusters formed by the American and European strains.

Conclusions. This study confirms the circulation of EV-D68 in Spain associated with mild/severe respiratory illnesses. Further surveillance studies are needed to improve our knowledge about the epidemiology and pathologies associated with EV-D68 infections.

(PO 38)

PESTE DES PETITS RUMINANTS VIRUS: EXPERIMENTAL INFECTION WITH A VIRULENT STRAIN AND VACCINE PROTECTION STUDY IN A SPANISH SHEEP BREED

C. CANO-GÓMEZ[1]; F. LLORENTE[1]; P. FERNÁNDEZ-PACHECO[1]; A. ROBLES[1]; A. VILLALBA[1]; M. C. BARBERO[1]; G. LIBEAU[2]; M. A.JIMÉNEZ-CLAVERO[1]; J. FERNÁNDEZ-PINERO[1]

[1]INIA-CISA, Valdeolmos, Spain; [2]CIRAD, Montpellier, France.

Peste des petits ruminants (PPR) is an acute animal disease affecting small ruminants, included in the OIE list of notifiable diseases due to its high economic impact. PPR is a highly contagious disease which spreads rapidly by direct contact through excretions/secretions from sick animals, caused by a Morbillivirus of the Paramyxoviridae family, PPRV. The severity of the disease depends upon the virulence of the strain and the susceptibility of the species/breed affected. The acute form may kill up to 90% in 5-10 days upon onset of clinical signs in naïve populations. A subacute, milder form is known, in which the animals usually recover within a week of the onset of symptoms.

PPR is endemic in most of Africa, the Middle East, South Asia and China. PPR is controlled by vaccination, restriction of animal movements and efficient and rapid diagnosis. Attenuated vaccines, mainly Nigeria 75/1 strain, have been commonly

used, inducing a reported life-long protective immunity in sheep and goats against all known PPRV lineages. Recently, lineage IV PPRV expanded geographically, reaching Northern Africa, close to Southern European countries, concerned by the risk of PPR emergence in their territories. In this framework, we aimed to study the disease pattern produced in a Spanish native sheep breed ("Colmenareña") by a pathogenic strain of PPRV(Mor/08, lineage IV), and to evaluate the protection conferred by the immunization of this Spanish sheep breed with the live attenuated PPRV vaccine Nigeria75/1 (lineage II) against a challenge with the same pathogenic Mor/08 PPRV strain. For that, a group (n=4) of sheep was vaccinated (s.c.) and challenged (i.v.) 21 days post-vaccination, while other (n=4) was only challenged. Naïve or vaccinated sheep (n=2 each) were kept in contact with vaccinated/challenged animals or with just challenged sheep to evaluate contact transmission in the presence or absence of vaccination. Clinical follow-up and laboratory studies (viral load by qRT-PCR in blood, swabs, faeces, necropsy specimens, and serum antibodies by ELISA) were carried out. DIVA qRT-PCR was applied to differentiate vaccine from field PPRV strains.

As a result, the Spanish sheep breed "Colmenareña" inoculated with PPRV Mor/08 pathogenic strain showed generally mild clinical signs. Vaccination (Nigeria75/1) protected sheep against i.v. challenge with Mor/08 strain. This strain, however, was able to spread by direct contact from excretion/secretion sites to naïve and vaccinated contact sheep, the latter remained aviremic and protected from the disease.

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(PO 39)

PEPTIDES INTERFERING 3A PROTEIN DIMERIZATION DECREASE FMDV MULTIPLICATION

MÓNICA GONZÁLEZ-MAGALDI[1], ÁNGELA VÁZQUEZ-CALVO[1], BEATRIZ G. DE LA TORRE[2], JAVIER VALLE[2], KATHERINE I. CALDERÓN[1], DAVID ANDREU[2] AND FRANCISCO SOBRINO[1]

1. Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, 28049 Madrid, Spain

2. Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, 13 08003 Barcelona, Spain

Nonstructural protein 3A is involved in relevant functions in foot-and-mouth disease virus (FMDV) replication. FMDV 3A can form homodimers and preservation of the two hydrophobic α-helices (α1 and α2) that stabilize the dimer interface is essential for virus replication. In this work, small peptides mimicking residues involved in the dimer interface were used to interfere with dimerization and thus gain insight on its biological function. The dimer interface peptides α1, α2 and that spanning the two hydrophobic α-helices, α12, impaired in a dose dependent manner in vitro dimer formation of a peptide containing the two α-helices, this effect being higher with peptide α12. To assess the effect of dimer inhibition in cultured cells, the interfering peptides were N-terminally fused to a heptaarginine (R7)

sequence to favor their intracellular translocation. Thus, when fused to R7, interference peptides (100 µM) were able to inhibit dimerization of transiently expressed 3A, the higher inhibitions being found with peptides α1 and α12. The 3A dimerization impairment exerted by the peptides correlated with significant, specific reductions in the viral yield recovered from peptide-treated FMDV infected cells. In this case, α2 was the only peptide producing significant reductions at concentrations lower than 100 µM. Thus, dimer interface peptides constitute a tool to understand the structure-function relationship of this viral protein and point to 3A dimerization as a potential antiviral target. A similar approach is being followed to study the effect of coxsackievirus

(PO 40)

MEMBRANE TOPOLOGY OF FOOT- AND-MOUTH DISEASE VIRUS 3A PROTEIN

MÓNICA GONZÁLEZ-MAGALDI[1], MIGUEL A. MARTÍN-ACEBES[1], LEONOR KREMER[2] AND FRANCISCO SOBRINO[1]

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, 28049 Madrid, Spain[1]. Centro Nacional de Biotecnología (CNB-CSIC), Cantoblanco, 28049 Madrid, Spain[2].

Foot-and-mouth disease virus non-structural protein 3A plays important roles in virus replication, virulence and host-range; nevertheless little is known on the interactions that this protein can establish with different cell components. Here, we describe a nonintegral membrane protein topology of transiently expressed FMDV 3A. This topology was supported by the lack of glycosylation of versions of 3A in which each of the protein termini was fused to a glycosylation acceptor tag, as well as by their accessibility to degradation by proteases. According to this model 3A would interact with membranes through its central hydrophobic region exposing its N- and C- termini to the cytosol, where interactions between viral and cellular proteins required for virus replication are expected to occur. This 3A topology is novel among picornaviruses, highlighting that in FMDV this non-structural protein shows characteristics and functions that differ from those of other virus family members.

(PO 41)

MONOCYTE PORCINE CELL LINES FOR PRODUCTIVE AFRICAN SWINE FEVER VIRUS INFECTION

EG. SÁNCHEZ, P. FERNÁNDEZ, ML.NOGAL, Y.REVILLA.

Virology Department, Centro de Biología Molecular "Severo Ochoa", Madrid, Spain

ASFV is highly pathogenic double-stranded DNA virus with a marked tropism for cells of the monocyte-macrophage lineage. Although monkey cell lines such as Vero or COS allow the adaptation of ASFV strains after several passages, a suitable porcine cell line able to efficiently support ASFV infection is necessary to develop models for cell-host interaction and vaccine studies. For this purpose, four different porcine cell lines from monocyte-macrophage origin (IPAM WT, IPAM-CD163, CΔ2+, WSL) have been tested in order to set up the most similar conditions to the infection in primary alveolar macrophages (PAM) in terms of

phenotype, ASFV infection susceptibility and viral production. To achieve this, we analyzed on these lines the presence of CD163 and CD169 cellular surface receptors since they are linked to differentiation and maturation of the macrophages and seem to beclosely related to ASFV infection. ASFV susceptibility was analyzed in the infected cells by the expression of the viral late protein p72 and viral production by titration on plaque assays. Results showed that although all porcine cell lines analyzed were susceptible to ASFV infection, none of them was as efficient as PAM in terms of virus production. Future experiments will be focus on describing which cellular factors are related with the ability of porcine cell lines to support an ASFV productive infection in order to establish a suitable model of study.

(PO 42)

THE AMINO ACID SUBSTITUTION Q65H IN 2C PROTEIN OF SVDV INCREASES RESISTANCE TO BREFELDIN A.

A. VÁZQUEZ CALVO, F. CARIDI, F. SOBRINO, M.A. MARTÍN ACEBES.

Centro de Biología Molecular Severo Ochoa (UAM-CSIC), Madrid, Spain.

Swine vesicular disease virus (SVDV) is a porcine pathogen and a member of the PICORNAVIRIDAE family. It is included into the ENTEROVIRUS genus and is closely related to the human pathogen coxsackievirus B5 (CVB5). Brefeldin A (BFA), an inhibitor of the cellular protein GBF1 (a guanine nucleotide exchange factor for small cellular GTPases Arf), induces Golgi complex disassemblly and alters the cellular secretory pathway. BFA has been shown to inhibit the RNA replication of different enteroviruses including SVDV. In this study we have analyzed the effect of several GBF1 inhibitors on SVDV production and isolated a SVDV mutant with increased resistance to BFA. A single amino acid substitution, Q65H, in the 2C protein was found to be responsible for the increased BFA resistance.

(PO 43)

IDENTIFICATION OF CD8 T CELL EPITOPES IN VP2 AND NS1 PROTEINS OF BLUETONGUE AND AFRICAN HORSE SICKNESS ORBIVIRUSES IN IFNAR(-/-) 129/SV MICE

B. FERNÁNDEZ-RETUERTO[1], A. MARÍN-LÓPEZ[1], F. DE LA POZA[1], E. CALVO-PINILLA[2], J. ORTEGO[1].

[1]. Centro de Investigación en Sanidad Animal, INIA-CISA, Valdeolmos, Madrid, Spain.

[2]. The Pirbright Institute, Pirbright, Surrey, United Kingdom.

Bluetongue virus (BTV) and African horse sickness virus (AHSV) are *Orbivirus* of the family *Reoviridae* that cause severe disease in ruminants and equids, respectively. Previous work in our laboratory has shown the presence of CD8+ T cells specific of BTV and AHSV antigens in mice immunized with recombinant Modified Vaccinia Ankara (rMVA) expressing VP2 and NS1 proteins of both orbivirus. We also observed that the induction of a strong CD8+ T cell response is critical to induce multiserotype protection. We have now selected potential CD8 T cell epitopes (MHC-class I binding peptides) for the 129 mouse strain

corresponding to the VP2 and NS1 proteins of BTV-4 and AHSV-4, using a combination of four epitope prediction algorithms (SYFPEITHI, BYMAS, NetMHC I and NetMHCpan). ELISPOT and Intracellular Cytokine Staining (ICS) analysis showed that peptides NS1 (152) (GQIVNPTFI) of BTV-4 as well as peptides VP2 (1052) (YTFGNKFLL) and NS1 (92) (CVIKNADYV) of AHSV-4 elicited IFN-γ production in splenocytes of MVA-VP2 and MVA-NS1 immunized mice and were identified as CD8 T cell epitopes. In addition, these three MHC-class I-binding peptides induced the surface expression of CD107a in CD8+ T cells, an indirect marker of cytotoxic activity. Importantly, NS1 (152) epitope of BTV-4, and VP2 (1052) and NS1 (92) epitopes of AHSV-4 are highly conserved among the 27 BTV and 9 AHSV serotypes, respectively. The characterization of BTV and AHSV specific CD8 T-cell epitopes provides useful information for the design of novel multiserotype vaccines against these two orbivirus.

(PO 44)

DETECTION AND MOLECULAR CHARACTERIZATION OF ZOONOTIC VIRUSES IN SWINE ISOLATED IN ITALIAN PIG HERDS

M. MARINA[1], I. DI BARTOLO[1], G. IANIRO[1], G. ANGELONI[1], C. MAGISTRALI[2], F. OSTANELLO[3], F. M. RUGGERI[1].

[1] *Department of Veterinary Public Health & Food Safety, Viral Zoonoses Unit, Istituto Superiore di Sanità, Rome, Italy.*

[2] *Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy*

[3] *University of Bologna, Dep. of Veterinary Medical Sciences, Ozzano dell'Emilia (BO), Italy*

Gastrointestinal disease is frequent in pigs, and among the different etiological agents involved viruses are considered the leading cause of diarrhea in this animal species. Furthermore about half of newly identified swine pathogens are viruses, most of which may be transmitted to humans by direct contact or by indirect transmission pathways. In this study, the prevalence of astrovirus (AstV), group A rotavirus (RVA), norovirus (NoV) and hepatitis E virus (HEV) infections in pigs were investigated. During 2012-2014 a total of 242 fecal samples were collected from pigs at different production stages (from 0 to 180 day-old) in eight swine farms located in northern, central and southern Italy.

Seven out of 8 farms analyzed were positives for AstV, which was detected in 163/242 (67.4%) samples and represented the most prevalent virus; 61 animals (37.4%) showed diarrhea. HEV, was detected in 6 farms and in 45/242 (18.6%) of the samples analyzed. Twenty-three HEV infected pigs had diarrhea (45%). A lower prevalence was observed for RVA, only three farms resulted positives, it was found in 10/242 samples (4.13%), 6 out of these showed diarrhea (60%). On the contrary, no swine samples were found to be positive for NoV. This study compares for the first time in Italy the occurrence of astroviruses, rotaviruses and hepatitis E in a same population of pigs, and reports the molecular characterization of viral strains detected.

The presence of enteric viruses also in asymptomatic swine identifies a possibly underscored risk of virus spreading among animals and addresses a potential source of infection for humans. Further studies are required in order to understand the

role of these viruses in gastrointestinal diseases of pigs. Sequence analyses may permit to assess the zoonotic potential of the viruses detected in animal clinical samples.

(PO 45)

HEPATITIS E VIRUS IN PORK LIVER SAUSAGES SOLD IN ITALY

I. DI BARTOLO[1], G. ANGELONI[1], E. PONTERIO[1], F. OSTANELLO[2] AND F. M. RUGGERI[1].

[1].Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy

[2]. Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (BO), Italy

Hepatitis E is an acute human disease caused by the hepatitis E virus (HEV). In low-income countries, the virus has been involved in waterborne outbreaks. Autochthonous hepatitis E cases are increasingly reported in developed countries, where sporadic cases and small outbreak have been reported. The disease is normally self-limiting (mortality rate 1%), but chronic infections have recently been observed in transplanted patients. The etiological agent HEV is a small RNA virus infecting both humans and animals. Pigs and possibly other animal species are reservoir for HEV, and the consumption of raw contaminated animal meat and meat products has been linked to sporadic cases and small outbreaks of hepatitis E in humans. In the present study, we investigated the presence of HEV and fecal cross-contamination in both fresh and dry pork liver sausages in Italy bought at a grocery store in Italy. The genome of HEV was detected by qRT-PCR in both raw (10 out of 45 slices, 250 mg each, 22.2%) and dry (1 of 23 slices, 4.3%) liver sausages, but viability of the virus was not demonstrated. A phylogenetic tree was drawn using both RdRp and MTase fragments. Results confirmed presence of genotype 3 HEV strains and a correlation between the HEV genomes detected in liver sausages in this study with swine and human HEV strains reported in Europe, including Italy. This pilot study fosters more investigations on HEV presence in pork-derived food, to assess the possible risk for the consumers.

(PO 46)

DEVELOPMENT OF A NOVEL LATERAL FLOW ASSAY FOR DETECTION OF ASFV IN BLOOD

P. SASTRE ANTORANZ[1], T. PÉREZ ESCODA[1,2], C. GALLARDO FRONTAURA[3], M. ARIAS NEIRA[3], A. MONEDERO MARCOS[1], T. RUIZ GONZÁLEZ[1], P. RUEDA PÉREZ[1].

1. Inmunología y Genética Aplicada S. A. (INGENASA), Madrid, Spain

2. Current address: Urano vet, Barcelona, Spain

3. Centro de Investigación en Sanidad Animal, INIA, Madrid, Spain

African swine fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages, causing a wide range of syndromes from mild disease to lethal hemorrhagic fever. The causative agent of the infection, ASF virus (ASFV), is a large, enveloped, icosahedral double-stranded DNA virus that belongs to the Asfarviridae family. The disease is endemic in Sub-Saharan Africa and Sardinia. Since 2007, several cases have been declared in Georgia, Armenia, Azerbaijan, and in the

Russian Federation, where the continued spread presents a serious threat to the swine industry worldwide. Rapid detection of infected animals is of paramount importance for early detection of outbreaks, reducing the transmission of the viruses to uninfected animals and subsequently spreading of the disease. Current diagnosis of ASF is based on direct identification of the virus by polymerase chain reaction (PCR) or virus isolation, and detection of antibodies by either ELISA, immunoblotting or immunofluorescence assay. However, these methods are still rather time consuming and require well equipped laboratories and personnel, delaying the disease diagnosis in remote areas.

Ingenasa has developed a Lateral Flow Assay (LFA) for antigen detection based on the use of MAbs against VP72 protein of ASFV, the major viral capsid protein and considered the most immunogenic protein of the virus. First experiments using VP72 recombinant protein or inactivated virus from tissue culture showed promising results with a sensitivity similar to that a commercially available DAS-ELISA (11.PPA.K.2, Ingenasa). Moreover, these strips were tested with blood from experimentally infected pigs at CISA-INIA Level 3 Laboratory. The animals were inoculated with different viral isolates and blood was collected at different days post infection (pi). The sensitivity of the test allowed detecting viral loads from 10^4 HAU corresponding with day 4-7 pi. Further validation is currently ongoing in different countries in Europe, Asia and Africa.

This novel pen-side test offers a rapid, economic and simple-to-use diagnostic tool suitable or field application, allowing the early diagnosis of ASF. It does not require any kind of equipment, nor skilled users to perform the test. Furthermore, the test has been designed to be used with blood, thus making the sample processing quite easy and feasible even at field level. All these features make these devices very suitable for small field labs or task forces, supporting in many cases local decisions, especially in countries where laboratory infrastructure is under development or even missing.

Acknowledgements

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GENERATION OF PRRS VLPS BY MULTIPLE PROTEIN CO-EXPRESSION IN THE BACULOVIRUS SYSTEM

M. GARCÍA DURÁN, S. COSTA, J. SARRASECA, N. ROJA, J. GARCIA MIGUET, ISABEL GARCÍA, M.J. RODRIGUEZ

Inmunologia y Genetica Aplicada, S.A., Madrid, Spain.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in pig industry, causing high economic losses worldwide. Live-attenuated viruses are the most commonly used PRRSV vaccines, but they are not fully effective. Protective immune response in PRRS is based on neutralizing antibodies and cellular response but, until now, there is not a clear epitope or protein considered the only responsible of the protective mechanisms. Moreover, the results seem

to point out that several structural proteins are contributing to the global immune response. This work aimed at the construction of PRRS Virus Like Particles (VLPs) that incorporate Gp5, M, Gp2, Gp3, Gp4 and E proteins.

The cDNA sequences corresponding to the six structural proteins were amplified from Olot virus strain and cloned into the multiple expression baculovirus vector pBAC4x-1 in two separate plasmids: Gp5 and M in one plasmid (pBAC4x-1A) and Gp2, Gp3, Gp4 and E in another one (pBAC4X-1B). The expression of the different proteins was assessed by Western blot. The culture supernatants after single infection with pBAC4x-1A or after co-infection with pBAC4x-1A plus pBAC4x-1B were semi purified with a sucrose cushion. Subsequently, were loaded on the top of a sucrose layer gradient. The formation of VLPs was confirmed, in single infections and in co-infections, by electron micrograph of the gradient fractions corresponding to PRRSV density (1,15-1,16 g/cc).

Gp5 and M proteins were easily detected in the two types of VLPs with the specific mAbs, showing an apparent molecular weight similar to the one observed in the virus. The minor structural proteins are, in general, more difficult to detect both in the VLPs and in the virus. This fact could be reflecting the stoichiometry of the virus, whose envelope is mainly composed of Gp5/M complexes whereas the Gp2-Gp3-Gp4 complexes and E protein are nestled (Dokland 2010). In this work, Gp3 protein had an apparent size smaller than the one in the wild type virus, which can be indicating a lower glycosylation level.

The system presented here has the flexibility to easily add or remove complete structural proteins to the basic Gp5/M VLPs, providing a useful approach to study the implication of particular proteins. Besides, the possibility to exchange individual proteins to generate PRRS VLPs from different strains would be a helpful tool for the development of specific vaccines. Nevertheless, further work is needed to clarify important aspects of the VLPs generated as their immunological abilities.

Acknowledgements

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LIMITED SUSCEPTIBILITY OF MICE TO USUTU VIRUS (USUV) INFECTION AND INDUCTION OF FLAVIVIRUS CROSS-PROTECTIVE IMMUNITY

A.B. BLÁZQUEZ[1], E. ESCRIBANO-ROMERO[1], M.A. MARTÍN-ACEBES[1,2], T. PETROVIC[3], J.C. SAIZ[1]

[1]*Departamento de Biotecnología. INIA, Madrid, Spain.*

[2]*Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, Madrid, Spain.*

[3]*Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia*

Flaviviruses (*Flaviviridae* family) are RNA viruses that constitute a worrisome threat to global human and animal health. Until recently, West Nile virus (WNV) was the only mosquito-borne flavivirus circulating in Europe, being responsible of numerous outbreaks that have dramatically increased in number and severity in recent years,

with dozens of human and horse deaths and a high avian mortality across the continent. In 2001, another flavivirus, the Usutu virus (USUV), was detected for the first time in Austria and, since then, the virus has quickly spread across Europe, causing a considerable number of bird deaths and neurological disorders in a few patients. Even though USUV infects multiple avian species, there is little information about USUV susceptibility, pathogenicity and cross-reactive immunity. In the present report, the susceptibility of suckling and adult mice to USUV infection and the induction of cross-protective immunity against WNV challenge was addressed. All adult mice infected with either 10^2 or 10^4 pfu/mice of USUV survived to the infection, while only 16.6% and 8.3%, respectively, of those infected with similar doses of WNV did it. On the other hand, in suckling mice survival rates against USUV infection were dose dependent (84.2% and 40%, respectively), but also higher than that recorded (18%) after WNV (10^4 pfu/mice) infection. Except 6 adult mice infected with the lower USUV dose (10^2), all the remaining surviving animals either adults or suckling resulted protected against challenge with a high dose of WNV. No USUV-RNA could be detected in any of the adult mice analysed between 4 and 35 days post-infection (d.p.i.). In contrast, USUV-RNA was amplified form suckling mice 7 d.p.i., but not early (4 d.p.i.) or later (15 d.p.i.). These findings demonstrate that mice susceptibility to USUV infection is age dependent and that the elicited antibodies are cross-reactive and protective against other flaviviruses infection, such as that of WNV.

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DETECTION OF PAN/FOOT AND MOUTH DISEASE VIRUS BY A MULTI-CHECK rRT-PCR STRATEGY

L RIOS[1], CL. PERERA[1], L CORONADO[1], D RELOVA[1], AM ÁLVAREZ[2], L GANGES[3], H DÍAZ DE ARCE[4], LJ. PÉREZ[1], JI. NÚÑEZ[3].

[1]. Centro Nacional de Sanidad Agropecuaria (CENSA), La Habana, Cuba.

[2] Instituto Nacional de Investigaciones Agricolas (INIA), Maracay, Venezuela.

[3]. Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, Bellaterra, Cerdanyola del Vallès, Spain.

[4] Hospital italiano de Buenos Aires, Buenos Aires, Argentina

The objective of this study was to develop and validate a multi-check strategy using rRT-PCR (multi-rRT-PCR) based on SYBR-Green I for pan/foot and mouth disease virus (pan/FMDV) diagnosis. Based on the *in silico* analyses, different primer pairs were selected and addressed in order to reduce the probability of viral escape and possible failures in the pan/FMDV detection due to the high variability of the virus. The analytical parameters were assessed on a large representative number of viral strains. The repeatability of the test and its performance on field samples were also evaluated. The multi-rRT-PCR was able to detect novel emergent strains of FMDV which had circulated in South America during the period 2006-2010 and on which the individual assays failed when they were applied independently. We demonstrate that the system proposed is a reliable and rapid diagnosis method for sensitive and specific detection of FMDV. Therefore, a validated multi-rRT-PCR assay based on SYBR Green I detection coupled to melting

curves analysis for pan/FMDV diagnosis on clinical samples is proposed. This work also highlights the need to incorporate the multi-target detection principle in the diagnosis of viral pathogens with highly variable genomes as FMDV.

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GENERATION OF VIRUS-LIKE PARTICLES (VLPs) FROM THE NEW VARIANT RABBIT HAEMORRHAGIC DISEASE VIRUS (RHDV2) AND ITS APPLICATION FOR SEROLOGICAL STUDIES

J. BARCENA[1], Y. GONZÁLEZ, B., GUERRA[1], J.R. CASTON[2], E. BLANCO[1] AND A. ALEJO[1]

[1]. Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, 28130 Madrid, Spain.

[2]. Dpto. Estructura de Macromoléculas, (CNB-CSIC), Cantoblanco, 28049 Madrid, Spain.

The new variant of rabbit haemorrhagic disease virus (RHDV2) has distinct biological properties and may be replacing the previously more prevalent RHDV virus in several European countries. Importantly, the RHDV vaccine does not confer complete protection against RHDV2 induced disease, which has prompted the development and adoption of a novel inactivated virus vaccine. ELISA methods for the diagnosis of classical RHDV infection are well characterized, but currently, there are no available specific serological tests for RHDV2.

Here we describe the expression of the complete VP60 major coat protein of RHDV2, which self assembles into virus like particles (VLPs). These VLPs have been used to study the specific antibody response in RHDV and RHDV2 vaccinated animals, confirming their differential antigenic properties. Finally, screening of sera obtained from a wild rabbit population suggests an elevated prevalence of the novel RHDV2, circulating among apparently healthy individuals.

The VLPs from RHDV2 provide important tools to monitor circulation of the novel virus variant and to discriminate between RHDV and RHDV2 infection.

(PO 51)

COMPLETE GENOME SEQUENCE OF A GENOTYPE 3 HEPATITIS E STRAIN IDENTIFIED IN A SWINE FARM IN ITALY

L. DE SABATO[1], I. DI BARTOLO[2], G. ANGELONI[2], E. PONTERIO[2], M. MONINI[2], F.M. RUGGERI[2], F. OSTANELLO[1]

[1]. Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (BO), Italy

[2].Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy

Hepatitis E is an acute disease of humans caused by a small RNA virus, the hepatitis E virus (HEV). Four mammalian HEV genotypes are recognized. Genotype 1 and 2 are restricted to humans and consist of epidemic strains circulating in developing countries, mainly associated to waterborne outbreaks. Genotype 3 and 4 infect both humans and many animal species (pig, deer, wild boar, and rabbit) and circulate in developed countries. These two latter genotypes are considered zoonotic, for which pigs and less frequently other animal species (wild boar, deer) are reservoirs. Based on sequence analysis and intra-genotype variability, genotypes are divided into sub-genotypes. Thegenotype 3 can be divided into 10 sub-genotypes (3a–3j). In this study, hepatitis E infection was

investigated in piglets affected with diarrhea in two small farms in Italy. The virus was detected in 11 out of 14 animals tested. Based on sequence analysis the 6 Italian strains examined belonged to two clusters that contain both swine and human strains from Europe and Japan, belonging to genotype 3 sub-genotypes e and f. The two Italian clusters shared a nucleotide identity of 81.8% in the 400bp ORF2 (capsid protein) fragment and 87.5% in the 400bp ORF1 (RdRp) fragment, confirming that genotypes 3 circulating in pigs in Italy are heterogeneous. The complete genome of a g3e strain and the complete coding regions (partial ORF1, complete ORF2 and ORF3) of a representative g3f strain were obtained and compared to other HEV full length or partial sequences available on line. Results obtained revealed that porcine strains clustered together with human and swine strains detected in Europe. The analyses conducted showed that most changes in the coding regions correspond to synonymous mutations, whereas only a small ORF1 region and the ORF3 showed sites subjected to positive selection. Further analyses are needed to understand the possible different clinical significance of HEV genotypes and sub-genotypes.

(PO 52)

THERAPEUTIC MVA-B VACCINE IMPROVES THE MAGNITUDE AND QUALITY OF THE T CELL IMMUNE RESPONSES IN HIV-1 INFECTED SUBJECTS ON HAART

C. E. GÓMEZ[1], B. PERDIGUERO[1], J. GARCÍA-ARRIAZA[1], V. CEPEDA[1], C. O. SÁNCHEZ-SORZANO[1], B. MOTHE[2], J. L. JIMÉNEZ[3], M. A. MUÑOZ-FERNÁNDEZ[3], J. M. GATELL[4], J. C. LÓPEZ BERNALDO DE QUIRÓS[3], C. BRANDER[2], F. GARCÍA[4] AND M. ESTEBAN[1]

[1] Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain. [2] IrsiCaixa-HIVACAT, Hospital Universitari Germans Trias i Pujol, Badalona, Spain.[3] Hospital Gregorio Marañón, Madrid, Spain. [4] Hospital Clinic-HIVACAT, IDIBAPS, Barcelona, Spain.

Previous studies suggested that poxvirus-based vaccines might be instrumental in the therapeutic HIV field. A phase I clinical trial was conducted in 30 HIV-1-infected patients on highly active antiretroviral therapy (HAART) with CD4 T cell counts above 450 cells/mm^3 and undetectable viremia which were randomized to receive 3 intramuscular injections of MVA-B vaccine (10^8 PFU/ dose) (coding for clade B HIV-1 Env, Gag, Pol and Nef antigens) or placebo, followed by interruption of HAART. Here, the magnitude, breadth, quality and phenotype of the HIV-1-specific T cell responses were assayed by intracellular cytokine staining (ICS) in 22 out of 30 volunteers pre- and post-vaccination. Furthermore, a sub-study of HIV-1 viral rebound dynamics was performed in 9 out of 22 subjects during the first 12 weeks after HAART interruption. MVA-B vaccine significantly induced the expansion and also the

appearance of new HIV-1-specific CD4 T cell responses (mostly against Gag and GPN antigens) that were high in magnitude, broad, with an enhanced polyfunctionality and of T effector memory (TEM) phenotype, while maintained the magnitude and quality of the preexisting HIV-1-specific CD8 T cell responses. In addition, the MVA-B-induced immune responses were associated with a delayed HIV-1 plasma viral rebound in 50% of the vaccinees analyzed. Thus, MVA-B vaccination represents a feasible strategy to improve T cell responses in individuals with preexisting HIV-1-specific immunity.

(PO 53)

ANALYSIS OF DUAL ANTIVIRAL ACTIVITY AGAINST HIV-1/HSV-2 OF NEW POLYANIONIC CARBOSILANE DENDRONS WITH FATTY ACIDS AT THE CORE AS A TOPICAL MICROBICIDE

C. GUERRERO-BELTRÁN[1], R. CEÑA-DIEZ[1], D. SEPÚLVEDA-CRESPO[1,2], J.L. JIMÉNEZ[2], R. GÓMEZ[3], F. J. DE LA MATA[3], Mª A. MUÑOZ-FERNÁNDEZ[1,2,*]

[1]. Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain.Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.Spanish HIV HGM BioBank, Madrid, Spain.

[2]. Plataforma de Laboratorio, Hospital General Universitario Gregorio Marañón, Madrid, Spain. IISGM, Madrid, Spain. CIBER-BBN, Madrid, Spain

[3]. Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, Alcalá de Henares, Madrid, Spain. CIBER-BBN, Madrid.

The HIV pandemic continues its spread at a rate of over 15,000 new infections every day. Nowadays, only in sub-Saharan Africa heterosexual transmission represent the 80% of the new infections, mainly in women. The development of new preventive treatments in the last years has been carried out. Among them, topical microbicides and nanotechnology play an important role. We introduce the main steps of new potential microbicides screening *in vitro* and *in vivo*, as well as the methodology used to achieve effective polyanionic carbosilane dendrons microbicides. We research the potential topical microbicide activity against HIV-1 and HSV-2 infection of six new different polyanionic carbosilane dendrons named BDCG044, BDCG046, BDCG048, BDCG050, BDCG052 and BDCG054.

The dendrons were synthesized by the group of Inorganic chemistry of Alcalá de Henares. We focused on carbosilane branches dendrons from first to third generation, with palmitic or hexanoic fatty acids as core and capped with sulfonate groups. We evaluated cytotoxicity in different cell lines *in vitro* (TZM.bl, PBMCs and VERO), inhibition of HIV-1 (X4-HIV-$1_{NL4.3}$ or R5-HIV-1_{NLAD8}) replication and HSV-2 333, time-of-addition experiments, establishment of IC_{50}, cell fusion, HIV-1 internalization and binding assays, vaginal irritation test and subsequent histological analysis. Different data analyses were performed using Calcusyn software.

BDCG048 and BDCG054 showed high biosafety in primary cells and different cell lines derived from vagina and uterus (maximum safe concentration at a range of 10 µM in TZM.bl cells). Moreover, these dendrons showed a great broad-spectrum antiviral activity achieving inhibitions of 99% using X4-HIV-$1_{NL4.3}$ in the

presence/absence semen, high inhibition against HSV-2, blocked the entry of different HIV-1 strains, and protected the epithelial monolayer cells from cell disruption. IC_{50}-values were at the order of nanomolar concentration. Additionally, no irritation was detected in female mice after dendron vaginal administration.

We conclude that BDCG048 and BDCG054 both third generation dendrons with hexanoic or palmitic fatty acids as core, respectively could be effective to inhibit HIV-1 and HSV-2 infection and transmission within genital mucosa. We can provide promising outcomes to encourage BDCG048 and BDCG054 as a hopeful microbicides. Although this promising results, further assays are needed to be performed in order to lead these results to clinical trials.

(PO 54)

SYNERGISTIC ACTIVATION OF LATENT HIV-1 EXPRESSION BY NOVEL HISTONE DEACETYLASE INHIBITORS AND BRYOSTATIN-1

M. MARTÍNEZ-BONET[1], M.I. CLEMENTE[1,2], M.J. SERRAMÍA[1], E. MUÑOZ[3], S. MORENO[4], M.A. MUÑOZ-FERNÁNDEZ[1,*]

[1] *Laboratorio de InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón; Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN); C/ Dr. Esquerdo 46, 28007 Madrid, Spain*

[2] *Plataforma de cultivos celulares, Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), C/ Dr. Esquerdo 46, 28007 Madrid, Spain*

[3] *Departamento de Inmunología, Facultad de Medicina, Universidad de Córdoba, Avda. Menéndez Pidal, s/n. 14004, Córdoba, Spain*

[4] *Servicio de Enfermedades Infecciosas, Hospital Universitario Ramón y Cajal and IRYCIS, Madrid.*

Effective combination antiretroviral therapy (cART) has improved the quality and life expectancy of HIV-infected patients; nevertheless achieving the cure of HIV is still an unattainable challenge for the scientific community. Although cART achieves undetectable plasma viral RNA and the normalization of CD4 T cell levels in almost all patients, several studies have shown that HIV remains incurable owing to the persistence of latently infected cells. The persistence of HIV-1 involves numerous overlapping cellular pathways, which are interesting from the pharmacological point of view. Thus, targeting multiple steps within the virus latency mechanisms is important to optimize the reactivation effect. Thus, recent therapeutic interventions to eradicate HIV are focused on the activation of viral production from latently infected cells. We evaluated the effect of different combinations of bryostatin-1 (BRY) and novel histone deacetylase inhibitors (HDACIs) in HIV reactivation and compared the toxicity and phenotype modifications induced by single or combined treatment. The lymphocyte or monocyte/macrophage latently infected cell lines J89GFP and THP89GFP, respectively, were treated with BRY, panobinostat (PNB) and romidepsin (RMD) alone or in combination and the viral reactivation effect was assessed as EGFP expression. We calculated the combination index (CI) for each drug combination to determine synergy. Primary CD4 T cell viability, activation and proliferation profile was analyzed after single or combined drug treatment. In

terms of latent HIV reactivation, we demonstrated a synergistic activity in the BRY/HDACIs combinations tested, whereas nonsynergistic or additive effects were observed when PNB was mixed with RMD. The EC_{75} of BRY, PNB and RMD were reduced in these combinations, showing a decrease from 20 to 4-fold respectively. Primary CD4 T cells treated with drug combinations presented better activation and proliferation profiles in comparison with single drug at their EC_{75} value treated cells. In summary, the combination between BRY, PNB and or RMD presented a synergistic profile inducing virus expression in both lymphocyte and monocyte/macrophage HIV latently infected cells. Additionally, the combinatorial strategy presented herein could lead to a reduction in the concentrations of LRAs used in vivo, resulting in a diminution of adverse effects, limiting the local injuries, the toxicity, and the inflammation, making this combinations an attractive novel option for future clinical trials.

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BIFUNCTIONALIZED CARBOSILANE DENDRONS FOR THE PREVENTION OF HIV-1 INFECTION

S.MORENO[1,2], F. J. DE LA MATA[1], R. GÓMEZ[1], Mª A. MUÑOZ-FERNÁNDEZ[1,2,*]

[1]. Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain. Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain.Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.Spanish HIV HGM Biobank, Madrid, Spain.

[2]. Departamento de Química Inorgánica, Universidad de Alcalá, Campus Universitario, Alcalá de Henares, Madrid, Spain. CIBER-BBN, Madrid, Spain

Due to the increased number of new HIV infections, alternative prevention strategies based on the use of topical vaginally products to inhibit HIV-1 infection in women has been established. Topical microbicides are used vaginally and/or rectally, and they act at an earlier stage of HIV-1 infection.[1] In the literature, polyanionic dendrimers and copper complexes have shown interesting biological properties as antiviral agents.[2,3]

Therefore, new bifunctionalized carbosilane dendritic systems have been designed. The topology of these systems allows them to have anionic peripheral groups for a therapeutic action, but in addiction, an excellent chelating agent at the focal point, which forms extremely stable complexes with a large number of metal ions.[4]

Firstly, copper complexes were synthesized, and in vitro studies were performed to evaluate the safety, biocompatibility, anti-HIV ability and mechanism of these bifunctionalized carbosilane dendrons. All compounds have been not toxic at the studied concentrations up to 20 µM in PBMC and TZM.bl cells and have demonstrated potent and a broad-spectrum anti-HIV-1 activity *in vitro*. Several experiments were carried out to study the mechanism of action, finding that these systems act entry level, joining the virus, meaning a virucidal activity.

The next step in a near future will be introduce other metals as gadollinium or gallium and these systems be used in

image for "*in vivo*" applications, obtaining diagnosis systems.

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(PO 56)

HUMAN IMMUNODEFICIENCY VIRUS TYPE 2 REVERSERANSCRIPTASE FIDELITY OF DNA-DEPENDENT DNA SYNTHESIS

A. SEBASTIÁN-MARTÍN[1], M. ÁLVAREZ[1], L. MENÉNDEZ-ARIAS[1]

[1] *Centro de Biología Molecular Severo Ochoa (CSIC/UAM), Nicolás Cabrera 1, Campus de Cantoblanco, 28049, Madrid, Spain*

In retroviruses, the reverse transcriptase (RT) is the enzyme responsible for the replication of the viral genome. RTs synthesize double-stranded DNA using RNA and DNA as templates. Their error rates have been estimated around 10-4-10-5 nucleotide substitutions per replication cycle, and this could explain in part the large genetic variability of the human immunodeficiency virus (HIV). The fidelity of the HIV type 2 (HIV-2) RT has been much less studied, compared with the HIV-1 RT. Available data are limited to nucleotide incorporation assays carried out with a limited number of template/primers. The aim of this work is to purify a prototypic HIV-2 RT (derived from the ROD strain), and analyze its fidelity of DNA-dependent DNA synthesis in M13mp2 *lac*Z-based assays.

The HIV-2 ROD RT was expressed and purified using a plasmid that encoded the sequences of the RT p68 subunit and the HIV-2 protease. Using this construction, p68/p55 heterodimers with His6 tags in their C-termini were purified. The fidelity was determined with a forward mutation assay, in which the M13mp2 phage genome lacking one strand of the *lac*Zα gene ("gapped DNA") was used as substrate of a gap-filling reaction. *Escherichia coli* MC1061 were electroporated with the product of the reaction and grown in M9 plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and isopropyl-1-thio-β-D-galactopyranoside, with *E. coli* CSH50 lawn cells. Mutants containing errors made by the RT while copying the *lac*Zα region of the M13mp2 DNA were identified as pale or colorless plaques and analyzed by nucleotide sequencing. For comparative purposes, mutant frequencies and error rates were determined in parallel for the HIV-1 BH10 RT.

The M13mp2-based assays revealed similar mutant frequencies for the HIV-2 ROD RT (1.26 x 10-2) and the HIV-1 BH10 RT (2.02 x 10-2). The slightly increased accuracy of the ROD RT resulted from a lower base substitution error rate, despite its higher tendency to introduce frameshifts. Mutational spectra of ROD and BH10 RTs showed common hot spots, such as the one located at position +88, and others found only in the mutational spectra of one of the enzymes. Hot spots in the HIV-2 ROD RT spectrum appeared in clusters (e.g. from positions +75 to +90, and +144 to +151) or at specific sites (e.g. positions +115 or +130).

Our results showed a similar accuracy for HIV-2 and HIV-1 RTs in DNA-dependent DNA synthesis reactions. Future studies will be focused on the analysis of fidelity while copying RNA templates.

(PO 57)

REDUCED CELL-ASSOCIATED HTLV-2 DNA IN ANTIRETROVIRAL TREATED HIV-1-HCV-COINFECTED PATIENTS WHO EITHER RECEIVED INTERFERON-A/RIBAVIRIN-BASED HEPATITIS C THERAPY OR HAD SPONTANEOUS HCV RNA CLEARANCE

ABAD-FERNÁNDEZ M, DRONDA F, MORENO A, CASADO JL, PÉREZ-ELÍAS MJ, QUEREDA C, MORENO S, **VALLEJO A**.

Department of Infectious Diseases, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Universitario Ramón y Cajal, Madrid, Spain.

Until recently, the standard treatment for hepatitis C virus (HCV) infection consisted mainly of a combination of interferon α (IFN-α) and ribavirin (RBV). It has been reported that IFN-α is effective in reducing HIV-1 RNA loads in naïve patients and in patients receiving antiretroviral treatment (ART). Also, a reduction of integrated HIV-1 DNA in CD4 T cells has been reported in two different works. One analyzed HIV-1-infected patients (not infected with HCV) who interrupted suppressive ART to receive treatment with IFN-α, and the other one analyzed HIV-1/HCV-coinfected patients under ART who received IFN-α and ribavirin as HCV treatment.

Patients and methods: We analyzed the level of cell-associated HTLV-2 DNA in i) 37 patients with HCV infection who had never received pegylated interferon alpha (IFNα)-based HCV treatment (HCV patients); ii) 15 patients who received pegylated interferon alpha (IFN-α) 2a or 2b combined with weight-based ribavirin, 10 of them with sustained virologic response (SVR), two patients who relapsed, and three patients who did not respond (IFN patients); and iii) 9 patients who had spontaneous HCV RNA clearance (SHC patients). Total cell-associatedHTLV-2 DNA was quantified by in-house real-time PCR. We also analyzed other immune factors, including T cell immune activation and plasma IL-6 levels.

Results: Either IFN patients or SHC patients had lower level of cell-associatedHTLV-2 DNA compared to HCV patients (p=0.022 and p=0.040, respectively). CD8 percentage and had received IFN-based treatment or had HCV clearance were independently associated to cell-associated HTLV-2 DNA. Immune activation and IL-6 level were higher in HCV patients.

Discussion: Our data indicate that patients treated with IFN-α have lower total cell-associated HTLV-2 DNA. On one hand, this observed effect reflects changes after HCV treatment and do not reflects a general decline of HTLV-2 DNA since HTLV proviral load is very stable over time. On the other hand, this work pointed out the importance of HCV infection upon the level of cell-associated HTLV-2 DNA, since patients with no HCV infection, due to spontaneous HCV RNA clearance had also lower level of cell-associated HTLV-2 DNA.

(PO 58)

DEVELOPMENT OF A NOVEL METHOD FOR TAXONOMIC CLUSTERING AND VISUALIZATION OF VIRAL GENOMIC SEQUENCES

S. DELGADO[1], F. MORÁN[2], A. MORA[3,4], J.J. MERELO[3,4], C. BRIONES[5,6]

[1].*Departamento de Sistemas Informáticos, Universidad Politécnica (UPM), Madrid, Spain.*

[2]. *Departamento de Bioquímica y Biología Molecular I, Universidad Complutense (UCM), Madrid, Spain.*

[3].*Departamento de Arquitectura y Tecnología de Computadores, Universidad de Granada (UGR), Granada, Spain.*

[4].*CITIC, Campanillas, Málaga, Spain.*

[5].*Departamento de Evolución Molecular, Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid, Spain.*

[6].*Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Spain.*

Over the last decade, the advent of next generation sequencing techniques has dramatically increased the amount of sequence data stored in databases. As a consequence, fast algorithms and classification methods are required to convert the growing number of nucleotide sequences into useful information (e.g., identification of data classes and clusters, characterization of relevant features or hidden relationships among data, etc.). Self-Organizing Map (SOM) is an unsupervised method used for clustering high-dimensional data without a previous knowledge of the class to which they belong (1). Also, SOM spatially orders the data on a two-dimensional map, what provides not only the clustering but a quantitative value of the similarity among the different groups. This fact could be very useful for clustering and organizing large databases of viral sequences, as well as for quickly and accurately classifying newly obtained data.

To process sequence data by SOM-based algorithms, DNA or RNA sequences must be previously transformed to fixed-size numeric vectors. Therefore, novel representation methods are required for automatically and bijectively transform aligned nucleotide sequences into numeric vectors, dealing with both nucleotide ambiguity and the presence of gaps derived from sequence alignment. We have developed a new DNA and RNA codification method based on Euclidean space, which has been tested using two SOM models: the classical Kohonen's SOM and Growing Cell Structures (GCS). The former is known for its usefulness in graphical exploratory data analysis (1) and, in turn, the latter produces better clustering results due to its flexible architecture (2).

A dataset composed of 44 complete sequences of the RT region of the HIV-1 *pol* gene belonging to the three phylogenetic groups of this virus (M, O and N), and to all the subtypes within the group M (A, B, C, D, F, G, H, J, K), has been used to test the developed codification method. The algorithm has revealed that the most important factor affecting the accuracy of the sequence clustering is the assignment of an extra weight to the presence of alignment-derived gaps. Our results show that clustering of HIV-1 sequences is quick and straightforward, and the retrieved classification is in agreement with traditional sequence-based phylogenetic reconstructions (3). This suggests a broad

applicability of such a novel codification method in different fields of virology.

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2. Fritzke (1994). Neural Networks, 7, 1441.

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(PO 59)

VIRAL EVOLUTION IN A HIV-1 DUAL LTNP INFECTED PATIENT

A. MOYANO[1], N. PEDREÑO[1], T. ÁLVARO[1], C. CASADO[1], I. OLIVARES, R. FUENTES [1], C. RODRIGUEZ[2], J. ROMERO[2], C. LÓPEZ GALÍNDEZ[1] AND M. PERNAS[1].

1. Servicio de Virología Molecular, CNM, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

2. Centro Sanitario Sandoval (CSS). Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid 28010, Spain.

Background. Long term controller patients (LTNP) constitute a group of HIV-1 infected patients without clinical symptoms for more than 10 years without antiviral therapy. Dual infection (DI), infection by two or more different viral strains in LTNP has been described only in a few sporadic cases and there are no studies concerning viral evolution in this group of patients. In this work, we analyse the viral envelope gene evolution in a DI LTNP and its relation with clinical and immunological parameters.

Methods. Samples from a LTNP dual infected patient were taken between 17-28 years after the first HIV-1 positive**.** In order to study viral evolution**,** proviral DNA was amplified by limiting dilution nested PCR in the c2-v5 region of *env* gene. Clinical parameters were compared with the diversity of the quasispecies in the different samples during follow-up. Fluctuations of different viral populations were also analyzed. HIV-1 was isolated by co-culture in different samples and its replicative capacity was measured in TZMbl cells. The relation between the replicative capacity and viral evolution, calculated as distance to the most recent common ancestor (MRCA), was estimated.

Result. Viral diversity in the quasispecies fromthe samples taken during the follow up was related to clinical parameters. We observed a statistical significance ($p<0,5$) in the relation between the increase in viral diversity and years after diagnosis and a decrease in the % of CD4+ T cells in relation with diversity ($p<0,5$). No relation between viral diversity and viral load and % CD8+T cells were observed. The replicative capacity of recombinant viruses with envelopes from the patient virus was related with the distance to the common ancestor but no relation was observed.

We studied the fluctuation of the different viruses and viral populations with time. There was a fluctuation in the presence of the populations of the two viruses in the patient. We detected that the viral strains “a” decrease along the study, while the second strain “b” became predominant.

Conclusions. These results show the presence of the different patterns of evolution of the two strains in a DI infected LTNP patient, and an increase in viral diversity which could be correlated with clinical markers**.**

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SORAPHEN A: A NATURAL PRODUCT FROM MYXOBACTERIA THAT INHIBITS HIV THROUGH BLOCKING HOST FATTY ACID SYNTHESIS

E. FLETA-SORIANO[1], C. LORCA-ORÓ[2], G. MIRAMBEAU[2], C. LOPEZ-IGLESIAS, G. KOUTSOUDAKIS[4], J. DIEZ[4], M. BRÖNSTRUP[5], J. P. MARTINEZ[1] & A. MEYERHANS[1,6]

[1]*Infection Biology Group, DCEXS, Universitat Pompeu Fabra, Barcelona, Spain*

[2]*AIDS Research group, IDIBAPS, Barcelona, Spain*

[3]*Cryo-Electron Microscopy Unit, CCiTUB, Universitat de Barcelona, Barcelona, Spain*

[4]*Molecular Virology Group, DCEXS, Universitat Pompeu Fabra, Barcelona, Spain*

[5]*Department of Chemical Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany*

[6]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

Introduction: Human Immunodeficiency Virus (HIV) infections continue to threaten global human health. They require life-long treatment with a combination of antiviral drugs. Co- infections with the hepatitis C virus (HCV) add a further level of complexity as HCV-specific drugs have to be given in addition leading to intricate drug-drug interactions. Targeting shared host factors involved in the replicative processes of both viruses could simplify co-infection treatments. Soraphen A (SorA) is a myxobacterial metabolite that inhibits the host acetyl-CoA carboxylase, a key enzyme in fatty acid biosynthesis. The anti-HIV activity of SorA was first identified in a high-throughput screen of myxobacterial metabolites. Recently, we have also described the SorA-mediated inhibition of HCV. The aim of this study was to determine the mechanism by which SorA inhibits HIV.

Methods*:* The anti-HIV effective concentration 50 (EC_{50}) and the cellular cytotoxicity 50 (CC50) of SorA were determined by titration using a TZM-bl cell infection assay. HIV production was analyzed by measuring p24 in the supernatant or by immunofluorescence. EGFP-Gag assembly and HIV maturation were detected with confocal and transmission electron microscopy, respectively. Viral supernatants from latently infected ACH2 cells with or without SorA were analyzed for infectivity, p24 and gp120 content, and viral-RNA. HIV binding to CD4 and HIV cell fusion was tested to analyze the entry capacity of viruses produced from SorA-treated cells.

Results*:* SorA inhibits mainly late steps of HIV-1 *in vitro* with an EC_{50} between 0.14 and 1.8 µM. Neither Gag assembly nor HIV maturation were inhibited by SorA as shown by confocal detection of gag assembly-spots and virus particle inspection with transmission electron microscopy. Rather the amount of HIV envelope proteins per particle were reduced. This correlated with a reduction of virus host- cell fusion.

Conclusions*:* SorA inhibits HIV by altering the composition of virus produced in cells treated with the drug. Together with its anti-HCV activity, SorA is an interesting candidate for HIV-HCV co-infection treatments.

(PO 61)

SORAPHEN A: A BROAD-SPECTRUM ANTIVIRAL NATURAL PRODUCT WITH POTENT ANTI-HEPATITIS C VIRUS ACTIVITY

G. KOUTSOUDAKIS[1], I. ROMERO-BREY[2*], C. BERGER[2*], G. PÉREZ-VILARÓ[1], P. MONTEIRO PERIN[3,4], F. W. RUDOLF VONDRAN[4,5], M. KALESSE[6], K. HARMROLFS[7], R. MÜLLER[7], J.P. MARTINEZ[8], T. PIETSCHMANN[3], R. BARTENSCHLAGER[2,9], M. BRÖNSTRUP[6], A. MEYERHANS[8,10], J. DÍEZ[1]

[1]*Molecular Virology Group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.*

[2]*Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany.*

[3]*TWINCORE - Institute of Experimental Virology, Centre for Experimental and Clinical Infection Research, Hannover, Germany.*

[4]*German Centre for Infection Research (DZIF), partner site Hannover-Braunschweig, Germany.*

[5]*ReMediES, Department of General, Visceral and Transplantation Surgery, German Centre for Infection Research Hannover Medical School, Hannover, Germany.*

[6]*Department of Chemical Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany.*

[7]*Helmholtz Institut für Pharmazeutische Forschung Saarland, Saarbrücken, Germany.*

[8]*Infection Biology Group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.*

[9]*German Centre for Infection Research (DZIF), partner site Heidelberg, Germany.*

[10]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

**equally contributed to this work*

Background & Aims: Co-infections by the hepatitis C virus (HCV) and human immunodeficiency virus (HIV) represent a significant challenge for treatment due to the necessity of combining diverse antiviral compounds that often leads to complex drug-drug interactions. Soraphen A (SorA) is a myxobacterial metabolite that inhibits the acetyl-CoA carboxylase, a key enzyme in lipid biosynthesis. We have previously identified SorA to efficiently inhibit HIV. The aim of the present study was to evaluate the capacity of SorA and analogues to inhibit HCV infection.

Methods: SorA inhibition capacity was evaluated *in vitro* using cell-culture derived HCV, HCV pseudoparticles and subgenomic replicons. Infection studies were performed in the hepatoma cell line Huh7/Scr and in primary human hepatocytes. The effects of SorA on membranous web formation were analyzed by electron microscopy.

Results: SorA potently inhibits HCV infection at nanomolar concentrations. Obtained EC_{50} values were 0.70 nM with a HCV reporter genome, 2.30 nM with wild-type HCV and 2.52 nM with subgenomic HCV replicons. SorA neither inhibited HCV RNA translation nor HCV entry, as demonstrated with subgenomic HCV replicons and HCV pseudoparticles, suggesting an effect on HCV replication. Consistent with this, evidence was obtained that SorA interferes with formation of the membranous web, the site of HCV replication. Finally, a series of natural and synthetic SorA analogues helped to establish a first structure-activity relationship.

Conclusions: SorA has a very potent anti-HCV activity. Since it also inhibits HIV, SorA is a promising candidate for the

development of simplified treatments of HCV/HIV co-infection.

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MAPPING THE TRANSCRIPTION INITIATION SITES IN THE *IN VIVO* RNA CONFORMATIONS OF BOTH POLARITY STRANDS OF EGGPLANT LATENT VIROID

A. LÓPEZ-CARRASCO[1], S. GAGO[2], R. FLORES[1], S. DELGADO[1]

[1]*Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia, Valencia, Spain*

[2]*Leibniz Institut für Pflanzenbiochemie, Halle (Saale), Germany*

Having a genome just composed by a small (250-400 nt) circular RNA without protein-coding ability, viroids depend on sequence/structure motifs that are recognized by the host proteins mediating their infectious cycle (replication, movement and suppression of defensive responses). These motifs are integrated in the compact secondary structures that viroid RNAs adopt as a consequence of their extensive self-complementarity, in which double-stranded segments are flanked by loops usually stabilized by arrays of non-canonic interactions. Viroid RNA structure can be essentially tackled: i) *in silico,* with algorithms searching the most stable conformations, ii) *in vitro,* by RNase and bisulphite probing in aqueous solutions and, more recently, by SHAPE ("selective 2'-hydroxyl acylation analyzed by primer extension"), and iii) *in vivo,* by identifying either natural covariations that preserve the double-stranded segments or substitutions that leave unaffected the loop shape according to isostericity matrices predicting recurrent three-dimensional motifs more conserved in structure than in sequence. In the present work we have applied these approaches to eggplant latent viroid (ELVd), of the family *Avsunviroidae* encompassing viroids with hammerhead ribozymes that replicate in plastids (mostly chloroplasts). Data from the three approaches are consistent and indicate that (+) and (-) genomic RNAs fold into rod-like conformations with a bifurcation at both termini. These two conformations, even if similar, are not identical as revealed by their different electrophoretic mobility in non-denaturing polyacrylamide gels. One functional aspect strongly depending on the structure of viroid RNAs is transcription, which in the family *Avsunviroidae* is catalyzed by a nuclear encoded polymerase (NEP) translocated into chloroplasts. Moreover, since RNA folds cotranscriptionally, the initiation sites of the nascent RNA strands may influence the adoption of metastable, although functionally relevant structures, like the hammerhead ribozymes that mediate self-cleaving of the oligomeric RNA intermediates generated in replication through a symmetric rolling circle mechanism. Applying RLM-RACE ("RNA ligase mediated-rapid amplification of cDNA ends") and primer extension methodologies to ELVd RNAs isolated from infected tissue, we have also determined in the present work the *in vivo* initiation sites for both ELVd strands. Within the conformations that these genomic RNAs adopt *in vivo*, the initiation sites map at positions close to one of the two bifurcations, which might serve to recruit

the NEP or some associated transcription factor.

(PO 63)

LOG-INCREASING THE ABILITY TO SENSE HUMAN THROMBIN RECEPTOR ANTIBODIES USING RECOMBINANT ELONGATED FLEXUOUS PLANT-MADE VIRUS-LIKE PARTICLES

I. GONZÁLEZ GAMBOA, P. MANRIQUE, M. SÁEZ, F. SÁNCHEZ, F. PONZ.

Centro de Biotecnología y Genómica de Plantas (CBGP; UPM-INIA). Pozuelo de Alarcón (Madrid). Spain

Plant Viral Nanoparticles (VNPs) have been recently exploited in nanobiotechnology for multiple applications, and their physical and biological characterization is also being actively carried out. In many cases, plant VNPs present characteristics quite advantageous in comparison with animal or bacterium viruses. Most advances in the deployment of plant VNPs have been performed on viruses with icosahedral virions (CPMV, CCMV), or elongated rigid rods (TMV). Flexuous elongated VNPs (family *Flexiviridae* or *Potyviridae*, for instance) offer some specific traits worth exploring, but have hitherto received less attention comparatively.

We are developing the potyvirus *Turnip mosaic virus* (TuMV) as a source of VNPs with different purposes. Displaying foreign peptides genetically fused to the N-terminus of the structural viral coat protein (CP) in infectious virus constructs, allowed to show log-increases in peptide immunogenicity and the ability of the recombinant virus to increase its ability to be used as a peptide antibody sensor, also at the log-level. However, we also found that some foreign peptides severely impaired the ability of the recombinant virus to infect host plants. To overcome this problem, we have approached the production of recombinant VNPs of the VLP type, trough transient high-level expression of the recombinant CP in plants.

Since no previous production of TuMV VLPs has been reported in biofactory plants, we undertook the expression of non-modified viral CP and of a recombinant CP fused to a peptide strongly interfering with virus infection. This was achieved in *Nicotiana benthamiana* plants agroinfiltrated with *Agrobacterium tumefaciens* bearing high-expression vectors of the pEAQ family. As a proof-of-concept peptide we selected one derived from the human thrombin receptor (TR), whose ability to impair virus infection was assessed. High level expressions of both CP forms were achieved in plant leaves. The CPs assembled into VLPs detected under the electron microscope. The ability of the recombinant TR-VLP to log-increase the sensitivity to sense antibodies specific to the TR-derived peptide was confirmed. These results allow overcoming the biological restriction imposed by peptides interfering with virus infectivity, opening the door to the exploitation of plant-made flexuous elongated VNPs devoid of infectious nucleic acids.

(PO 64)

ECO-EVOLUTIONARY DYNAMICS OF PEPINO MOSAIC VIRUS IN TOMATO PLANTS

P. GÓMEZ[1*], M. JUÁREZ[2], M.A. SÁNCHEZ-PINA[1], J.M. GARCÍA-VILLALBA[1], C. ALCAIDE[1] AND M.A. ARANDA[1]

[1] *Centro de Edafología y Biología Aplicada del Segura (CEBAS)- CSIC, Departamento de Biología del Estrés y Patología Vegetal, PO Box 164, 30100 Espinardo, Murcia, Spain*

[2] *Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche, Ctra. de Beniel km 3.2, 03312 Orihuela, Alicante, Spain*

** Correspondence: pglopez@cebas.csic.es*

While individual plants are often infected in nature with more than one related or unrelated virus, the extent to which mixed infections can modulate the evolutionary dynamics of these viruses is unclear. *Pepino mosaic virus* (PepMV) is an emerging RNA virus known to be one of the most important tomato pathogens worldwide. Phylogenetic analyses showed that PepMV populations in Spain were composed of isolates of two types (PepMV-CH2 and PepMV-EU), and here, we show that they appear to be still co-circulating after 10 years from their first detection with high prevalence and genetic variability. We then examined how viral interaction among both PepMV types and also between PepMV and other important RNA viral pathogen of tomato (i.e., *Cucumber mosaic virus*, CMV), could affect their evolutionary dynamics. We found that an antagonistic interaction among both PepMV types may explain mixed-infections prevalence, and this interaction was neither host-nonspecific nor affected by the presence of CMV. These results suggest a close relationship between both PepMV types that face a strong interference competition. Furthermore, our microscopical preliminary resultsbased on RNA in situ hybridization indicate that these viruses are able to infect the same cell types, and combining ultra-structural microscopy with biological analyses *in planta* we will identify whether this antagonistic interaction comprises a direct competition within the same infected cell for common plant and/or viral resources. Our results suggest that beyond the epidemiological circumstances that initiate epidemics, viral interactions by mixed infections in plants could profoundly impact the outcome of viral diseases.

(PO 65)

THE *POTYVIRIDAE* P1a LEADER PROTEASE CONTRIBUTES TO HOST RANGE SPECIFICITY

H. SHAN[1], F. PASIN[1], A. VALLI[2] , C. CASTILLO[3], C. RAJULU[1], A. CARBONELL[4], C. SIMÓN-MATEO[1], J A. GARCÍA[1], B. RODAMILANS[1].

[1].*Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología (CNB-CSIC), Darwin 3,Madrid, Spain*

[2]. *Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK*

[3].*Laboratorio de Biotecnología y Desarrollo, Instituto Nacional de Higiene "Rafael Rangel", Caracas, Venezuela*

[4]. *Donald Danforth Plant Science Center, St. Louis, MO 63132, USA*

The P1a protein of the ipomovirus *Cucumber vein yellowing virus* is a serine protease phylogenetically related to potyviral P1. It is located at the N-terminal end of the polyprotein and requires an

unknown host factor for its proteolityc activity; this might be related to host specificity. To help elucidate the role of P1a cleavage in host range definition, a series of constructs and chimeric viruses were built. In this work we demonstrate that separation of P1a from the polyprotein is essential for viral RNA silencing suppression and infection. We also show that this separation is host dependent. These findings support the role of viral proteases as important factors of host adaptation.

(PO 66)

A FACTOR COOPERATING WITH HCpro IN THE STABILIZATION OF ITS COGNATE CAPSID PROTEIN IS PLACED IN THE P3-6K1 CODING REGION

A. GALLO GALLARDO[1], J.J. PÉREZ[1], A. VALLI[2] AND J.A. GARCÍA[1].

[1]. Centro Nacional de Biotecnología, Campus de la Universidad Autónoma de Madrid, Madrid, Spain.

[2]. Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, United Kingdom.

Plum pox virus (PPV) is a member of the genus *Potyvirus* (*Potyviridae* family). The helper component proteinase (HCPro) of potyviruses is a multifunctional protein that is involved in diverse steps of the viral infection, such as, polyprotein processing, aphid transmission, and suppression of host antiviral RNA silencing. Recently, it has been described a new function of HCPro by which this viral factor enhances the stability of its cognate capsid protein (CP) and the yield of virus particles. However, when PPV CP is expressed in plants from an mRNA lacking other viral coding sequences, it is less stable upon in vitro incubation than when it is expressed from a polycistronic RNA encoding other viral proteins, and it can not be stabilize efficiently by PPV HCPro. These results indicate that the effect of HCPro in the stabilization of CP is more pronounced with the cooperation of other viral proteins and/or the corresponding RNA. In this work, we have analyzed different deleted forms of the PPV RNA in search for a viral factor that contributes to CP stabilization. As result, we have identified the factor that cooperates with HCPro in the region encoding the proteins P3, P3N-PIPO and 6K1.

(PO 67)

DISSECTING THE MULTIPLE ROLES OF PEPINO MOSAIC VIRUS CAPSID PROTEIN

F.E. MÉNDEZ[1], L. RODRÍGUEZ-MORENO[1], R.N. SEMPERE[1], M.A. SÁNCHEZ-PINA[1], M. VALLE[2] AND M.A. ARANDA[1]

[1]. Dpto. de Biología del Estrés y Patología Vegetal, CEBAS-CSIC, Murcia, Spain

[2]. Structural Biology Unit, CIC bioGUNE, Derio, Spain

Pepino mosaic virus (PepMV; genus *Potexvirus,* family *Alphaflexiviridae*) is a widespread plant virus that causes a major disease in tomato crops worldwide. The PepMV genome consists of a single stranded RNA of approximately 6.4 kb containing five open reading frames, including a replicase gene, a triple gene block (TGB) encoding TGBp1, TGBp2 and TGBp3, and a coat protein (CP) gene. Apart for its structural role, the PepMV CP is known to be the elicitor of the *Rx* resistance, to modulate the nature and severity of PepMV-induced symptoms, to be required for virus cell-to-cell movement

and to be an RNA silencing suppressor. To have a better understanding of the mechanisms underlying PepMV CP multiple roles, we are following a double approach. On the one hand, we are characterising CP single mutants in relation to their ability to sustain PepMV cell-to-cell movement and viral particle formation and/or stability. On the other hand, we have carried out genetic (Y2H, yeast-two-hybrid) and biochemical (affinity chromatography) screenings to identify tomato proteins that interact with PepMV CP. For the Y2H, a PepMV-infected tomato cDNA normalized library was built and screened against the CP, providing three different interacting proteins in addition to the CP itself: a Glutathione-S-transferase (GST), a Receptor-like serine/threonine kinase (STK) and a Ribosomal protein L13 (L13). Using the commercial One-Strep-tag fused to the CP of a PepMV agroinfectious clone, following affinity chromatography with tomato and *N. benthamiana* extracts, an additional interacting protein, the Heat shock cognate 70.3 (Hsc70.3), was identified. *In vivo* interactions were validated in *N. benthamiana* plants by bimolecular fluorescence complementation assay (BiFC) except for the STK/CP pair that did not produce yellow fluorescence emission. Finally, in order to identify a hypothetical role for each interactor in the viral infective cycle, all candidates were assessed in virus-induced gene silencing (VIGS) assays in tomato plants. Diverse phenotypes were observed for the different interactors, including repression of systemic PepMV accumulation.

(PO 68)

THE ROLE OF TRANSLESION POLYMERASES IN THE GENERATION OF GENETIC VARIABILITY OF AN EMERGENT ssDNA PLANT VIRUS

DÍAZ MARTÍNEZ, LUIS[1,2]; VIGUERA MÍNGUEZ[1], ENRIQUE; GRANDE PÉREZ, ANA[1,2]

[1] *Área de Genética, Universidad de Málaga, Campus de Teatinos, Málaga, Spain.*

[2] *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Málaga, Spain*

Single-stranded DNA (ssDNA) viruses such as animal circoviruses or plant geminiviruses are important emergent viruses. SsDNA viruses are as variable as their RNA equivalents and evolve quickly, with high mutation rates and mutation frequencies around 10-3-10-5 mutations/nt. Several factors are responsible for the elevated substitution rates of ssDNA viruses including polymerase replication fidelity, mismatch repair, exogenous and endogenous DNA damage, nucleotide imbalances and the action of other cellular DNA modifying enzymes. Indels can also be introduced during replication or as a consequence of recombination. Unlike RNA viruses, which owe their genetic variability in part to their error prone RNA-dependent RNA polymerases, ssDNA viruses do not encode DNA polymerases. They are replicated by unknown cellular DNA polymerases in the host nucleus via a rolling circle mechanism. Mutation bias compatible with the deamination and oxidation of single-tranded DNA has been observed in

geminiviruses. This kind of DNA damage is a substrate for Translesion Polymerases (TLS), involved in lesion bypass. TLS pols of the Y family lack proofreading activity and have low nucleotide selectivity, and thus exhibit high error rates for base substitutions and indels, even in the absence of damage. Here, we have addressed the involvement of TLS polymerases in the replication of the Tomato Yellow Leaf Curl Virus (TYLCV) geminivirus in *Arabidopsis thaliana*. To this end, wt *A. thaliana*, homozygous *AtRev1* and *AtPolK*, and heterozygous *AtPolH*TLS mutants were infected with TYLCV. TLS expression and viral loads were analysed at 7, 14, 21 and 28 days post infection (dpi). We observed an absence of AtRev1 transcripts, a significant reduction in AtPolK expression, while AtPolH was expressed at wt levels in the corresponding mutants. In each mutant, expression of the other TLS polymerases was unaffected. In addition, viral loads in *AtRev1*, *AtPolH*and *AtPolK*were comparable to those of wt *A. thaliana* TYLCV infections. Preliminary results of the effect of altered TLS levels on TYLCV variability measured by next eneration sequencing will be shown.

(PO 69)

ECOSYSTEM BIODIVERSITY AS A DETERMINANT OF PLANT VIRUS INFECTION RISK: PLANT SPECIES RICHNESS VERSUS COMMUNITY COMPOSITION

C. RODRÍGUEZ-NEVADO[1], M. GARCÍA-LOMANA[1], R. GAVILÁN[2], I. PAGÁN[1]

[1]. Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Madrid, Spain.

[2]. Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Spain.

Ecosystem biodiversity provides fundamental services for human welfare. It has been proposed that one of such services is the ability to reduce virus infection risk. According to this theory, the reduction in the number of species present in an ecosystem would increase the density of those that are virus hosts, resulting in higher virus prevalence. Experimental analyses have supported this prediction as often as not. This has led to hypothesize that other factors, such as the ecosystem composition (identity and relative abundance of species), may also determine virus infection risk. However, this hypothesis has been seldom analyzed, mainly due to the lack of well-characterized wild ecosystems.

To address this subject, we characterized the number, identity, and relative abundance of plant species in five locations of evergreen oak forests and five of riparian forests of the Iberian Peninsula; two ecosystems that account for 75% of the wild landscape in Spain. At each location, we analyzed the prevalence and host range of plant virus species of the genus *Potyvirus* in spring, summer and autumn of 2013-2014.

Our results indicated that in all seasons plant species richness was higher in riparian than in evergreen oak forests, with *Potyvirus* prevalence being also higher in the former than in the later ecosystem. This result is compatible with an effect of the number of host species in virus infection risk. Interestingly, in both ecosystems most host species were perennial, although community composition was evenly distributed

between perennial and annual plants. This suggests that host identity is also important in determining virus infection risk. Finally, within each ecosystem, virus prevalence across seasons correlated with the relative abundance of host species, even when the number of host species did not significantly change.

Hence, the present study, which is one of the first of its kind, provides evidence that not only plant species richness but also the identity and relative abundance of host species may be important determinants of virus infection risk.

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MIXED INFECTIONS OF POTYVIRUSES AND CRINIVIRUSES IN TWO PATHOSYSTEMS: SWEET POTATO AND CUCURBITS

A.B. MORENO, A. MINGOT, J.J. LÓPEZ-MOYA

Center for Research in Agricultural Genomics CRAG, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, Cerdanyola del Vallès, 08193-Barcelona, Spain

Plant-pathogenic viruses are responsible of relevant economic losses in agriculture worldwide. In addition to numerous individual diseases caused by a single viral infection, certain combinations of viruses are known to result in severe synergistic effects. A good example is the Sweet Potato Viral Disease (SPVD), caused by the simultaneous infection of host plants by the potyvirus *Sweet potato feathery mottle virus* (SPFMV) and the crinivirus *Sweet potato chlorotic stunt virus* (SPCSV). Metagenomics studies are revealing the frequent occurrence of mixed infections in plants that in many cases have remained unnoticed because no observable exacerbation of symptoms appear in the multiple-infected hosts.

A similar combination of two relevant viruses affecting cucurbits resembles the mentioned SPVD: the potyvirus *Watermelon mosaic virus* (WMV) and the crinivirus *Cucurbit yellow stunting disorder virus* (CYSDV) can coinfect the same host plants. The combined presence of the two viruses has been shown in field surveys, but a detailed evaluation of their interaction is missing. In particular, the putative effect of this mixed infection might be relevant in two essential processes of the pathogen cycles, like their natural dissemination by insect vectors (aphids and whiteflies for potyviruses and criniviruses, respectively), and their capacity to deal with the plant defence mechanisms, including RNA silencing responses.

The experimental approaches adopted to study mixed infections of WMV and CYSDV will be presented, including standardized inoculation methods with the natural insect vectors, and efficient molecular tools for viral load quantification in real time RT-PCR assays. For the controlled inoculation procedures, the use of double clip-on cage devices that allows easy transfer of insects from infected viral sources to test plants will be described. Finally, the comparison with the best-known SPVD case will include the divergences between the two pathosystems, like the presence of specific RNA silencing suppressors in the sweet potato infecting viruses, not found in the cucurbit-infecting counterparts. The purpose of the work is to gain a better knowledge on the combination of WMV and CYSDV, which could eventually result

in new management strategies and recommendations for reducing the damages caused by these viruses.

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INTERACTION BETWEEN A VIRAL COAT PROTEIN AND THE BHLH TRANSCRIPTION FACTOR ILR3 PROMOTES PLANT DEFENCE AND DROUGHT STRESS TOLERANCE IN NICOTIANA SPP.

F. APARICIO AND V. PALLÁS

Department of Molecular and Evolutionary Plant Virology.Instituto de Biología Molecular y Celular de Plantas (IBMCP) (UPV-CSIC). Ingeniero Fausto Elio s/n, 46022 Valencia, Spain.

Address correspondence to vpallas@ibmcp.upv.es; faparici@ibmcp.upv.es

During virus infection, the interaction of specific viral components with host factors elicits the transcriptional reprogramming of diverse cellular pathways. These alterations can lead to the development of disease symptoms, although recent evidence has revealed that some pathogenic viruses might benefit the plant host under specific extreme environmental conditions. The molecular bases of this phenomenon are, however, poorly understood. In this work we show that the coat protein of *Alfalfa mosaic virus* directly interacted with ILR3, a transcription factor (TF) belonging to the basic helix–loop–helix (bHLH) family which, in combination with other members of the family, has been proposed to participate in diverse metabolic pathways. Our findings indicate that in Nicotiana ssp this interaction interfered with the ILR3 subcellular location and that a fraction of this TF relocated from the nucleus to the nucleolus. This interaction also modulated its transcription activity since the expression of a member of the plant NEET proteins, which has been proposed to be regulated by ILR3, is reduced in infected plants. NEET down-regulation was associated with changes in reactive oxygen species production which, in turn, affected salicylic acid and ABA signalling pathways. Remarkably, infected plants showed increased ABA biosynthesis and higher water content under drought stress conditions. Our results establish a molecular link between the viral infection mechanism and host adaptation to specific extreme environmental conditions, and thus shed light on the mechanisms driving these beneficial interactions.

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INSIGHTS INTO VIROID RNA STRUCTURE AS REVEALED BY ATOMIC FORCE MICROSCOPY

M. MORENO[1], L. VÁZQUEZ[2], M.A. LÓPEZ-CARRASCO[3], J.A. MARTÍN-GAGO[1,2], R. FLORES[3], C. BRIONES[1,4]

[1]. *Department of Molecular Evolution, Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid, Spain*

[2]. *Instituto de Ciencia de Materiales de Madrid (CSIC), Cantoblanco, Madrid, Spain*

[3]. *Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain*

[4]. *Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas. (CIBERehd), Spain*

Viroids are small (250-400 nt), non-protein-coding, circular RNAs that depend on sequence/structure motifs for recruiting

the host plant proteins they need for replication, movement, and circumvention of defensive barriers (1, 2). Data derived from *in silico* and *in vitro* approaches, together with *in vivo* evidence in specific cases, support that viroid RNA genomes are largely self-complementary, folding up on themselves into collapsed secondary structures in which stretches of nucleotides forming Watson-Crick pairs are flanked by loops without apparent structure. However, sound evidence shows that complex arrays of non-canonical pairs stabilize such loops, in particular those appearing in the rod-like secondary structure characteristic of potato spindle tuber viroid (PSTVd) and most other members of the family *Pospiviroidae,* which are critical for replication in the nucleus and systemic trafficking. In contrast, members of the family *Avsunvioidae* like eggplant latent viroid (ELVd) and peach latent mosaic viroid (PLMVd), which replicate in plastids, adopt bifurcated or clearly multibranched conformations occasionally stabilized by kissing loop interactions required for viroid viability *in vivo.* However, data on the three-dimensional (3D) structure of viroid RNA genomes are still required.

To get a deeper insight into viroid structure, we have used atomic force microscopy (AFM) to analyze genomic viroid RNAs in native conditions. AFM is a nanotechnology-based tool particularly well suited for the structural characterization of a wide range of biological entities, including RNA molecules of different lengths, RNA-RNA and RNA-protein complexes (3). One of the main advantages of AFM over electron microscopy techniques is that it provides a 3D surface profile of the imaged sample without requiring any staining or coating, thus minimizing the structural disruption of the biological entity under study. This feature, together with its nanometer resolution, makes AFM an increasingly used technique in different fields of virology (4). Based on our previous optimization of AFM-based protocols for analyzing structured RNA molecules (5), we have been able to conduct a comparative structural analysis of three different viroid RNAs belonging to the families *Pospiviroidae* (PSTVd) and *Avsunvioidae* (ELVd and PLMVd) in different ionic conditions. The main results obtained will be presented.

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(PO 74)

EVOLUTION OF GII.4 NOROVIRUS VARIANTS IS DRIVEN BY IMMUNE PRESSURE AS DETERMINED BY MONOCLONAL ANTIBODY REACTIVITY PATTERNS

N. CARMONA VICENTE, S. VILA VICENT, J. RODRÍGUEZ DÍAZ, J. BUESA

Departamento de Microbiología, Facultad de Medicina, Universidad de Valencia, Avda. Blasco Ibáñez 17, 46010 Valencia, España

Human noroviruses are responsible for most epidemic outbreaks and many sporadic cases of acute gastroenteritis

worldwide. Noroviruses are also the main water- and foodborne viruses. In the last years, norovirus GII.4 has emerged as the most prevalent genotype. This high prevalence might be explained by several causes, including viral fitness and evolutionary mechanisms that promote the escape from the immune system.

The aim of the present work was to determine the ability of GII.4 noroviruses to escape the immune response during its evolution process. Due to the lack of a robust human norovirus replication 'in vitro' system we cloned and expressed several epidemic variants of norovirus GII.4 P particles in *E. coli*, including variants from 1996 to 2012. We also produced VLPs from the GII.4-2006b variant in the baculovirus expression system. Mice were immunized with VLPs from the 2006b variant. After fusion of splenocytes and clonal selection of hybridomas, the 3C3G3 monoclonal antibody was obtained. A rabbit was also immunized with the P particles of the VA 387 GII4 variant (1996) to obtain a rabbit polyclonal antiserum. The antibodies were characterized by ELISA and Western blot against the repertoire of P particles. The ability of these antibodies to block the binding of norovirus VLPs to their receptors was also assayed by ELISA.

Although the polyclonal antibody showed a wide recognition capability, differences in its reactivity towards the non-homologous P particles and the homologous P particles were found. More interestingly, the monoclonal antibody 3C3G3 was very effective recognizing its homologous P particles (GII.4-2006b) and the related GII.4-2008 P domain, but reacted very poorly against the earlier (1996) and newer variants (2012). Furthermore, this antibody showed a great blocking activity of viral binding to their receptors in saliva and in Caco-2 cells, suggesting that the epitope recognized by this antibody is associated to the receptor binding region of the virus.

These results demonstrate that the immune system drives the evolution of GII.4 noroviruses, selecting new epidemic variants that are able to escape from blocking antibodies. More interestingly, we show that both older and newer variants are not blocked by our monoclonal antibody, giving the opportunity to the re-emergence of older variants. This fast evolving profile of noroviruses to escape the immune system might also explain the poor protection observed after natural infections and the high rate of reinfections caused by noroviruses.

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ROLE OF VIMENTIN FILAMENTS IN VACCINIA VIRUS ASSEMBLY AND MATURATION

M.J. RODRÍGUEZ[a], J.CHICHÓN[a], S. GUTIÉRREZ-ERLANDSSON[c], J.J.CONESA[a], M.ESTEBAN[a], JOSÉ L. CARRASCOSA[a,b]

[a] *Department of Structure of Macromolecules, Centro Nacional de Biotecnología-CSIC (CNB-CSIC), Madrid, Spain*

[b] *Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia). Madrid, Spain.*

[c] *Servicio de Confocal, Centro Nacional de Biotecnología-CSIC (CNB-CSIC), Madrid, Spain.*

Vaccinia virus (VV) is one of the most studied members of the poxvirus family. The interaction of vaccinia with microtubules and actin cytoskeleton has been extensively studied. The involvement of vimentin intermediate filaments with

vaccinia virus as a support of the viroplasm foci of VV factories has also been previously suggested.

In our studies of cells infected with VV, the use of the steroidal lactone withaferin A, a potent vimentin inhibitor, has allowed us to demonstrate the crucial importance of this cytoskeletal intermediate filament in the establishment of the viral infection. We have combined confocal microscopy and the imaging of thin sections of cultured cells by transmission electron microscopy (TEM). These techniques allowed us to evaluate the role of vimentin in VV assembly and maturation both in the context of the whole cell and at an ultrastructural level. The confocal images show that, during the infection, a rearrangement of cytoskeletal vimentin intermediate filaments takes place around viral factories. The presence of vimentin bundles around the factories was confirmed by TEM using a specific embedding method of cell monolayers and serial sectioning, and it was further demonstrated by using vimentin-specific antibodies. In addition, the use of X-Ray tomography has solved the three dimensional cage of vimentin around the viral foci, in a near-to-native state of the infected cells. The inhibition of this vimentin network with withaferin A induced a significant reduction in the production of viral factories.

Our work demonstrates that the establishment of the viral factory requires an active role of the vimentin cytoskeleton, which must provide an scaffold to localize and concentrate viral components at the perinuclear site.

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EFFECT OF THE INTRODUCTION OF CHARGED RESIDUES AT THE DIMERIZATION INTERFACE OF FOOT AND MOUTH DISEASE VIRUS 3A PROTEIN

Y. A. VIEIRA, [1], F. SOBRINO, [1,2]; M. F. ROSAS, [1]

[1]*Centro de Biología Molecular Severo Ochoa, Madrid, Spain.*

[2]*Centro de Investigación en Sanidad Animal, INIA, Madrid, Spain.*

The FMDV non-structural 3A protein interacts with other viral and cellular proteins and can form intermolecular, antiparallel 3A dimers whose biological function is unknown. By homology with other picornavirus 3A proteins, the presence of two α-helices, located at residues 25-33 and 37-44, which form a dimerization interface is predicted. According to this model, the hydrophobic interactions established between the residues M29-L41, M29-I42 and L38-L38 contribute significantly to the stability of the dimer. Here we describe that replacements M29D, M29R, I42D and I42R, which result in acquisition of charged residues, did not significantly affect in vitro viral RNA translation and polyprotein processing or the cellular distribution and the ability to form dimers of transiently expressed 3A protein. However, preserving the hydrophobic character of residues 29 and 42 was shown essential for virus multiplication in cultured cells, since transfection with RNA of these mutants only allowed recovery of infectious viruses that selected amino acid replacements in the dimerization interface. Thus, following transfection of RNA with replacement

I42R, the substitution selected in the virus recovered, R42L, restored the hydrophobicity of the residue. Upon transfection with RNA containing replacement M29R the virus recovered selected first a mutation, I42L, in a residue predicted to interact with M29, being an additional substitution at the C-ter of the protein, S140F, observed after further growth of the virus.

(PO 77)

AFRICAN SWINE FEVER VIRUS INFECTS MACROPHAGES, THE NATURAL HOST CELLS, VIA CLATHRIN- AND CHOLESTEROL-DEPENDENT ENDOCYTOSIS

I. GALINDO[1], M.A. CUESTA-GEIJO[1], K. HLAVOVA[3], R. MUÑOZ-MORENO[2], L. BARRADO-GIL[1], J. DOMÍNGUEZ[1] AND C. ALONSO[1]

[1]*Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain*

[2] *Present address: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA*

3 Present address: Department of Immunology, Veterinary Research Institute, Brno, Czech Republic

The main cellular target for African swine fever virus (ASFV) is the porcine macrophage. Since early studies, it was known that ASFV entry in macrophages is mediated by saturable binding sites on the plasma membrane. However, the receptor/s for the virus is not yet characterized. This virus naturally replicates in porcine macrophages and monocytes and successful infection is linked to the expression of the CD163 scavenger receptor. In fact, cell lines used in previous entry studies, which were attributed to be of macrophage origin, lacked the characteristic expression profile.Then, we conducted our study after characterization of porcine macrophages obtained by alveolar lavage based on a set of specific surface markers including CD163 and CD169.

For efficient cell entry, animal viruses employ several strategies. Virus entry is a complex process in which virus particles should cross the cell membrane and to release their genome at the right location to complete efficient transcription and replication. In this study, we used chemical agents and molecular methods to investigate the cellular mechanism exploited for ASFV entry into the natural target cell and compared ASFV (including the ASFV adapted isolate Ba71V and a virulent field isolate (608 VR13) with low passage number in culture), with vaccinia virus (VV), which apparently involves different entry pathways. Our results show that ASFV uses endocytosis to infect host cells and takes advantage of several endocytic pathways to initiate infection. ASFV enters porcine macrophages by a dynamin-dependent endocytic pathway involving clathrin. The first steps of infection are also strongly pH-dependent. The presence of cholesterol in cellular membranes was found to be essential for a productive ASFV infection while actin-dependent endocytosis and the participation of phosphoinositide-3-kinase (PI3K) activity were other cellular factors required in the process of viral entry. These findings improved our understanding of the ASFV interactions with macrophages that allow for successful viral replication.

(PO 78)

GENERAL CHARACTERISTICS OF 100 PEDIATRIC PATIENTS WITH ACUTE RESPIRATORY INFECTION CAUSED BY BOCAVIRUS

J.REINA[1], A.IÑIGO[1], F.FERRÉS[2], V.LÓPEZ-COROMINAS[2]

[1]Virology Unit, Microbiology Service, [2]Pediatric Service. University Hospital Son Espases, Palma de Mallorca, Spain.

Introduction: Acute respiratory infections (ARI) of viral etiology are very frequent in children (<15 years) during the winter. Most are caused by RSV, adenovirus and influenza viruses. The Bocavirus have been implicated in this disease with varying frequency depending on the country.

Material and Methods: A prospective study (February 2013-February 2015) on this type of respiratory infections is presented. All patients with suspected IRA were taken a throat swab. The detection of bocavirus was performed by a commercial RT-PCR real time (Anyplex RV16; Seegen, South Korea).

Results: During the study period were analyzed 4,587 respiratory specimens, of which 2,243 (48.8%) were considered positive for respiratory viruses. We detected 100 cases of ARI caused by Bocavirus (2.1% of all processed samples and 4.4% of the positive samples). In 49 cases the bocavirus was detected as a single virus and 51 cases a mixed viral infection was detected, the main co-infecting virus were rhinovirus, RSV, adenovirus and influenza A. The 51% of cases were girls and 49% boys; the mean age of patients was 16.8 months (range 3 days-14 years). 69% of all cases occurred between the months of January (18%), February (37%) and March (14%), although cases were detected in every month of the year except June. The main symptoms were fever> 38 ° C (79%), cough (38%), cold symptoms (21%) and influenza-like symptoms (24%). The defintive clinical diagnoses were: 18% bronchiolitis, 15% pneumonia, and 11% bronchitis. 8% had some underlying disease, 3% were cancer, 2% premature and 2% asthmatics. 36 patients (58.4% female) required hospitalization with a mean age of 15.7 months. In these patients bocavirus detected exclusively in 55.5% of cases and as a mixed infection in the rest; the predominant virus in these cases was also rhinovirus. 44% of patients received antibiotic treatment (60% amoxiclin/clavulanic acid). No patient died directly or associated with the bocavirus infection.

Conclusions: The acute respiratory infections caused by bocavirus are not frequent (4.4%) which mainly affects the community infant population (<18 months). The predominant symptom fever and symptoms of upper respiratory tract.

(PO 79)

INFECTIOUS AETIOLOGY OF COMMUNITY ACQUIRED PNEUMONIA IN PAEDIATRIC HOSPITALIZED PATIENTS

M RODRÍGUEZ-DOMÍNGUEZ [1,2], C VÁZQUEZ [3], L MARTÍNEZ [1]; A COCA [3], D MARTÍN [4], A TAGARRO [5], R CANTÓN [1]; E OTHEO [3], JC GALÁN [1,2]

[1]Servicio de Microbiología. Hospital Universitario Ramón y Cajal. Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid.[2]Centro de Investigación Biomédica en Red en Salud Pública (CIBERESP). [3]Servicio de Pediatría. Hospital Universitario Ramón y Cajal. Universidad de Alcalá, Madrid. [4]Laboratorio de Microbiología. Laboratorio Central BR Salud. Hospital Universitario Infanta Sofía. San Sebastián de los Reyes, Madrid. [5]Servicio de Pediatría. Hospital Universitario Infanta Sofía. San Sebastián de los Reyes, Madrid.Spain.

BACKGROUND/OBJECTIVE; Community acquired pneumonia (CAP) is a major cause of morbidity in paediatric population in developed countries and an important cause of infant death in developing ones. Our aim was to define current bacterial and/or viral causes of CAP in paediatric patients admitted to two University Hospitals in Madrid.

METHODS: For two years (April 2012-April 2014), 66 patients (2months-17 years old), with clinical of lower respiratory tract infection and a radiographic pulmonary infiltrate or condensation, were studied with a large microbiological diagnostic yield. *Streptococcus pneumoniae* (Sp) PCR in blood and urinary antigen detection was performed at admission. Sixteen respiratory viruses were investigated by a multiplex PCR (Luminex xTAG RVP). Microbial agents associated to atypical pneumonia, *Mycoplasma pneumoniae* (Mp) and *Chlamydia pneumoniae*(Cp) were screened using serological and molecular approaches in plasma or in respiratory samples. Blood or pleural fluid cultures (when thoracocentesis was indicated) were also performed. Disease severity was evaluated according to clinical, analytical and outcome data.

RESULTS: An infectious agent could be associated to CAP in 52/66 (78%) patients Virus were detected in 49/66 patients (74 %), being more than one virus detected in nearly 40% (19/49). Rhinovirus was the most common virus (20/49, 40%), followed by adenovirus (8/49, 16%), RSV and metapneumovirus (MPV) (7/49, 14%, each). Isolated bacterial pathogens were only found in 5/66 (7.5%) cases. The microorganisms detected in these bacterial infections were 2 Sp, 2 Cp and 1 Mp. In 6/66 samples (9%) viral and bacterial agents were detected simultaneously. Interestingly, 4/5 infections caused by Mp were also associated with viral infection. Serological test used for Cp yielded IgM positive in 9 cases but seroconversion was not observed and only 2/9 were PCR positive for this pathogen. Regarding disease severity, mixed infections of virus-bacteria were significantly associated to complicated pneumonia (p=0,047). Among virus-virus coinfection, the presence of MPV was associated to a worse outcome (complicated pneumonia and need for oxygen-therapy > 4 days) (p=0,043)

CONCLUSIONS:

- Viruses should be considered as a major cause of CAP in paediatric population.

- Mixed infections are commonly found. Viral-bacterial and the presence of certain viruses could be related with a worse

outcome, although these items are not clear nowadays.

- Attending to bacterial agents were only identified in 16% patients, a fast diagnosis based on multiplexed PCR may reduce antibiotic overuse.

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ESTABLISHMENT AND REPLENISHMENT OF THE VIRAL RESERVOIR IN PERINATALLY HIV-1-INFECTED CHILDREN INITIATING VERY EARLY ANTIRETROVIRAL THERAPY

M. MARTÍNEZ-BONET[1], M.C. PUERTAS[2], C. FORTUNY[3], D. OUCHI[2], J. MELLADO[4], P. ROJO[5], A. NOGUERA-JULIÁN[3], M.A. MUÑOZ-FERNÁNDEZ[1,*], J MARTINEZ-PICADO [2,6,7,*]

[1] *Hospital General Universitario Gregorio Marañón, Madrid, Spain. Instituto de InvestigaciónSanitaria Gregorio Marañón, Madrid. Networking Research Center on Bioengineering,Biomaterials and Nanomedicine (CIBER-BBN). Spanish HIV HGM BioBank, Madrid, Spain*

[2] *AIDS Research Institute IrsiCaixa, Institut d'Investigació en Ciències de la Salut GermansTrias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain*

[3] *Unidad de Enfermedades Infecciosas, Servicio de Pediatría, Hospital Sant Joan de Déu,Universitat de Barcelona, Esplugues del Llobregat, Spain*

[4] *Servicio de Pediatría Hospitalaria y E. Infecciosas y Tropicales Pediátricas. Hospital Universitario Infantil LA PAZ- H. Carlos III, Madrid.*

[5] *Servicio de Pediatría. Hospital 12 de Octubre, Madrid, Spain*

[6] *Universitat de Vic – Universitat Central de Catalunya (UVic-UCC), Vic, Spain*

[7] *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain*

AIDS-related mortality in children has decreased significantly with the wide availability of combination antiretroviral therapy (cART). Initially, cART was strongly recommended only in infants with HIV-related symptoms. However, during recent years, multiple studies have suggested the benefit of starting early cART in all HIV-1-infected infants. Therefore, international guidelines are now recommending initiation of cART in all HIV-1-infected infants aged less than one year regardless of clinical and immunological conditions. Despite cART generally suppresses the replication of HIV-1, it does not cure the infection, because proviruses persist in stable latent reservoirs. In addition, it has been proposed that low-level proviral reservoirs might predict longer virologic control after discontinuation of treatment. Our objective was to evaluate the impact of very early initiation of cART and temporary treatment interruption on the size of the latent HIV-1 reservoir in vertically infected children. This retrospective study included 23 perinatally HIV-1-infected children who initiated very early treatment within 12 weeks after birth (n=14), or early treatment between week 12 and 1 year (n=9). We measured the proviral reservoir (CD4+ T-cell–associated HIV-1 DNA) in blood samples collected beyond the first year of sustained virologic suppression. We found a strong positive correlation between the time to initiation of cART and the size of the proviral reservoir. Children who initiated cART within the first 12 weeks of life showed a proviral reservoir sixfold smaller than children initiating cART beyond this time ($p<0.01$). Rapid virologic control after initiaton of cART also limits the size of the viral reservoir. However, patients who underwent transient treatment

interruptions showed a dramatic increase in the size of the viral reservoir after discontinuation. In summary, our study highlights the importance of very early initiation of cART, if possible within the first 12 weeks of life, and the benefit of optimal virologic control during the first years of life in order to limit the size of the viral reservoir. Moreover, this study suggests that treatment interruptions should be undertaken with caution, as they might lead to fast and irreversible replenishment of the viral reservoir.

(PO 81)

AGE DISTRIBUTION OF PEDIATRIC ACUTE RESPIRATORY INFECTIONS CAUSED BY RESPIRATORY SYNCYTIAL VIRUS

J.REINA[1], A.IÑIGO[1], F.FERRÉS[2], V.LÓPEZ-COROMINAS[2]

[1]Virology Unit, Microbiology Service, [2]Pediatric Service. University Hospital Son Espases, Palma de Mallorca, Spain.

Introduction: Acute respiratory infections (ARI) caused by RSV is the leading cause of this type of infection during the winter months, especially in the lower age at 2 years. Classically they preferably presented in children under 6 months and its spectrum is extended to 2 years old. In recent years we have detected a slight slip of the affected population to higher ages.

Material and Methods: From this observation a prospective study on the age distribution of ARI cases caused by RSV was conducted along epidemic seasons 2012/13, 2013/14 and 2014/15.

All patients attending the emergency department with a clinical compatible with ARI were taken a nasopharyngeal aspirate. The samples were subjected to the detection of different respiratory viruses by a commercial molecular amplification technique RT-PCR real time (Anyplex RV16, Seegen, South Korea).

Results: Over the three epidemic seasons we have detected 601 cases of acute respiratory infection caused by RSV. Although overall 83.2% of all cases occurred in children under two years, there have been differences in seasons with a possible trend towards older than this age.

Last season analyzed 78.6% of all cases occurred in children under 2 years compared to 83.3% and 87.3% of the previous two seasons. The 21.3% of patients with ARI caused by RSV in the 2014/2015 season had over 2 years of age. Also since last season has been observed, although not significant, increase in cases detected in patients over four years. The percentage of cases that occur in the first month of life, with 11.4% overall seems to have stabilized. This group together, that of children under 6 months (39.7%), represent 51.4% of all cases.

Conclusions: Studies of age distribution of RSV infections are very important to prevent the possibility of infection by vaccination of pregnant mothers. In our study, this type of vaccination would prevent hypothetically and with a vaccine efficacy of 100%, 51.4% of cases detected in the first six months of life, reducing by half the workload of this respiratory disease.

(PO 82)

EVIDENCE OF NOSOCOMIAL TRANSMISSION OF A ROTAVIRUS STRAIN TO TWO PREVIOUSLY VACCINATED INFANTS

P.F. SÁNCHEZ-LÓPEZ[1,2], C. SALVADOR GARCÍA[1], M. ITURRIZA-GÓMARA[3], K. JERE[3], S. VILA VICENT[4], J. BUESA GÓMEZ[4], M. SEGOVIA HERNÁNDEZ[1]

[1]. Hospital Universitario Virgen de la Arrixaca, Murcia, Spain

[2]. Servicio de Sanidad Ambiental. Dirección General de Salud Pública y Drogodependencias. Consejería de Sanidad y Política Social de la Región de Murcia, Spain

[3]. Institute of Infection and Global Health, University of Liverpool, UK

[4]. Universidad de Valencia, Spain

Contact: pedrof.sanchez2@carm.es

Nosocomial transmission of rotaviruses has been extensively documented. We describe the nosocomial transmission of a rotavirus strain to two previously vaccinated infants.

The first child was admitted to the University Hospital Virgen de la Arrixaca in Murcia, Spain, for asthma attack, and developed loose stools five days after admission. The child was discharged on eighth day post-admission. The second child was admitted to the hospital for eye swelling and fever, and was allocated the bed previously occupied by the first child two days ago. This second child started developing loose stools and vomiting six days post-admission, and was discharged after eight days in the hospital.

Both patients were positive for rotavirus antigen in stools by an immunochromatographic test (VIKIA® Rota-Adeno, bioMerieux), although both children were fully vaccinated with RotaTeq (Merck Sharp & Dohme BV). There was no known contact between them.

A genotyping assay by conventional RT-PCR, and subsequent semi-nested multiplex PCR was performed in stool samples from both children, G and P genotyping the strains, and partially amplifying 6 of the 11 fragments of the rotavirus genomic segments that encode the NSP1, NSP2, NSP4, VP4, VP6 and VP7 proteins. Forward and reverse amplicons were sequenced with Applied Biosystems BidDyeTM Fluorescent Terminator System, and the sequences were aligned using ClustalW. The partial sequences of the NSP1 (936 bp), NSP2 (930 bp), NSP4 (692 bp), VP4 (605 bp), VP6 (319 bp) and VP7 (836 bp) genomic fragments from both strains were analyzed using MEGA version 6. Genotypes were confirmed using the automated genotyping tool RotaC (htpp://rotac.regatools.be).

The genotype of both strains was G9P8. A 100% similarity between both strains was observed, except for the VP7 fragment, which differed in two nucleotides (99.76% similarity).

We conclude that both strains share a high degree of identity, and that the virus was probably transmitted within the hospital. The incubation time in both cases was relatively long, and rules out community acquisition. This strain caused disease in both children, despite the fact that RotaTeq contains the P8 type of the VP7 protein of the virus, suggesting a vaccine failure. Further studies are being conducted for fully characterize these strains, sequencing their whole genome.

This report highlights the risks of a nosocomial rotavirus transmission, even among vaccinated children. In the future, studies that also include monitoring asymptomatic shedding of rotavirus in the hospital environment, may be useful in order to assess the risk of transmission.

(PO 83)

FATAL MYOCARDITIS BY PARVOVIRUS B19 IN CHILDREN

J.M. CAPELO MÍGUEZ[1]; J. FONTENLA GARCÍA[1]; N. BALADO INSUNZA[1]; J. GARCÍA COSTA[1]; A. PÉREZ PEDROSA[1]; M. CABRERIZA[2]; J.E. ECHEVARRÍA MAYO[2].

[1]*Complejo Hospitalario Universitario de Ourense, Ourense, Spain*

[2]*ISCIII Majadahonda, Madrid, Spain*

Introduction: Cardiomyopathies are defined as"an acute or chronic inflammatory process in the absence of ischemia produced by a variety of toxins,drugs or infectious agents, in association with myocardial dysfunction,and confirmatory diagnosis established histopathological,histochemical and molecular criteria".

Incidence real of myocarditis in pediatric age is unknown because of its broad spectrum of clinical presentation.

Clinical suspicion is a challenge for the clinician due to the absence of a validated gold standard test for diagnosis,its insidious clinical presentation and its variable evolution.

Case Presentation

A 16month infant was admitted in the Intensive Care Unit with symptoms of24hours of evolution consisting on postration and vomiting.Exploration did not show erythema or exanthema.

Blood tests showed metabolic acidosis,leukocytosis and anemia,while chest x-ray showed an increased retrocardiac density and cardiothoracic ratio.Finally, sinus tachycardia and other signs consistent with right ventricular hypertrophy,together with Qwaves in DII were observed on the electrocardiogram.

After 6 hours,the patient started with a sharp respiratory deterioration with affectation of the overall state,hepatomegaly and impaired distal perfusion.

The echocardiogram showed a dilated left ventricle with impaired cardiac function,compatible with severe cardiogenic shock,followed by cardiorespiratory arrest requiring resuscitation maneuvers cardiopulmonary advanced without success.

Microscopic examination of the hearth tissue revealed intense areas of inflammatory lymphocytic infiltrates,with intense nuclear positivity.Parvovirus B19 DNA was amplified by PCR on myocardial tissue, as well as in ascetic, pleural and pericardial fluids.Inmunohistochemistry showed parvovirus B19antigens on endothelial cells of pericardial and epicardial vessels RT-PCR for human enteroviruses and parechoviruses were negative.All the findings were interpreted as indicative of multiorganic damage with signs of viral process of myocarditis by parvovirus B19.

Discussion and Conclusion: Myocarditis is a particularly important and heterogeneous entity in childhood. It is a

major cause of morbidity and mortality in children, being the major cause of unexplained sudden death.Parvovirus B19is currently the most common known etiologic agent of pediatric viral myocarditis.It affecting the coronary endothelium and causing dysfunction and myocardial ischemia.

The initial clinical diagnosis of myocarditis relied on the finding of lymphocytic infiltrate and myocardial degeneration,since there is no additional test diagnostic of noninvasive myocarditis.The pathologic examination confirmed the diagnosis of suspicion.The implication of B19DNA as the etiological agent was initially suggested by the positive PCR on myocardial tissue and fluids and confirmed by immunohistochemistry.The implication of other viruses associated with cardiac disease as enteroviruses was discarded.The initial clinical diagnosis is crucial to optimize the treatment and transfer of patients to a referral hospital with heart transplant program.

(PO 84)

RESPIRATORY SYNCYTIAL VIRUS: COINFECTIONS WITH RHINOVIRUS AND HUMAN BOCAVIRUS IN HOSPITALIZED INFANTS

C CALVO[1], ML GARCÍA-GARCÍA [1], F POZO [2], I CASAS[2].

[1] *Pediatrics Department.Severo Ochoa Hospital.Leganés. Madrid. Spain* [2]*Respiratory Virus and Influenza Unit. National Microbiology Center (ISCIII), Madrid, Spain.*

Background. It is not established if coinfections are more severe than single viral respiratory infections.

Objective. Studying and comparing simple infections and viral coinfections of respiratory syncytial virus (RSV) in hospitalized children.

Patients & Methods. From September 2005 to August 2013 a prospective study was conducted on children under 14, admitted with respiratory infection to the Pediatrics Department of the Severo Ochoa Hospital, in Spain. Specimens of nasopharyngeal aspirate were taken for virological study by using polymerase chain reaction, and clinical data was recorded. Simple RSV infections were selected and compared with double infections of RSV with rhinovirus (RV) or with human bocavirus (HBoV).

Results. 2993 episodes corresponding to 2525 children were analyzed. At least one virus was detected in 77% (2312) of the episodes. Single infections (599 RSV, 513 RV and 81 HBoV) were compared with 122 double infections RSV-RV and 61 RSV-HBoV. The RSV-RV coinfections had fever more often (63% vs. 43% p <0.001) (), and hypoxia (70% vs. 43%; p <0.001) than RV infections. Hypoxia was similar beween single or dual infectins (71%) . Bronchiolitis were more frequent in RSV simple group (p<0.001). The PICU admission was more common in RSV simple or RSV-RV group than in the RV monoinfection (p = 0.042).

Hospitalization was longer for both the RSV simple group and RSV-HBoV coinfection, about 1 day (4.7 vs 3.8 days, p <0.001) longer than in the simple HBoV infections. There were no differences in PICU

admission. RSV single group were of younger age than the other groups.

Conclusions: Coinfections between RSV-RV and RSV-HBoV are frequent. Overall viral coinfections do not provide greater severity, but have mixed clinical features.

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CLINICAL COMPARISON OF RESPIRATORY SYNCYTIAL VIRUS INFECTIONS SUBTYPE A VS SUBTYPE B IN HOSPITALIZED CHILDREN.

ML GARCÍA-GARCÍA[1], C CALVO[1], C LLORENTE[3], S DE BLÁS[3], F POZO [2], I CASAS[2].

[1] Pediatrics Department.Severo Ochoa Hospital.Leganés. Madrid. Spain [2]Respiratory Virus and Influenza Unit. National Microbiology Center (ISCIII), Madrid, Spain. [3]Estudiante de 6º Curso de Medicina. Universidad Alfonso X el Sabio. Madrid.

Background. Although respiratory syncytial virus (RSV) infections are the most important cause of hospitalization in infants and has been extensively studied, is not well established if subtype A or B are associated to different severity. Some authors consider than RSV B is more serious.

Objective. To evaluate the relationship between the severity of bronchiolitis and RSV subtype in hospitalized children.

Patients & Methods. From September 2005 to August 2014 a prospective study was conducted on children, admitted with respiratory infection to the Pediatrics Department of the Severo Ochoa Hospital, in Spain. Specimens of nasopharyngeal aspirate were taken for virological study by using polymerase chain reaction, and clinical data were recorded. Infections associated to RSV A and RSV B were selected and compared.

Results. 3278 episodes of viral respiratory infections were analyzed. RSV was detected in 1019 (31%); 619 (61%) cases were RSV A, 244 (24%) RSV B and 156 could not be typed. Infections were present mainly in November-January, the mean each was 1 year (median 6 months), and the most frequent diagnosis was bronchiolitis (57.4%). 586 (77%) of children had fever, and 611(70%) hypoxia. Infiltrate in Rx was present in 340 patients (49%). 35% of cases had a coinfection (mainly with rhinovirus). The hospitalization was of 4.7 $\pm$ 2.5 days. Only 23 (2.7%) infants needed PICU admission. We compared the clinical data of the total group, and also, the patients diagnosed of bronchiolitis, the patients diagnosed of recurrent wheezing and of pneumonia and we did not find any difference between the patients with RSV A or B. Single infections; 396 RSV A and 160 RSV B were also compared and no differences amongst them were detected.

Conclusions: Respiratory viral infections due to RSA virus in hospitalized children have no different clinical characteristics associated to type B or A. RSV B infections have not more severity.

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HUMAN BOCAVIRUS IN HOSPITALIZED CHILDREN AND COMPARISON WITH OTHER RESPIRATORY VIRUSES

C CALVO[1], ML GARCÍA-GARCÍA [1], D. CARBALLO[3], E MATÍNEZ-MONTESERÍN[3], F POZO [2], I CASAS[2].

[1] Pediatrics Department.Severo Ochoa Hospital.Leganés. Madrid. Spain [2]Respiratory Virus and Influenza Unit. National Microbiology Center (ISCIII), Madrid, Spain. [3]Estudiante de 6º Curso de Medicina. Universidad Alfonso X el Sabio. Madrid.

Background. The clinical characteristics of human bocavirus (hBoV) infections and its role are not yet well established.

Objective. Prospective study of infections associated to hBoV in hospitalized children and comparing simple infections with viral infections caused by the most frequent viruses; respiratory syncytial virus (RSV), rhinovirus (RV), and human metapneumovirus (hMPV) in the same population.

Patients & Methods. From September 2005 to August 2014 a prospective study was conducted on children under 14, admitted with respiratory infection to the Pediatrics Department of the Severo Ochoa Hospital, in Spain. Specimens of nasopharyngeal aspirate were taken for virological study by using polymerase chain reaction, and clinical data were recorded. Simple hBoV infections were selected and described. Single infections were compared with hBoV coinfections with other respiratory viruses and also with single RSV infections, RV infections and hMPV infections.

Results. 3275 episodes of viral respiratory infections were analyzed. At least one virus was detected in 76.5% (2504) of the episodes, 727 coinfections (22%). RSV and RV were the most frequent (31% each one). A total of 320 episodes were associated to hBoV (9.8%), 80 single infections, and 240 coinfections. Single hBoV infections were mainly in November and December (50%), in children of 24.7±24 months of age. 69% had fever, 52% hypoxia and 47% infiltrate in X-ray. Recurrent wheezing (60%) and pneumonia (22%) were the most common diagnoses. Coinfections of hBoV with other viruses had higher proportion of bronchiolitis 27.5% vs 15.5%, p<0.0001 and less pneumonia).

Single infections (665 RSV, 555 RV and 108 hMPV) were compared with single hBoV infections (80). RSV single infections affected younger children (9 vs 24 months, p<0,001), had more frequently hypoxia, (71% vs 52%, p<0.001), and the most frequent diagnosis was bronchiolitis (63%, vs 17%, p<0.001). Days of admission (0.002) at hospital and duration of hypoxia (0.04) was longer in RSV group. hBoV patients had more frequent pneumonia, higher C-reactive protein (p<0.001) and antibiotic treatment (41% vs 17%, p<0.001).

RV children had less frequently fever (69% vs 41%, p<0.001), and less pneumonia (11% vs 23%, p<0.001). hMPV children had also less proportion of pneumonia (8.4% vs 23%, p=0.14) and less CRP (35±41 vs 67 ±79, p=0.004) and leukocytosis (p=0.019).

Conclusions: HBoV infections are frequent in hospitalized children and associate to fever, pneumonia, increased CRP and antibiotic treatment. There are

significantly differences with other respiratory virus infections.

(PO 87)

SURVEILLANCE OF HUMAN G12P[8] ROTAVIRUS IN SPAIN

JAVIER BUESA[1], SUSANA VILA VICENT[1], MANUEL FERNÁNDEZ JIMÉNEZ[1], CRISTINA SANTISO BELLÓN[1], AINARA ARANA SALABERRÍA[2], MILAGROS MONTES[2],GUSTAVO CILLA[2], JOSEP PRAT FORNELLS[3], ROSA ESCOMS TRULLENQUE[3], ROSA BARTOLOMÉ COMAS[4], RAUL ORTÍZ DE LEJARAZU[5], JOSÉ M. EIROS BOUZA[5], CRISTINA SERAL GARCÍA[6], F. JAVIER CASTILLO GARCÍA[6],PEDRO F. SÁNCHEZ-LÓPEZ[7], CARME SALVADOR GARCÍA[8], JESÚS RODRÍGUEZ DÍAZ[1], MIREN ITURRIZA GÓMARA[9]

[1] *Dpto. de Microbiología, Facultad de Medicina, Universidad de Valencia, Spain*

[2] *Servicio de Microbiología,Hospital Universitario Donostia, San Sebastián, Spain*

[3] *Laboratorio de Microbiología, Hospital de Sagunto (Valencia), Spain*

[4] *Servicio de Microbiología, Hospital Val d'Hebrón, Barcelona, Spain*

[5] *Depto. de Microbiología, Facultad de Medicina, Universidad de Valladolid, Spain*

[6] *Servicio de Microbiología, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain*

[7] *Servicio de Sanidad Ambiental. Consejería de Sanidad y Política Social de Murcia, Spain*

[8] *Servicio de Microbiología. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain*

[9] *Institute of Infection and Global Health, University of Liverpool, Liverpool, Inglaterra*

Group A rotaviruses are one of the leading causes of acute gastroenteritis in young children worldwide. Rotavirus displays a seasonal pattern of infection in countries with temperate climate, with epidemic peaks occurring in winter and spring. Rotavirus vaccination may influence on the fluctuations of circulating rotavirus genotypes, especially in areas with low vaccine coverage. Significant annual changes in genotype distribution have been frequently detected even in the pre-vaccine era.

We participate in the European Rotavirus Network, EuroRotaNet, which was established in January 2007 to perform epidemiological surveillance of rotavirus strains by characterizing their G and P types. Uncommon strains of epidemiological importance are further characterized by analyzing the subgroup (VP6) and NSP4 genotype or by whole genome sequencing. Overall, 6 genotypes circulate in Europe with a prevalence $\geq$ 1% and included G1P[8], G4P[8], G2P[4], G9P[8], G3P[8] and G12P[8], making up these six genotypes to 91% of all characterized strains.

A notorious emergence of G12P[8] strains was detected in the Basque Country (Northern Spain) in 2004-05, being the predominant genotype in the 2010-11 (65% of all strains) and 2011-12 seasons (81.6%). Whereas the prevalence of this genotype declined in the Basque Country to very low levels in 2012-13 (1.2%) and 2013-14 (2.3%), an increase of G12P[8] strains was detected during 2013-14 in other Spanish regions (Castilla-León, Aragón, Catalonia, Valencia and Murcia), accounting overall for 15.3% of 466 typed strains. During the 2013-14 season, G12P[8] strains were detected in Valencia in 27.5% of rotavirus-positive samples. G12P[8] strains had also been detected in the feces of pigs in farms of Aragón in

2006. This finding is interesting, as it suggests G12 rotavirus transmission between humans and pigs. Phylogenetic analyses of the VP7 and VP4 genes demonstrated that they belong to lineages III of both genotypes. These strains display the typical human Wa-like gene constellation, and this may be the key to their recent emergence and spread. Rotavirus G12[P8] should be considered as an emerging genotype in Spain causing seasonal epidemics like the common human rotavirus genotypes G1–G4 and G9. After its emergence, G12[P8] genotype distribution fluctuates year to year and across different geographic regions. Continued surveillance of circulating rotavirus strains will allow us to know the future evolution of this genotype.

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DETECTION AND CHARACTERIZATION OF UNCOMMON HUMAN G3P[6] ROTAVIRUS A STRAINS CAUSING DIARRHEA IN ITALIAN CHILDREN IN 2009

G. IANIRO[1], R. DELOGU[2], L. FIORE[2], F.M. RUGGERI[1]

[1].*Dept. of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy*

[2].*National Center for Immunobiologicals Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy*

Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis (AGE) in young children, causing up to 450.000 deaths worldwide, mostly in developing countries. Most of RVA infections in humans across developed areas of the planet are related to five major G/P combinations: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. During the surveillance activity of RotaNet-Italy, three uncommon G3P[6] RVA strains, designated as RVA/Human-wt/ITA/NA01/2009/G3P[6], RVA/Human-wt/ITA/NA06/2009/G3P[6], and RVA/Human-wt/ITA/NA19/2009/G3P[6], were identified in stool specimens from children with diarrhea hospitalized in Southern Italy in 2009.

After PCR genotyping following standardized EuroRotaNet protocols, samples NA01, NA06 and NA19 showed the G3P[6] genotype. Sequencing of the eleven genomic segments was planned and performed to characterize the three uncommon RVA strains further and investigate their origin. RVA strains with a P[6] P-genotype in association with several G-genotypes have been isolated frequently in Africa, and sporadically also in developed countries. P[6] RVA strains have been detected in both patients with gastroenteritis and asymptomatic children, and P[6] has been established as a major P-genotype among porcine RVAs.

G- and P- genotyping was performed by reverse transcription-nested polymerase chain reaction (RT-nPCR), using mixtures of primers for either gene 9 or 4. For sequence analysis, RT-PCR reactions included primers specific for each gene investigated. Multiple sequence alignments and phylogenetic tree construction were performed with MEGA6, applying the Maximum-Likelihood (ML) method.

NA01, NA06 and NA19 RVA strains were found to possess the unusual genotype constellation G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2. This study reports the first

detection of uncommon G3P[6] RVA strains in human patients in continental Italy. The phylogenetic analysis of the eleven genomic segments showed no evidence of zoonosis or inter-species reassortment, revealing complete DS-1-like genomic constellations previously associated to human cases in Africa and Europe. The analysis of the hypervariable regions of VP7 and VP4 (VP8*) revealed high amino acid identity between the G3P[6] RVA strains involved in this study.

The comparison of the G3 RVA strains investigated in this study and other G3 RVAs characterized previously in Italy reveals that a large variety of G3 genomic variants have been reported throughout Italy, which might be partially related to the persisting massive immigration from across the Mediterranean sea. The detection of exotic RVA strains also in developed countries highlights the importance of surveillance activity on rotaviruses, similar to other imported and emerging pathogens, in order to prepare and control public health threats.

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THE ITALIAN ROTANET SURVEILLANCE PROGRAM, 2007-2014: DETECTION OF THE EMERGING GENOTYPE G12

R. DELOGU[1], G. IANIRO[2], F.M. RUGGERI[2], L. FIORE[1], AND THE ROTANET-ITALY STUDY GROUP

[1]. *National Center for Immunobiologicals Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy*

[2]. *Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy*

Rotavirus is the major cause of acute gastroenteritis in infants worldwide, causing every year up to450.000 deaths, mostly in developing countries. In Italy, the Istituto Superiore di Sanità has implemented a nationwide laboratory-based surveillance of acute rotavirus gastroenteritis to investigate the diversity of rotavirus strains circulating before the introduction of large-scale vaccination. RotaNet-Italy is linked to the EuroRotaNet network, which includes 17 European diagnostic laboratories.

Since 2007, approximately 9390 rotavirus positive stool samples were collected from pediatric patients with acute diarrheahospitalized in 14 Italian Regions, and were genotyped, following EuroRotaNet protocols. Significant variation in the frequency of different rotavirus genotypes was observed between different years and areas of Italy. Most strains belonged to genotypes G1-G4 and G9, and P[8] or P[4], commonly found in humans worldwide.

Overall, the predominant genotype detected during the seven rotavirus seasons was G1P[8](51%), followed by G9P[8] (16%), G4P[8] (9%), G2P[4] (8%) and G3P[8] (3%). However, unusual or emerging strains, such as G3P[19], G6P[6], G8P[4] and G12P[8], were also detected, suggesting either gene reassortment events between rotaviruses of different origin or importation of strains from other countries. In particular, during the 2012-13 surveillance the spread of the emerging G12P[8] rotavirus genotype was unexpectedly detected in the Central Italian region of Umbria (75%), and in different regions during the following season 2013-14 (9%).All G12 strains were

subjected to phylogenetic analysis. Sequence analysis showed a close nucleotide identity of both the VP4 and VP7 genes among the G12P[8] strains. The VP7 gene was similar to other G12 strains circulating in different years and countries, except for the Spanish G12 strains that showed a lower correlation with the Italian G12 strains, and clustered separately in the phylogenetic tree.The VP4 gene was closely related to other local and global P[8] strains showing different G-types. Overall findings suggest the introduction and evolution of a single G12 VP7 gene into the local Wa-like rotavirus population.

Most rotavirus infection occurred in children <2 years of age, but cases were also reported in older subjects, identifying risks of infection through contact with infected children and increased susceptibility of the elderly population to rotavirus.

Data from 7-year RotaNet-Italy surveillance confirm the genetic diversity of rotaviruses circulating in Italy, and the existence of remarkable differences between Regions and years. Continuous rotavirus strain surveillance in different countries is important to obtain a better understanding of rotavirus genotype evolution, particularly after vaccine introduction.

(PO 90)

INFLUENZA A VIRUS NS1 PROTEIN MIMICS ONCOGENIC PI3K RESULTING IN ISOFORM SPECIFIC CELLULAR REDISTRIBUTION AND ACTIVATION

J. AYLLON[1, 4], B. G. HALE[2], M. T. SÁNCHEZ-APARICIO[1, 4] AND A. GARCÍA-SASTRE[1, 3, 4]

[1]Department of Microbiology, [3]Department of Medicine and [4]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, USA

[2]Institute of Medical Virology, University of Zürich, Zürich, Switzerland

The non-structural protein 1 (NS1) of influenza virus performs a broad variety of pro-viral activities in the infected cell, mostly mediating the evasion from the host innate immune response by being the main viral interferon antagonist. However, among the multiple interactions described for this small, multifunctional protein there are several whose biological relevance remains obscure, such as NS1 ability to bind to and activate class IA phoinositide-3-kinases (PI3K). PI3K are highly regulated lipid kinases that act as critical nodes in multiple cell signaling networks that regulate cellular physiology, including differentiation, growth, survival, trafficking and immune function. As such, PI3K are also important proto-oncogenes whose deregulation lies behind a great number of different human cancers. Structurally, class IA PI3K are heterodimers formed by a regulatory (p85) and catalytic (p110) subunits, of which there are several isotypes described, adding further layers of complexity to their activity.

In order to unravel the cellular relevance of NS1-activated PI3K, we have developed a

bimolecular fluorescence complementation (BiFC) assay to selectively track the different regulatory and catalytic isotypes of PI3K and their behavior upon activation. Using this system we found that NS1 induces an isotype-specific relocation and activation of the different PI3K heterodimers. However, the effects of other known activators of PI3K such as Ras, Src and receptor tyrosine kinases were different from those induced by NS1. By contrast, clinically relevant, oncogenic hyper-activating mutations in both catalytic and regulatory subunits of PI3K recapitulate the effect caused by NS1. We postulate that by mimicking an oncogenic deregulation of the PI3K pathway influenza virus induces a transient, transformed-like status in the infected cell to stimulate virus replication.

(PO 91)

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS RECOMBINANT VACCINE INCLUDING SAFETY GUARDS IN E AND NSP1 GENES

JA. REGLA NAVA[1], M. LOPEZ DEDIEGO[1], JL. NIETO TORRES[1], JM. JIMENEZ GUARDEÑO[1], C. CASTAÑO RODRÍGUEZ[1], R. FERNANDEZ DELGADO[1], CRAIG FETT[2], STANLEY PERLMAN[2], AND LUIS ENJUANES[1].

[1]*Department of Molecular and Cell Biology, Centro Nacional de Biotecnología (CNB-CSIC), Darwin 3, Campus Universidad Autónoma de Madrid, 28049 Madrid, Spain*

[2]*Department of Microbiology, University of Iowa, Iowa City, Iowa, USA*

Coronavirus such as severe acute respiratory syndrome coronavirus (SARS-CoV) and middle east respiratory syndome (MERS-CoV) cause high case fatality rates and remain as major human public health threats. No specific therapy for any human coronavirus is available, making vaccine development critical for protection against these viruses. Previously, we demonstrated that a mouse-adapted SARS-CoV (SARS-CoV-MA15) lacking the envelope (E) protein (rSARS-CoV-MA15-ΔE) is attenuated *in vivo*. To identify E protein regions and host responses that contribute to rSARS-CoV-MA15-ΔE attenuation, several mutants (rSARS-CoV-MA15-E*) containing point mutations or deletions in the amino-terminal or the carboxy-terminal regions of the E protein were generated. We showed that small deletions and modifications within E protein led to virus attenuation, causing minimal lung injury, limited neutrophil influx to the lungs, reduced expression of proinflammatory cytokines, increased anti-inflammatory cytokine levels, and enhanced CD4+ and CD8+ T cell counts in vivo. These data suggests that the described mutant phenotype contributed to virus attenuation. The attenuated mutants fully protected mice from challenge with virulent virus. A major problem of using live attenuated viruses as vaccines is the possibility of reversion to virulence. To overcome this limitation, we introduced additional attenuating mutations into the nsp1 protein to generate a safer vaccine candidate. Nsp1 gene was selected as a target because it is located at a distal position (>20kb) from that of E gene in the viral genome, making the generation of a virulent virusthrough a single recombination event with circulating coronaviruses highly unlikely. To identify

nsp1 protein regions that contribute to rSARS-CoV-MA15 attenuation, several mutants (rSARS-CoV-MA15-nsp1*) containing small deletions in of the nsp1 protein were generated. Deletion of 121 to 129 and 154 to 165 aminoacids in the carboxy terminal region of nsp1 protein led to virus attenuation. Immunization with single SARS-CoV mutants protected mice against challenge with the lethal parental virus. A recombinat virus including safety guards in E and nsp1 genes was generated. This mutant virus was completely attenuated and protected mice against challenge with the lethal parental virus, indicating that this virus is promising vaccine candidate.

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INEFFICACY OF A LIVE ATTENUATED VACCINE IN CLASSICAL SWINE FEVER VIRUS POSTNATALLY PERSISTENTLY INFECTED PIGS: IS THE CONTROL OF DISEASE ON THE LINE?

S. MUÑOZ-GONZÁLEZ[1], M. PEREZ-SIMÓ[1], M. MUÑOZ[1], J.A.BOHORQUEZ[1], R. ROSELL[1,2], A. SUMMERFIELD[3], M. DOMINGO[1,4], N. RUGGLI[3] AND L. GANGES[1*]

[1.] *Centre de Recerca en Sanitat Animal (CReSA), IRTA-Universitat Autònoma de Barcelona (UAB), Campus de la UAB, 08193 Bellaterra, Barcelona, Spain*

[2.] *Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, (DAAM), Generalitat de Catalunya, Spain*

[3.] *Institute of Virology and immunology (IVI), Mittelhäusern, Switzerland*

[4.] *Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, UAB, 08193 Bellaterra-Barcelona, Spain*

Classical swine fever (CSF) causes major losses in pig farming, with various degrees of disease severity. Efficient live attenuated vaccines against Classical swine fever virus (CSFV) are used routinely in endemic countries. However, despite intensive vaccination programs in these areas for more than 20 years, CSF has not been eradicated. Molecular epidemiology studies in these regions suggests that the virus circulating in the field has evolved under the positive selection pressure exerted by the immune response to the vaccine, leading to new attenuated viral variants. Recent work by our group demonstrated that a high proportion of persistently infected piglets can be generated by early postnatal infection with a low and a moderate virulent CSFV strains. Here we studied the immune response to a Hog Cholera Lapinized virus vaccine (HCLV), C-strain, in 6-week-old persistently infected pigs following post-natal infection. CSFV-negative pigs were vaccinated as control. The humoral and interferon gamma responses as well as the CSFV RNA load were monitored during 21 days post vaccination. The experiments were approved by the Ethics Committee for Animal Experiments of the Autonomous University of Barcelona (UAB), according to existing national and European regulations.A complete lack of detection of the vaccine viral RNA was found in the serum samples and in the tonsils from CSFV postnatal persistently infected pigs during 21 days post vaccination. Furthermore, lack of response to E2 specific antibodies and absence of neutralizing antibody titres were shown in CSFV persistently infected-vaccinated animals. Likewise, absence of IFN-gamma

producing cells response against CSFV or PHA was also observed. To our knowledge, this is the first report demonstrating the absence of response to vaccination in CSFV persistently infected pigs.

(PO 93)

ISG15 INHIBITS RESPIRATORY SYNCYTIAL VIRUS INFECTION THROUGH PROTEIN ISGYLATION AT EARLY STAGES OF THE VIRAL CYCLE

R. GONZÁLEZ-SANZ[1,2], M. MATA[2,3], J. BERMEJO-MARTÍN[4], A. ÁLVAREZ[1], J. CORTIJO[2,3], J.A. MELERO[2,5] AND I. MARTÍNEZ[1,2]

[1] Unidad de Infección Viral e Inmunidad. Centro Nacional de Microbiología, Instituto de Salud Carlos III. Madrid, Spain. [2] Centro de Investigación Biomédica en Red. Enfermedades Respiratorias (CIBERES). Instituto de Salud Carlos III. Madrid, Spain. [3] Fundación Investigación Hospital General Universitario de Valencia. Valencia, Spain. [4] Hospital Clínico Universitario de Valladolid. Valladolid, Spain. [5] Unidad de Biología Viral. Centro Nacional de Microbiología, Instituto de Salud Carlos III. Madrid, Spain

Human Respiratory Syncytial Virus (RSV) is the leading cause of severe lower respiratory tract infections in children but also a significant cause of morbidity and mortality in the elderly and immunocompromised individuals. Despite an intense research, neither a vaccine nor an effective therapeutic treatment is currently available. A better understanding of the complex interactions between RSV and the host may help to find new therapeutic targets against this virus. Interferon stimulated gen 15 (ISG15) is an ubiquitin-like protein that is highly induced during viral infections and has been reported to play either an antiviral or a proviral activity, depending on the virus. ISG15 can exert its function either through conjugation to target proteins in a process termed ISGylation, or in a conjugation independent manner. Previous studies from our laboratory demonstrated a strong ISG15 up-regulation during *in vitro* RSV infection. In this work, a more detailed analysis of the ISG15 role in RSV infection is presented. ISG15 overexpression and siRNA silencing experiments, along with ISG15 knockout cells demonstrated an anti-RSV effect of this molecule at early stages of the virus cycle. Conjugation inhibition assays revealed that ISG15 exerts its antiviral activity via protein ISGylation. However, this antiviral activity occurs only when high levels of ISG15 are present in cells before RSV infection, as in the case of cells previously stimulated with interferon. Finally, ISG15 is also up-regulated in human respiratory pseudo-stratified epithelia and in nasopharyngeal washes from infants infected with RSV, suggesting a possible antiviral role of this molecule *in vivo*. These results improve our understanding of the innate immune response induced by RSV and demonstrate the antiviral activity of ISG15 against this virus for the first time, thus opening new possibilities for infection control.

(PO 94)

MODULATION OF HOST INNATE IMMUNE RESPONSE BY BERNE VIRUS M AND N STRUCTURAL PROTEINS

G. NIEVES MOLINA, S. PLAZUELO, D. RODRÍGUEZ AGUIRRE

Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain

Type I IFN system acts as the first line of defense to control the replication and dissemination of invading viruses, and to promote the adaptive immune responses. The ability of the viruses to evade this innate response is crucial to ensuring their progeny survival. Toroviruses are enteric positive-sense, single stranded RNA viruses that belong to the *Coronaviridae* family, *Torovirinae* subfamily, of the *Nidovirales* order. Unlike the coronaviruses, the toroviruses have been poorly studied, and many issues about their interactions with the host remain unexplored. In this study we demonstrated that the equine torovirus Berne virus (BEV), prototype member of the subfamily, antagonizes the transcription and production of the IFN-α/β cytokines. Also, BEV infection reduces the expression of the IFN-stimulated genes MxA, ISG15, and ISG56 induced upon stimulation with IFN, SeV, and poly I:C. Next we analyzed the implication of the membrane (M) and nucleocapsid (N) BEV structural proteins in this process. Using an IFN-β promoter-luciferase reporter assay we observed that both proteins block the induction of this gene mediated by SeV. The activation of the IFN-β promoter depends on both the activation of the transcription factors IRF-3 (interferon regulatory factory factor 3) and NF-κB (nuclear factor-kappa B). With a similar approach we showed that the M and N proteins inhibit the IRF-3 activation. Nonetheless, only the M protein is able to suppress the NF-κB reporter activation mediated by the TNF-α. Then, we wanted to determine at which step of the IFN-β pathway the M and N proteins were interfering. For this, we stimulated IFN-β transcription with different proteins involved in the signaling, including the RIG-I and MDA5 helicases, the mitochondrial adapter protein MAVS, the TBK1 and IKKε kinases, and the IRF-3 transcription factor. The results indicated that the M protein inhibits the IFN-β induction mediated by RIG-I, MDA5, MAVS, TBK1, and IKKε, while the N protein blocks its induction only after MAVS stimulation. Neither of these two BEV proteins suppresses the transcription of IFN-β mediated by IRF-3, suggesting that the block in signaling is prior to IRF-3 activation. Finally, we observed that the M and N proteins interact with a complex formed by TBK1/IKKε/IRF-3 proteins and this association could be a mechanism for disrupting IFN-β expression. In summary, these results indicate that M and N proteins play a crucial role in the IFN antagonism exerted by BEV, and probably in its pathogenicity.

(PO 95)

RECOMBINANT ADENOVIRAL VECTOR EXPRESSING IFN-TAU INDUCES PROTECTIVE IMMUNE RESPONSE IN MICE CHALLENGE WITH A LETHAL DOSE OF INFLUENZA VIRUS

V. MARTIN[1*], E. PASCUAL[1], M. AVIA[1], G. RANGEL[1], A. DE MOLINA[2], E. BLANCO[1], A. ALEJO[1] AND N. SEVILLA[1]

[1]*Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain*

[2]*Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain*

Virus-infected cells secrete a broad range of interferon (IFN) subtypes, which in turn initiate the expression of antiviral factors that confer host resistance. Type I IFNs (IFN-α, IFN-β, IFN-, IFN, IFN-δ) signal act through a common universally expressed cell surface receptor (IFNRI). Interferon-tau (IFN-τ) constitutes a new class of type I IFN identified in ruminants that is structurally related to IFN-α -β and-ω and capable of inducing an antiviral activity in a similar manner, although, unlike the former it is not inducible by dsRNA. While usually type I IFNs are highly species-specific, IFN-τ displays high antiviral, anti-proliferative and immunomodulatory activities across species with a prominent lack of cytotoxicity at high concentrations in cell culture and possibly *in* vivo. IFNs play a central role in the defence of vertebrates against viral infections leading the cells to an antiviral state that effectively limits virus replication.

We present here the expression of IFN-τ from a second-generation human recombinant adenovirus 5, a vector widely used for vaccine delivery with excellent properties as vehicle due to its genetic stability, safety, thermostability and activation of innate immune response. To evaluate the potential of this approach as an antiviral treatment *in vivo*, we chose to use the murine influenza model. Thus, congenic B6.A2G-Mx1 mice, expressing the murine Mx1 protein, were challenged with the FLUAV strain, A/PR/8/34 (H1N1) (designated hv-PR8). This variant is closely related to but distinct from the Cambridge strain of A/PR/8/34 and is highly virulent in $Mx1^{+/+}$ mice. We hypothesised that, in $Mx1^{+/+}$ mice, the expression of IFN-τ could induce a strong innate immune response and a more robust resistance to influenza by activating the Mx1 gene in addition to other antiviral genes. This animal model was previously used to demonstrate that IFN-α might be used to prevent disease induced by highly lethal human H5N1 influenza viruses. Using this experimental system we demonstrate here that a single dose of intranasal administration of a recombinant adenovirus vector expressing IFN-τ could protect against a highly virulent influenza strain (hv-PR8) in B6.A2G-Mx1 mice, making it a good antiviral candidate for this and other similar viruses. Moreover, the results show that IFN-τis active in other, non-ruminant species indicating its potential cross species applicability *in vivo*. Furthermore, no toxic effects were noted throughout the course of treatment. In summary, we present a useful tool for the administration of a non-toxic type I interferonwithpotential applicabilityin the treatment of several viral diseases.

(PO 96)

AN IMPROVED BACULOVIRUS EXPRESSION VECTOR INCREASES THE SECRETION OF THE RECOMBINANT HEMAGGLUTININ FROM INFLUENZA VIRUS

E. GUIJARRO- PARDO[1*], S. GÓMEZ-SEBASTIÁN[2*], S. MARTINEZ-PULGARIN [2], MIGUEL CID[2], C. ALVARADO[2] J.M. ESCRIBANO[1]

[1] *Departamento de Biotecnología, INIA, Madrid, Spain*

[2] *Alternative Gene Expression S.L. (ALGENEX), Pozuelo de Alarcón, Madrid, Spain*

** E. Guijarro- Pardoand S. Gómez-Sebastiánhave equally contributed to this work.*

To simplify the manufacturing process of the influenza hemagglutinin (HA) and circumvent the weaknesses in conventional and reverse genetics based vaccines, the improvements on recombinant-based production technologies are highly relevant. The baculovirus vector expression system (BEVS) is one of the most reliable and effective production methodology which has been selected by several companies producing new generation vaccines against influenza. However, the transient expression mediated by the baculovirus infection affects the cells secretion pathways due to the lytic nature of the system, reducing the recovered yields of recombinant proteins correctly processed from infected cells supernatants. Recently, it has been described the TopBac® baculovirus expression cassette. Baculovirus vectors modified by TopBac® have unprecedented production yields, increasing also the quality of recovered recombinant proteins because they are produced in insect cells with an increased viability late after infection. This increase in cell viability is due to a delay in the virus-induced apoptosis produced after infection with the vector. The prolonged cell integrity significantly contributes to avoid the typicalaberrant forms and proteolysis found in the BEVS. The present study consisted in the determination of the level of preservation of the secretion pathway in cells infected by a TopBac®-modified baculovirus expressing the HA in comparison to a conventional baculovirus. For this purpose, we obtained 2 recombinant baculoviruses (modify or not by the TopBac® cassette) expressing this protein fused to the signal peptide of melittin to facilitate the protein secretion. The recombinant baculoviruses obtained were used to infect insect cells and *Trichoplusia ni*larvae. The results obtained in insect cells showed that at optimal production times (48-72 hpi), when cellular viabilities are above 80%, a secretion increment approximately of 2 times was obtained by using the TopBac®-modified baculovirus in comparison to the conventional vector. This increment in secretion was accompanied by the absence of tubulin in the cell media, as an indirect measurement of the cellular integrity. Similar results of secretion were obtained in the analysis of the hemolymph of infected larvae, with increments of 2 times of HA secreted when using the TopBac®-modified baculovirus. This result constitutes a step forward in the scaling-up production of the HA protein using the BEVS, providing a broad-based strategy for the simplification of the production of recombinant subunit vaccines against

seasonal or pandemic influenza at high yields, avoiding the cumbersome egg-based production conventionally used.

(PO 97)

ROLE OF THE CYSTEINE-RICH DOMAINS OF POXVIRUS TNF RECEPTORS IN THEIR IMMUNOMODULATORY ACTIVITY

L. MESTRE, SM. PONTEJO, S. BLANCO, P. LANUZA, E. PRIEGO, A. ALCAMÍ

Centro de Biología Molecular "Severo Ochoa" (CBMSO) Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Autónoma de Madrid (UAM), Cantoblanco, 28049, Madrid, Spain

Viral tumour necrosis factor receptors (vTNFRs) are soluble proteins secreted after poxvirus infection characterized by mimicking the extracellular domain of TNF superfamily receptors. Thus, vTNFRs bind to and inhibit the signalling induced by the host TNF superfamily ligands as a mechanism of immune evasion. Up to four different vTNFRs expressed by poxvirus have been described and named CrmB, CrmC, CrmD and CrmE. It was reported that while CrmC and CrmE only bind to TNF□, CrmD and CrmB were also able to inhibit *in vitro* the biological effects of lymphotoxin alpha (LT□).

The N-terminal region of these proteins is related to the TNF-binding site of cellular TNFRs and is characterized by three cysteine-rich domains (CRDs) named CRD1, CRD2, CRD3. CRD1 contains a conserved preligand assembly domain critical for ligand binding and receptor trimerization, while CRD2 has been proved necessary for the TNF-vTNFR interaction. It has been suggested that the specificity of this interaction is provided by CRD3. Nevertheless, the role of CDR3 is still unclear.

The aim of the present work was tostudy the involvement of CRD3 in the binding specificity and biological activity of the vTNFRs. With this purpose, we have expressed in the baculovirus system twelve mutant proteins of CrmB, CrmC, CrmD and CrmE exchanging CRD3 between them and analysed their ability to inhibit the cytotoxic effect of TNF□ or LT□. Our results show that the specificity of the interaction site depends on the vTNFR studied. While both CRD3 from CrmC and CrmE are not responsible for blocking cytotoxic effect induced by TNF□, CRD3 from CrmD is necessary to protect L929 cells from TNF□ induced-cell death. Moreover, CRD3-CrmB is involved in the inhibition of cytotoxicity induced by LT□.

This work contributes to the understanding of strategies used by viruses to evade the immune response, which provide us with a potent immunomodulatory tool that could have therapeutic potential.

***Keywords**: poxvirus, vTNFRs, immune system.*

(PO 98)

THE PDZ-BINDING MOTIF OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS ENVELOPE PROTEIN IS A DETERMINANT OF PATHOGENESIS AND VIRAL FITNESS

JM. JIMENEZ-GUARDEÑO, JL. NIETO-TORRES, ML. DEDIEGO, JA. REGLA-NAVA, C. CASTAÑO-RODRIGUEZ, R. FERNANDEZ-DELGADO AND L. ENJUANES

Department of Molecular and Cell Biology, Centro Nacional de Biotecnología (CNB-CSIC), Darwin 3, Campus Universidad Autónoma de Madrid, 28049 Madrid, Spain

Severe acute respiratory syndrome coronavirus (SARS-CoV) is the etiological agent of a worldwide epidemic that appeared in China in 2002, infecting 8000 people worldwide with an average mortality of about 10%. Previously, we demonstrated that a recombinant SARS-CoV lacking the multifunctional envelope (E) protein generated in our laboratory was attenuated *in vivo* in three different animal models, being a promising vaccine candidate. Here we report that the E protein PDZ-binding motif (PBM), a domain potentially involved in the interaction with more than 400 cellular proteins, what highlights its relevance in modulating host-cell behavior, is a major determinant of SARS-CoV virulence. Removal of PBM in SARS-CoV E protein using reverse genetics, drastically diminished lung damage, leading to virus attenuation. Cellular protein syntenin was identified to bind the E protein PBM during SARS-CoV infection by using three complementary strategies: yeast two-hybrid, reciprocal coimmunoprecipitation and confocal microscopy assays. In addition, syntenin redistributed from the nucleus to the cell cytoplasm, colocalizing with E protein during infection with SARS-CoV containing the E protein PBM. The relocalization of syntenin activated p38 MAPK and led to the overexpression of inflammatory cytokines. In fact, silencing of syntenin using siRNAs led to a decrease in p38 MAPK activation in SARS-CoV infected cells, reinforcing their functional relationship. Furthermore, active p38 MAPK was reduced in the lungs of mice infected with SARS-CoVs lacking E protein PBM as compared with those infected with viruses containing this motif, leading to a decreased expression of inflammatory cytokines and to virus attenuation. Interestingly, administration of a p38 MAPK inhibitor increased mice survival up to 80% after infection with SARS-CoV, indicating the relevance of this signaling pathway in SARS-CoV pathogenesis. The impact of E protein PBM during SARS-CoV infection was supported by showing that recombinant viruses lacking the E protein PBM incorporated novel PBMs after serial passages. This data confirms that the PBM of E protein is a virulence factor that increases virus fitness.

(PO 99)

EFFECT OF HERPES SIMPLEX VIRUS TYPE 1 SHUT-OFF PROTEIN (VHS) AND INTERFERON ON THE GROWTH OF A HERPES SIMPLEX VIRUS DEFECTIVE IN ICP0

R. GARCÍA UTRILLA, B. MORENO, S. GUERRA, E. TABARÉS

Preventive Medicine and Public Health and Microbiology Department, Medicine Faculty Autonomous University of Madrid, Madrid Spain

Herpes Simplex Virus Type 1 (HSV-1) affects more than 80% of the human population causing both acute infections, characterized by a wide range of clinical manifestations, and latent infections. Due to their structural and genomic characteristics as well as the HSV-1 viral life cycle, these viruses have been widely used as genetic tools to study and treat many diseases with considerable social, economic and cultural impact, such as central nervous system disorders or cancer. While promising, the development of new recombinant HSV-1-based viruses is still warranted and needed, specifically the development of viruses that are not susceptible to host cytokine inhibition, a factor the limits the therapeutic use of many viruses.

The primary goal of this study was to produce enhanced green fluorescent protein (EGFP) HSV-1 reporter viruses defective in the immediate early protein ICP0, which renders HSV-1 hypersensitive to treatment with interferon (IFN). The ability to visualize and track the virus throughout the entire infection process via EGFP fluorescence highlights the potential of these viruses as powerful genetic tools in cytokine sensitivity assays. The mutant viruses dl41GF (ICP0-, *vhs*-, EGFP+) and dlTKGF (ICP0-, TK-, EGFP+) were constructed by homologous recombination using the parental virus dl1403 (ICP0-). In addition, we also constructed FTKGF (TK-, EGFP+) using the HSV-1 parental strain F, to serve as a reference viruses for the all assay performed. These three recombinant viruses were characterized by PCR and direct fluorescence microscopy. Once characterized, the growth kinetics, sensitivity to IFN and the influence of the viral shut-off (*vhs*) protein on viral growth were assessed in Vero and U2OS cells. Replication of dlTKGF and dl41GF was 15.7 and 12.2 fold higher in U2OS cells, respectively, compared to Vero cells. These orders of magnitude were significantly higher than those observed for the reference viruses HSV41GF (*vhs*-, EGFP+) and FTKGF, which we contribute to the absence of ICP0 protein in the first mutant viruses. The virus dl41GF was twice as sensitive to IFN and exhibited 7.5 fold increased viral replication compared to the dlTKGF virus in U2OS cells. In conclusion, the results of this study suggest a possible cooperative action between HSV-1 ICP0 and shut-off proteins, both at the level of viral growth and IFN sensitivity carried out on U2OS cells.

(PO 100)

ANALYSIS OF IMMUNOPROTECTION OF gD AND THE CHIMERIC GLYCOPROTEIN gD-gB (gDB2) OF HERPES SIMPLEX VIRUS TYPE 2 EXPRESSED BY PSEUDORABIES VIRUS RECOMBINANTS AND AMPLICONS

E. RUBIO FERNÁNDEZ[1], L. VARELA[1], A. L MUÑOZ[1], B. MORENO[1], E. TABARÉS[1], I. GADEA[2]

[1]. Departamento de Medicina Preventiva y Salud Pública y Microbiología, Facultad de Medicina Universidad Autónoma de Madrid, Madrid, Spain

[2]. Servicio de Microbiología Médica, Fundación Jiménez Díaz, Madrid, Spain

More than 500 million people worldwide are infected with herpes simplex virus type 2 (HSV-2), and 23 million new HSV-2 infections are developed each year. HSV-2 can produce from recurrent genital ulcers to potentially lethal infections and the need for a vaccine is widely recognized. Over the last decades, numerous efforts have been made to develop effective vaccines against HSV-2; however, most studies have not given satisfactory results in human clinical trials. The main goal of this study was to analyze in BALB/c mice, the immunoprotection properties of gD (gD2) and a chimeric glycoprotein gD-gB (gDB2) of herpes simplex virus type 2 by using pseudorabies virus (PRV) recombinant and amplicons as expression vectors. Three groups of mice were immunized subcutaneously with recombinant virus PRV-HgD, PRV-HgDB and PRV-BT90. PRV-BT90 was used as reference, which it does not express any HSV-2 glycoprotein constructions.Humoral immune response was analyzed by ELISA test, which showed the presence of anti-HSV-2 antibodies in the groups immunized with PRV-HgD and PRV-HgDB. A survival study was carried out over a 21-day period after mice were challenged with a lethal dose of an HSV-2 clinical isolate by intravaginal infection. The results of this analysis were compared with groups of BALB/c mice immunized with plasmids (PpD and PpDB) or amplicons (AmpgD2 and AmpgDB2) expressing the same HSV-2 glycoproteins constructions that PRV recombinants. All the groups studied showedstatistically significant differences in survival with the control group, except for the groups of mice immunized with PRV-HgDB and AmpgDB2. The best results of survival were achieved with the plasmid PpDB and the amplicon AmpgD2, whose survival percentages were 85% and 75%, respectively.

(PO 101)

A CHIMERIC HIV-1 GP120 FUSED WITH VACCINIA VIRUS 14K (A27) PROTEIN AS AN HIV IMMUNOGEN

ANEESH VIJAYAN[1], JUAN GARCÍA-ARRIAZA[1], SURESH C. RAMAN[1], JOSÉ JAVIER CONESA[2], FRANCISCO JAVIER CHICHÓN[2], CÉSAR SANTIAGO[3], CARLOS ÓSCAR S. SORZANO[4], JOSÉ L. CARRASCOSA[2] AND MARIANO ESTEBAN[1§]

[1]Department of Molecular and Cellular Biology, [2]Department of Structure of Macromolecules, [3]X-ray Crystallization Unit, [4]Biocomputing Unit, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CNB-CSIC), 28049, Madrid, Spain.

In the HIV vaccine field, there is a need to produce, in considerable quantities, soluble and stable forms of the Env protein with the capacity to trigger broad B and T-

cell responses. Here, we report the generation and characterization of a chimeric HIV-1 gp120 protein (termed gp120-14K) by fusing gp120 from clade B with the vaccinia virus (VACV) 14K oligomeric protein (derived from A27L gene). Stable CHO cell lines expressing HIV-1 gp120-14K fusion proteins were generated, and characterized by size exclusion chromatography, electron microscopy and binding to conformational antibodies. In human monocyte-derived dendritic cells (moDCs), gp120-14K protein upregulates the levels of several proinflammatory cytokines and chemokines associated with Th1 innate immune responses (IL-1β, IFN-γ, IL-6, IL-8, IL-12, RANTES). Moreover, we showed in a murine model, that a heterologous prime/boost immunization protocol consisting of a DNA prime with a plasmid expressing the gp120-14K protein followed by a boost with MVA-B [a recombinant modified vaccinia virus Ankara (MVA) expressing HIV-1 gp120, Gag, Pol and Nef antigens from clade B], generates stronger, more polyfunctional, and greater effector memory HIV-1-specific CD4+ and CD8+ T-cell immune responses, than immunization with DNA-gp120/MVA-B. The DNA/MVA protocol was superior to immunization with the combination of protein/MVA and the latter was superior to a prime/boost of MVA/MVA or protein/protein. In addition, all of these immunization protocols enhanced antibody responses against gp120 of the class IgG2a and IgG3, together favoring a Th1 humoral immune response. These results demonstrate that fusing VACV 14K with HIV-1 gp120, forms an oligomeric protein with apparent native-like structure, triggering a Th1 innate immune response in human moDCs, and enhancing HIV-1-specific adaptive and memory T-cell immune responses, as well as humoral responses, in immunized mice. This novel HIV-1 gp120-14K immunogen might be considered as an HIV vaccine candidate for broad T and B-cell immune responses.

(PO 102)

VIROLOGICAL AND IMMUNOLOGICAL CHARACTERIZATION OF NOVEL NYVAC-BASED HIV/AIDS VACCINE CANDIDATES EXPRESSING CLADE C TRIMERIC SOLUBLE GP140(ZM96) AND GAG(ZM96)-POL-NEF(CN54) AS VIRUS-LIKE PARTICLES

B. PERDIGUERO[1],C. E. GÓMEZ[1],V. CEPEDA[1],L. SÁNCHEZ-SAMPEDRO[1],J. GARCÍA-ARRIAZA[1],E. MEJÍAS-PÉREZ[1], V. JIMÉNEZ[1],C. SÁNCHEZ[1],C. O. S. SORZANO[2],J. C. OLIVEROS[2],J. DELALOYE[3],T. ROGER[3], T. CALANDRA[3],B. ASBACH[4],R. WAGNER[4],K. V. KIBLER[5],B. L. JACOBS[5],G. PANTALEO[6] AND M. ESTEBAN[1]

[1] Department of Molecular and Cellular Biology and [2] Biocomputing Unit and Computational Genomics, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.[3] Infectious Diseases Service, Department of Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland. [4] University of Regensburg, Regensburg, Germany.[5] The Biodesign Institute at Arizona State University, Tempe, Arizona, USA. [6] Division of Immunology and Allergy, Department of Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland.

The generation of vaccines against HIV/AIDS able to induce long-lasting protective immunity remains a major goal in the HIV field. The modest efficacy (31.2%) against HIV infection observed in

the RV144 phase III clinical trial highlighted the need for further improvement of HIV vaccine candidates, formulation, and vaccine regimen. In this study, we have generated two novel NYVAC vectors, expressing HIV-1 clade C gp140(ZM96) (NYVAC-gp140) or Gag(ZM96)-Pol-Nef(CN54) (NYVAC-Gag-Pol-Nef), and defined their virological and immunological characteristics in cultured cells and in mice. The insertion of HIV genes does not affect the replication capacity of NYVAC recombinants in primary chicken embryo fibroblast cells, HIV sequences remain stable after multiple passages, and HIV antigens are correctly expressed and released from cells, with Env as a trimer (NYVAC-gp140), while in NYVAC-Gag-Pol-Nef infected cells Gag-induced virus-like particles (VLPs) are abundant. Electron microscopy revealed that VLPs accumulated with time at the cell surface, with no interference with NYVAC morphogenesis. Both vectors trigger specific innate responses in human cells and show an attenuation profile in immunocompromised adult BALB/c and newborn CD1 mice after intracranial inoculation. Analysis of the immune responses elicited in mice after homologous

NYVAC prime/NYVAC boost immunization shows that recombinant viruses induced polyfunctional Env-specific CD4 or Gag-specific CD8 T cell responses. Antibody responses against gp140 and p17/p24 were elicited. Our findings showed important insights into virus-host cell interactions of NYVAC vectors expressing HIV antigens, with the activation of specific immune parameters which will help to unravel potential correlates of protection against HIV in human clinical trials with these vectors.

(PO 103)

HEAD-TO-HEAD COMPARISON OF VACCINIA VIRUS-BASED VECTORS NYVAC AND MVA EXPRESSING LEISHMANIA ACTIVATED C-KINASE IN MICE

E. MEJÍAS-PÉREZ[1¶]; L. SÁNCHEZ-SAMPEDRO[1¶]; C.O. S. SORZANO, [2]; M. ESTEBAN [1]

[1] *Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.*

[2] *Biocomputing Unit, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.*

[¶] *These authors contributed equally to this work.*

The poxvirus NYVAC and MVA vectors are used as vaccine candidates against HIV, but experimental information on a head-to-head comparison of immunogenicity and efficacy against a human disease is limited. Here we compared in mice the immune responses elicited and protection induced by NYVAC vectors expressing the leishmania activated C-kinase antigen (LACK) versus the well-known MVA-LACK vector. In a head-to-head comparison by DNA prime/virus boost protocols, we show that replication competent NYVAC-LACK expressing the C7L host range gene (NYVAC-LACK-C7L) induced the highest quality of $CD4^+$ and $CD8^+$ adaptive and effector memory T cell responses (IFNγ, TNFα, IL-2, CD107a) against LACK antigen. The $CD8^+$ T cell population induced by NYVAC-LACK-C7L also showed the highest proliferative capacity when stimulated with the LACK antigen. T cell differences

with MVA-LACK and between replication competent and replication defective NYVAC vectors were restricted to magnitude of the response. After subcutaneous *L. major* infection, challenge groups vaccinated with NYVAC-LACK-C7L showed higher protection than NYVAC-LACK group, and similar efficacy as those vaccinated with MVA-LACK. Our results revealed that the type of immune response and potency induced by NYVAC and MVA vectors is largely restricted to quantitative differences in T cells, with a replication competent NYVAC with C7L gene triggering the highest T cell adaptive, memory and proliferative immune responses.

(PO 104)

VIRUS-LIKE PARTICLES (VLPS) DERIVED FROM CALICIVIRUS AS A DELIVERY SYSTEM FOR THE PRESENTATION OF FOOT-AND-MOUTH DISEASE VIRUS EPITOPES

G.RANGEL[1], A. ALEJO[1], B.GUERRA[1], J.R. CASTON[2] J.BARCENA[1] AND E. BLANCO[1]

[1]. *Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, 28130 Madrid, Spain.*

[2]. *Dpto. Estructura de Macromoléculas, (CNB-CSIC), Cantoblanco, 28049 Madrid, Spain.*

Virus-like particles (VLPs) are appealing as vaccine candidates because their inherent properties (i.e., virus-sized, multimeric antigens, highly organised and repetitive structure, not infectious) are suitable for the induction of safe and efficient immune responses. In particular, VLPs from rabbit haemorrhagic disease virus (RHDV) have been shown to be good vaccine platforms (1).

Rabbit Hemorrhagic Disease virus (RHDV) is the prototype strain of the genus *Lagovirus* within the family *Caliciviridae*, a group of nonenveloped, icosahedral viruses which are composed of 180 copies of a single capsid protein, terms VP60.

Our research group has identified two independent locations within the RHDV capsid protein that can accomodate foreign of up to 42 aminoacids in length, without affecting the ability of the resulting chimeric protein to self-assemble into VLPs (2, 3). Our goal is to develop RHDV VLPs as a delivery system for the multimeric presentation of immunogenic epitopes derived from pathogens relevant for animal health.

Foot-and-mouth disease virus (FMDV) is a highly infectious disease of cloven-hoofed animals and probably the most important livestock disease in terms of economic impact. The aim of the present study was to analyze the potential of chimeric VLPs to induce specific immune responses against T- and B-cell epitopes from FMDV. To this end we generated chimeric VLPs harbouring, in different insertion sites, a neutralizing B-cell epitope derived from FMDV type O (currently the most widespread serotype), located around positions 140 to 160 of capsid protein VP1 (loop G-H), and a T-cell epitope highly conserved among FMDV serotypes from 3A non-structural protein (4).

Groups of mice were inoculated with the chimeric VLPs and we analyzed the humoral and cellular immune responses elicited. The results obtained indicated that the chimeric RHDV VLPs are able to induce potent antibody responses against FMDV B-cell epitope,especially when

inserted at an exposed site within the VLP structure.

Based on the outcomes, the potential suitability of these chimeric VLPs for new vaccine development against pig viral infections will be discussed.

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(PO 105)

EFFECT OF EPITOPE MULTIPLICITY AND CONNECTIVITY ON THE IMMUNOGENICITY AND PROTECTION OF DENDRIMERIC PEPTIDE VACCINES AGAINST FOOT-AND-MOUTH DISEASE VIRUS

E. BLANCO[1], B.GUERRA[1], B. G DE LA TORRE[2], D. ANDREU[2] AND F. SOBRINO [1,3]

[1.] *Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, 28130 Madrid, Spain.*

[2.] *Departament de Ciències Experimentals i de la Salut, Universitat Pompeu-Fabra, 08003 Barcelona, Spain.*

[3.] *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Cantoblanco, 28049 Madrid Spain.*

Foot-and-mouth disease (FMD) is a highly infectious disease of cloven-hoofed animals, admittedly the most important livestock disease in terms of economic impact. FMD control in endemic regions is implemented mainly by using chemically inactivated whole-virus vaccines,that show several disadvantages (requirement of a cold chain, periodic re-vaccinations , risk of virus release during vaccine production, etc).In this context, peptide-based vaccines are promising alternatives in the control of infectious diseases, offering several advantages, such as safety, accurate molecular delineation, ease of synthesis and scaleup or uncomplicated storage and transport.

Synthetic peptides incorporating protective B- and T-cell epitopes are candidates for new safer FMD vaccines. We have reported that dendrimeric peptides including four copies of a B- cell epitope (VP1 136 to 154) linked to a T-cell epitope (3A 21 to 35) of FMD virus (FMDV) elicit potent B- and T- cell specific responses and confer protection to type C FMDV challenge (1), while juxtaposition of these epitopes in a linear peptide induces less efficient responses. In order to extend this proof of concept to FMDV serotypes epidemiologically relevant at present, we designed new dendrimeric peptides harboring as B-cell epitopes sequences from FMDV serotype O (currently the most widespreaded serotype). To assess the relevance of B-cell epitope multivalency, downsized versions of the dendrimeric constructions, with two copies of the B epitope (B2T) were tested and compared to dendrimers bearing four copies (B4T), in the mice model, and we found that B2T constructions elicited similar or even better B- and T-cell specific responses than B4T (2). Interestingly, we also found that modifications on the conjugation chemistry used to attach B- and T-cell epitopes influenced the immunogenicity of the dendrimers, which was highest for maleimide-based conjugates.

In light of these results, in the present study we have compared the immunogenicity and protection against viral challenge elicited by B4T (thioether conjugate) and B2T (thioether or maleimide conjugates) in swine, an FMDV natural host. Efficient induction of neutralizing antibodies and optimal release of IFNγ was elicited by the three constructions, but in correlation with the mice model results, differences in immunogenicity and protection were again found. Taken together, our results provide useful insights for a more accurate design of FMD subunit vaccines.

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(PO 106)

ANALYSIS OF GROWTH OF HERPES SIMPLEX VIRUS TYPE 1 RECOMBINANT (HSV41GF) WITH EGFP EXPRESSION AND DEFICIENT IN "SHUT OFF" PROTEIN (*VHS*) IN VERO AND HELA CELLS

L. LERMA, B. MARTÍN, D. RODRÍGUEZ, S. GUERRA, E. TABARÉS.

Department of Preventive Medicine, Public Health and Microbiology.School of Medicine.Autónoma University of Madrid. Spain.

Herpes simplex virus type 1 (HSV-1) affects a significant percentage of the human population and is responsible for a wide variety of diseases. Due to the impact HSV-1 has on the general population and in the clinical setting, it is important to gain an in depth understanding of the cellular mechanisms involved in HSV-1 infections, including viral immune evasion mechanisms. For example, the double-stranded RNA-dependent protein kinase (PKR) is one of the core players involved in the cellular innate response to viral infections as it inhibits protein synthesis, thereby preventing disease progression. Herpesviruses, however, have developed strategies to interfere with and evade these antiviral cellular innate responses, allowing them to replicate in cells even when PKR is active. One of the strategies used by HSV-1 includes the viral "shut off" protein (*vhs*), encoded by the viral gene UL41, which functions to induce the degradation of cellular and viral mRNAs, leading to inhibition of protein synthesis. Understanding the exact role of *vhs* as a viral immune evasion protein would certainly increase our understanding of this virus and the mechanisms by which HSV-1 circumvents the innate intracellular immune response.

Towards this end, the virus HSV41GF, deficient in *vhs*, was constructed by homologous recombination, using HSV-1 strain F as a parental virus and EGFP as selection marker. The virus was characterized by PCR and western blot analysis using mono-specific antibodies obtained in rabbits by immunization with the fusion protein beta-galactosidase-*vhs* produced in *E. coli*. The viral growth of this mutant virus was analysed in Vero and HeLa cells and compared with the parental virus. While HSV-1 strain F showed similar growth in both Vero and HeLa cells, the growth of HSV41GF in HeLa was approximately 2 logs lower than in Vero cells. Analysis of the influence of PKR on

HSV41GF infection was carried out by infecting HeLa, HeLa SC and HeLa PKR$^-$ cells with HSV41GF and comparing infections kinetics across all cell lines and with the parental virus. Results showed that PKR is the main kinase involved in HSV-1 infection and *vhs* deficiency correlates with a decrease in eIEF2alpha factor phosphorylation levels, even in the presence of PKR.

(PO 107)

CHARACTERIZATION OF A NOVEL HEPATITIS C VIRUS (HCV) VACCINE CANDIDATE BASED ON MVA EXPRESSING THE NEARLY FULL-LENGTH HCV GENOME AND LACKING C6L VACCINIA VIRUS IMMUNOMODULATORY GENE

M. QUIRÓS[1], J. GARCÍA-ARRIAZA[1], C. E. GÓMEZ[1], C. O. S. SORZANO[2] AND MARIANO ESTEBAN[1]

[1] Department of Molecular and Cellular Biology and [2] Biocomputing Unit.Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CNB-CSIC), Madrid, Spain.

Hepatitis C virus (HCV) remains a global problem despite advances in treatment. Thus, the development of a safe and efficacious vaccine against HCV is one of the main goals for prevention and control of hepatitis C. In an effort to improve the immunogenicity of the previously described HCV vaccine candidate (termed MVA-HCV), based on the poxvirus MVA vector expressing the nearly full-length HCV genome from genotype 1a (Core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and a part of NS5B), we have generated a novel optimized MVA-HCV vaccine candidate (termed MVA-HCV ΔC6L) containing a deletion of C6L vaccinia virus immunomodulatory gene, which encodes for an inhibitor of IFN-β. Deletion of C6L had no effect on virus growth kinetics or on the expression of HCV antigens; hence, the C6L protein is not essential for MVA-HCV replication. The innate immune responses triggered by MVA-HCV and MVA-HCV ΔC6L in human macrophages and monocyte-derived dendritic cells showed an upregulation of IFN-β, proinflammatory cytokines and chemokines. Furthermore, we have analyzed the immunogenicity elicited by MVA-HCV ΔC6L following either homologous or heterologous prime/boost immunization protocols in C57BL/6 vaccinated mice. The results showed that MVA-HCV and MVA-HCV ΔC6L induced high, broad and polyfunctional HCV-specific CD4$^+$ and CD8$^+$ T-cell adaptive and memory immune responses. Most of the vaccine-induced T-cell responses were mainly mediated by CD8$^+$ T cells, being CD4$^+$ T cells also induced but at a lower magnitude. Homologous immunization protocols elicited HCV-specific CD8$^+$ T cells mainly directed against p7+NS2 antigens, whereas in heterologous immunization protocols the main target was the NS3 protein. Significantly, HCV-specific CD4$^+$ T cells directed against E1 and E2 antigens were induced in the heterologous regimens. Moreover, in the memory phase, HCV-specific CD8$^+$ T cells with an effector phenotype were predominant. These findings highlight the relevance of vaccinia virus immunomodulatory genes in HCV vaccine design.

(PO 108)

THE ANTIVIRAL AND IMMUNOSTIMULATORY EFFECTS OF SYNTHETIC RNAs CORRESPONDING TO THE FOOT-AND-MOUTH DISEASE VIRUS (FMDV) NON-CODING REGIONS (ncRNAs) RELY ON INNATE RESPONSES TRIGGERED BY RIG-I AND TLR ACTIVATION

B. BORREGO[1], M. RODRÍGUEZ-PULIDO[2], C. REVILLA[3], B. ÁLVAREZ[3], F. SOBRINO[2], J. DOMÍNGUEZ[3], M. SÁIZ[2]

1. Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos, Madrid, Spain

2. Centro de Biología Molecular Severo Ochoa (CISC-UAM), Cantoblanco, Madrid, Spain

3. Dpto. de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain

The innate immune system is the first line of defense against viral infections. Exploiting innate responses for antiviral, therapeutic and vaccine adjuvation strategies is being extensively explored. We have previously described the ability of small in vitro RNA transcripts, mimicking the sequence and structure of different domains in the non-coding regions of the foot-and-mouth disease virus (FMDV) genome (ncRNAs), to trigger a potent and rapid innate immune response. These synthetic non-infectious molecules have proved to have a broad-range antiviral activity and to enhance the immunogenicity of an FMD inactivated vaccine in mice. Here, we have studied the involvement of pattern-recognition receptors (PRRs) in the ncRNA-induced innate response and analyzed the antiviral and cytokine profiles elicited in swine cultured cells, as well as peripheral blood mononuclear cells (PBMCs). Our results show that the FMDV ncRNAs are able to trigger a broad innate immune response in swine PBMCs and provide evidence for the involvement of both Toll-like receptors (TLRs) and cytosolic sensing (RIG-I-like receptors) of the FMDV ncRNAs, accounting for the induced type-I IFN and cytokine response. Altogether, our findings suggest that these synthetic non-infectious molecules may have an immunostimulatory activity in livestock, as well as a potential application as immunomodulatory compounds in new antiviral and vaccine formulations.

(PO 109)

INTERFERENCE OF SINGLE AND DUAL BIOTIC STRESSES ON HOST DNA METHYLATION PATHWAYS

E.M. TORCHETTI[1], M. PEGORARO[2], B. NAVARRO[1], M. CATONI[3], E. NORIS[2], F. DI SERIO[1]

[1].Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, UOS Bari, Italy

[2].Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Torino, Italy

[3].Sainsbury Laboratory, University of Cambridge, Cambridge, UK

DNA methylation (DM) pathways play major roles in preservation of genome integrity, transposon stability and regulation of gene expression. In plants, DM has also been involved in responses to abiotic and biotic stresses, including defense against geminiviruses (GV), a large group of viruses with a single-stranded DNA genome that replicates in the nucleus forming minichromosomes associated with cellular histones. It is proposed that host DM machinery impairs viral accumulation

in the infected tissues by targeting GV DNA for methylation. In contrast, whether DM is involved in the molecular interplay between plants and nuclear replicating viroids, which are infectious non-protein-coding RNAs frequently inducing severe diseases in plants, is still unclear. Viroid RNAs are targeted by host enzymes involved in DM pathways, but whether the genes implicated in this pathways are differentially regulated in response to viroid infection is unknown. In addition, whether DM pathways may differentially target host and GV DNA depending on the presence or absence of a nuclear infecting viroid is also not known. To further explore the interference of single and dual infections by nuclear replicating infectious agents, we have developed an experimental system based on tomato plants infected by the geminivirus *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and/or the nuclear-replicating *Potato spindle tuber viroid* (PSTVd). DNA methylation profiles of TYLCSV DNA and of two host genomic targets were tested as molecular sensors of host DM under stress conditions. Moreover, expression of genes involved in DM was investigated at transcriptional level by quantitative RT-PCR assays. Our data show that both TYLCSV and PSTVd interfere with the regulation of most host genes involved in DM pathways and, interestingly, that the plant response to a single stress strongly differs from that to dual stresses, with synergistic effects.

(PO 110)

COMPARISON OF THE IMMUNE RESPONSES AND EFFICACY AFTER CHALLENGE ELICITED BY RECOMBINANT MVAs EXPRESSING SINGLE RIFT VALLEY FEVER VIRUS GLYCOPROTEINS

ELENA LÓPEZ-GIL, GEMA LORENZO, SANDRA MORENO, ALEJANDRO MARÍN-LÓPEZ, JAVIER ORTEGO AND ALEJANDRO BRUN

Centro de Investigación en Sanidad Animal (INIA). Valdeolmos, Madrid, Spain

Rift Valley Fever virus (RVFV), a mosquito-borne bunyavirus widely distributed in Sub-Saharan countries, Egypt and the Arabian Peninsula, causes disease in both human and livestock and is now considered an emerging threat for non-endemic countries due to the movement of infected animals and insect vectors including mosquitoes. The ample range of competent mosquito vectors for RVFV in many areas of the Mediterranean basin suggests that RVF outbreaks in non-endemic areas could potentially end-up in establishment of enzootic infection cycles. If this happen it would cause serious concern for both public and animal health. It is therefore desirable to develop control tools as well as enhance our knowledge about the immune mechanisms that correlate with the protection elicited by RVFV vaccines. In this work we have characterized the efficacy and immune response of recombinant MVA viruses expressing RVFV glycoproteins Gn and Gc. Previous data obtained in our laboratory showed that a single inoculation of MVA expressing both Gn and Gc was sufficient to induce a protective immune response in

mice after a lethal challenge with RVFV. The protection elicited by the MVA vaccination was related to the presence of glycoprotein specific CD8+ cells, in the absence of a consistent detection of neutralizing antibodies in vitro. To study the contribution of each glycoprotein antigen to protection a similar approach was extended to vaccines expressing only a single RVFV glycoprotein (either Gn or Gc). Our results suggest that protection of BALB/c mice upon RVFV challenge can be mediated by the activation of a strong cellular response (mainly against Gc epitopes) in the absence of a clear induction of neutralizing antibodies. However, this protection may be restricted to specific genetic backgrounds determining susceptibility to infection as shown by the lack of survival upon challenge of 129SvEv mice immunized with the same vaccines (MVAGn or MVAGc). Our data also point out that the expression of both glycoproteins enhances humoral immunogenicity perhaps explaining the higher protection rates in MVAGnGc vaccinated 129 SvEv mice. The detection of IL-2 and IL-6 supports the induction of cellular responses since both cytokines play a role in T-cell survival and activation. Thus, the identified Gc specific CD8+ T-cell population may act as a key component in the protection after challenge observed in the MVA immunized mice, contributing to the elimination of infected cells and reducing morbidity and mortality.

(PO 111)

ANALYSIS OF THE ANTIVIRAL ACTIVITY OF SILVER NANOPARTICLES AGAINST RIFT VALLEY FEVER VIRUS IN VITRO AND IN VIVO

B. BORREGO[1], G. LORENZO[1], H. ALMANZA[2], F. MATEOS[1], E. LÓPEZ-GIL[1], N. DE LA LOSA[1], V. A. BURMISTROV[3], A. N. PESTRYAKOV[4], A. BRUN[1], N.BOGDANCHIKOVA[5]

[1] *Centro de Investigación en Sanidad Animal, INIA, Valdeolmos, Spain.*

[2] *FMyP- Universidad Autónoma Baja California, Tijuana, Mexico*

[3] *Vector-Vita Ltd, Novosibirsk, Russia*

[4] *Tomsk Polytechnic University, Tomsk, Russia*

[5] *Centro de Nanociencias y Nanotecnología, UNAM, Ensenada, Mexico*

Rift Valley Fever virus (RVFV) is a mosquito borne pathogen causing an important disease in ruminants often transmitted to humans after epizootic outbreaks, thus becoming a very relevant pathogen for animal health due to the economic losses associated, and also for human health. Currently there is no available treatment or licensed Rift Valley fever vaccine for human use, therefore the development of new approaches able to inhibit viral replication and transmission allowing an efficient control of the disease is a must. Silver nanoparticles have been described to exert some inhibitory effect against some enveloped viruses belonging to different families. Compared to the classical antiviral approaches, the use of metal nanoparticles poses many advantages, mainly the non-emergence of resistant variants, as well as their safety and low cost.

In this work we have tested the antiviral potential against RVFV infection, both in cell culture and in animal models, of silver nanoparticles formulated as Argovit. Though the ability of silver nanoparticles to control an ongoing RVFV infection in the conditions tested seems to be limited, the incubation of virus with Argovit before the infection leads to a reduction of viral infectivity both in vitro and in vivo. Our results reveal the potential application of the microbicidal properties of silver nanoparticles to control the infectivity of this important zoonotic pathogen.

(PO 112)

ANTI-INFLAMMATORY PROPERTIES OF THE SECRET DOMAIN FROM POXVIRUS TNF RECEPTORS

C. SÁNCHEZ[1], A. ALEJO[2], S.J. MARTIN PONTEJO[1], P. FALLON[3] AND A. ALCAMI[1].

[1] *Centro Biología Molecular Severo Ochoa, (CBMSO). Madrid. Spain.*

[2] *Centro de Investigación en Sanidad Animal (INIA). Madrid. Spain*

[3] *Trinity College. Dublín. Ireland.*

Poxviruses encode numerous proteins devoted to the control of the host's immune response, including a set of secreted, cytokine binding proteins that act mainly as competitive inhibitors of their ligands. Amongst these, a family of virally encoded TNF receptors (vTNFRs) with homology to their cellular counterparts are thought to have important roles during infection. Ectromelia virus, the causative agent of mousepox, encodes a single active vTNFR named CrmD which is known to block effectively TNFa. Additionally, CrmD contains a structurally distinct domain termed, the SECRET domain, that can bind and block the activity of a reduced set of chemokines. This domain was identified in several other poxviral secreted proteins. To address the possible concerted anti-inflammatory role of both domains, we generated recombinant ectromelia viruses lacking CrmD or expressing a truncated version that blocked TNF but not chemokine activity. We found CrmD to block the inflammatory footpad swelling reaction in vivo, with the SECRET domain contributing significantly to this activity. We next tested the ability of recombinant CrmD or a truncated version lacking the SECRET domain to block inflammation in a murine model of rheumatoid arthritis. Both approaches confirmed that the presence of a chemokine binding domain enhanced the anti-inflammatory potential of a vTNFR in vivo. Because secreted human TNFRs are currently used in the clinic for the treatment of several inflammatory conditions, we reasoned that addition of a chemokine binding domain to such a protein might enhance is activity. Therefore, we generated a set of secreted hTNFRs fused to SECRET domains derived from different viral proteins and screened them for correct TNF and chemokine inhibitory activity. One selected construct was further purified and its binding and inhibitory activity characterized in vitro and in cell culture. Finally, we determined the anti-inflammatory activity of this recombinant hTNFR-SECRET protein in vivo, showing its ability

to block the development of a rheumatoid arthiritis like disease.

(PO 113)

SECRETOR STATUS DETERMINES SUSCEPTIBILITY TO DIARRHEA BUT NOT TO INFECTION IN A FAMILY NOROVIRUS OUTBREAK

N. CARMONA VICENTE, M. FERNÁNDEZ JIMÉNEZ, S. VILA VICENT, J. RODRÍGUEZ DÍAZ, J. BUESA

Departamento de Microbiología, Facultad de Medicina, Universidad de Valencia, Avda. Blasco Ibáñez 17, 46010 Valencia, España

Human noroviruses (NoVs) are the main cause of non-bacterial gastroenteritis worldwide. Several studies have associated NoV susceptibility to human histo-blood group antigens (HBGAs), namely to the secretor status (*FUT2* gene expression), and to Lewis antigens (Le^a and Le^b) determined by the *FUT3* gene. Earlier volunteer and outbreak studies showed that only secretor-positive individuals were infected, either symptomatically or asymptomatically. However, more recent studies have demonstrated that secretor-negative individuals may also be infected by NoVs, and that the susceptibility to infection can be genotype-specific. A NoV gastroenteritis outbreak occurred in a household of 9 family members early January 2010, giving us the opportunity to study the susceptibility to NoVs of the different individuals involved. To reach our aim we recruited 22 volunteers including 8 members of the family affected by the outbreak and their secretor status, ABO and Lewis antigens were analyzed. The binding of different NoV VLPs to their saliva samples and the NoV-specific salivary and serum IgA and IgG antibody titers and their capacity to block virus binding to their receptors were assayed.

The results showed that the outbreak was caused by a GII.4-New Orleans- 2009 variant. The most relevant finding was that an asymptomatic non-secretor individual also shed NoVs in his stools.

We observed that different NoV VLPs showed different binding patterns determined by HBGAs and that the non-secretors saliva was poorly recognized by any of the VLPs GII.4 variants. Interestingly, anti-NoV IgA antibody levels both in saliva and in serum samples, from secretor and non-secretor individuals, showed no differences, while only high norovirus-specific IgG antibody titers were found in both convalescent sera (collected 14 days post-infection) and in memory sera (collected 1 year post-infection) in secretor positive individuals.

It was examined the capability of the different sera (both convalescent and memory sera) to block the binding of VLPs to the saliva of a secretor-positive O blood type donor, using GII.4-2006b variant VLPs. As expected, the binding to receptors present in the saliva was blocked efficiently by secretor positive sera, up to 68%. In contrast, non-secretor sera did not block the binding at all.

This results reinforce the idea that susceptibility to human NoVs is both dependent on HBGAs profile of the individuals, as well as on the NoV genotype and variant. We also show that the immunity to NoV lasts for at least one year after infection, showing that symptomatic infection strongly stimulates the immune system.

(PO 114)

THE INTERFERON-INDUCED ANTIVIRAL FACTOR MxA DOES NOT INHIBIT VACCINIA VIRUS REPLICATION

M M. LORENZO, J.M. SANCHEZ-PUIG, AND R. BLASCO

Departamento de Biotecnología, INIA. Carretera de la Coruña km 7.5, 28040 Madrid, Spain

Mx proteins contribute to the antiviral response induced by type I and type III interferons. They belong to the dynamin family of large GTPases, but their molecular mecanism of action is currently unknown. Interestingly, different Mx proteins display widely different ranges of activities when assayed on unrelated viruses. For instance, human MxA has antiviral activity against many RNA viruses. The activity of different Mx proteins seems to depend on the subcellular localization of the protein. Nuclear forms (like mouse Mx1) protect against viruses that replicate in the cell nucleus, while cytoplasmic forms (like mouse Mx2) inhibit replication of VSV and some other viruses that replicate in the cytoplasm. Remarkably, the human MxA protein, which is localized to the cytoplasm, has a broad antiviral spectrum irrespective of the virus replication compartment.

Interestingly MxA has been reported to have inhibitory activity against some large DNA viruses like African Swine Fever virus and Monkeypox virus (Netherton et al 2009 Inhibition of a large double-stranded DNA virus by MxA protein. J Virol. 83:2310-20; Johnston et al. 2012 In vitro inhibition of monkeypox virus production and spread by Interferon-β. Virol J. 2012, 9:5). However, Vaccinia virus (VV) can grow efficiently in cells pretreated with IFN-I, that express high levels of MxA, suggesting that virus replication is not blocked by these, or other, IFN-induced antiviral proteins.

We have constructed a cell line inducibly expressing human MxA, and showed that MxA expression was able to block replication of RNA viruses like Vesicular Stomatitis virus (VSV). Vaccinia virus was able to replicate unabated in those cells, in conditions in which VSV replication was severely inhibited, indicating that Vaccinia virus was not being affected by the presence of MxA. Further, a Vaccinia virus recombinant overexpressing MxA from viral promoters was able to grow to wild-type levels and form wild-type sized virus plaques, further demonstrating the lack of effect of MxA on Vaccinia virus replication.

We considered the possibility that Vaccinia virus resistance might be the result of virus- encoded MxA counteracting factor(s). To test this hypothesis, we carried out coinfections of Vaccinia virus and VSV in conditions of MxA blockage of VSV replication. Consistently, we failed to detect cross protection of VSV from the action of MxA by the coinfecting Vaccinia virus, suggesting that Vaccinia virus resistance to Mx is due to lack of susceptibility of the virus and not to counteraction by trans-acting factors expressed by vaccinia virus.

(PO 115)

IMMUNE RESPONSE ELICITED BY DENDRIMERIC PEPTIDES AGAINST CSFV IN DOMESTIC PIGS. A NEW PATH TO DIVA STRATEGY

J. A. BOHÓRQUEZ[1], S. MUÑOZ-GONZÁLEZ[1], S. DEFAUS[2], B.G. DE LA TORRE[2], M. PEREZ-SIMÓ[1], R. ROSELL[1,3], M. DOMINGO[1,4], D. ANDREU[2], L. FRAILE[5] AND L. GANGES[1]*

[1]*Centre de Recerca en Sanitat Animal (CReSA), Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus de la UAB, Bellaterra, Barcelona, Spain*

[2]*Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, 08003 Barcelona*

[3] *Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, (DAAM), Generalitat de Catalunya, Spain*

[4]*Departamento de Sanitat i d'Anatomia Animals, Facultat de Veterinària, UAB, Bellaterra-Barcelona*

[5]*Departament de Producció Animal, ETSEA, Universidad de Lleida, 25198, Spain*

Classical swine fever virus (CSFV) impairs the immune system of the host. The degree of immune compromise is one of the determining aspects in the outcome of the disease. Previous studies have shown the existence of B and T cell epitopes into CSFV, mainly in the E2 and NS3 proteins. Dendrimers represent a promising tool for the multimeric presentation of epitopes in candidate vaccines. This strategy can be useful for basic investigations of the mechanisms governing the induction and control of immunity. The aim of this work was to evaluate the CSFV specific immune response generated by different epitopes within E2 and NS3 CSFV proteins, combining them with a T helper epitopes reported from Foot and mouth disease virus (FMDV) and Peste des petits ruminants virus (PPRV), using the multimerisation strategy. Five dendrimeric constructs in different conformations were formulated and inoculated in five groups (four animals each) of six-week old pigs (Landrace x Large White), while another group (control group) was inoculated with NaCl 0.9%. Two doses of 2 mg each of the corresponding construct, mixed with Montanide v206 adjuvant (Seppic), were administered at days 0 and 21 of the trial. An experimental challenge with CSFV was performed with 10^5 $TCID_{50}$ of CSFV (strain Margarita) 15 days after the second immunisation. Additionally, four pigs (vaccination controls) were immunized with one dose of a commercial live-attenuated vaccine (C-strain) and were challenged with the same inoculum at 16 days post vaccination (dpv). Humoral as well as cellular immune response were evaluated in the 28 pigs at 8 different dates after vaccination and challenge. Different levels of partial protection from clinical signs were observed in the five dendrimeric immunized groups. Interestingly, the best clinical protection was found in two groups inoculated with the same peptides in different conformations (B4T or B2T). In terms of CSFV specific humoral response analysed against the E2 protein, one of the dendrimeric peptides developed a faster humoral response at 8 days post challenge. The cellular response was evaluated through INF-γ producing cells, with two groups showing INF-γ levels after stimulation with PHA on the day of Challenge (0 DPI) and one of this groups increasing this response even further at 8 DPI. The specific humoral and cellular response against every peptide will be

presented. This results show the capacity of a previously reported epitope for a monoclonal antibody to induce immune response in pigs.

(PO 116)

INTERFERENCE OF MERS-CoV ACCESSORY GENES WITH THE INNATE IMMUNE RESPONSE AND ITS CONTRIBUTION TO VIRULENCE

I. SOLA[1], J. CANTON[1], F. J. GUTIERREZ-ALVAREZ[1], L. MORALES[1], S. ZUÑIGA[1], M. T. SANCHEZ-APARICIO[2], A. GARCIA-SASTRE[2], AND L. ENJUANES[1]

[1]*Department of Molecular and Cell Biology.National Center of Biotechnology (CNB-CSIC).Campus Universidad Autonoma Madrid. Madrid. Spain*

[2]*Department of Microbiology.Icahn School Medicine at Mount Sinai. New York. USA*

Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging coronavirus infecting humans, associated with acute pneumonia, occasionally renal failure, and a high mortality rate (as of February 28th 2015, 1030 laboratory-confirmed cases have been reported, including at least 381 related deaths), which is considered a public health threat. A reverse genetics system for MERS-CoV has been developed by the construction of an infectious cDNA clone inserted into a bacterial artificial chromosome, providing a tool to study the virus molecular biology and to develop attenuated viruses as vaccine candidates. A collection of recombinant MERS-CoVs deficient in the genus-specific genes 3, 4a, 4b and 5 was generated from cDNA clones. The growth kinetics of mutant viruses was similar to that of the wild-type virus, indicating that accessory genes were not essential for MERS-CoV replication in cell cultures. Infection with rMERS-CoV-Δ4ab deficient in 4a and 4b proteins significantly increased the expression of cytokines regulating the inflammatory and innate immune responses, suggesting their contribution to the inhibition of the NF-□B signaling pathway during infection. Consequently, these genes might modulate pathogenesis. rMERS-CoV-Δ4ab infection induced the formation of stress granules (SG), suggesting the involvement of 4a-4b proteins in the inhibition of the SG-mediated antiviral response. In contrast to the deletion of accessory genes, an engineered virus lacking the structural envelope, E, protein (rMERS-CoV-ΔE) was not successfully rescued, since viral infectivity was lost at early passages. Interestingly, rMERS-CoV-ΔE was rescued and propagated in cells transiently or stably expressing E protein in trans, indicating that rMERS-CoV-ΔE virus was replication-competent and propagation-defective. Therefore, the rMERS-CoV-ΔE is potentially a safe and promising vaccine candidate.

(PO 117)

REGULATION OF EBOLA VIRUS MATRIX PROTEIN BY SUMO

P. ALVARIÑO[1], C.F. DE LA CRUZ-HERRERA[2], A. EL MOTIAM[1], M. BAZ-MARTÍNEZ[1], P. RUIBAL[3], J.V. PÉREZ-GIRÓN[3], V. LANG[4], M.S. RODRÍGUEZ[4], C. MUÑOZ-FONTELA[3], C. RIVAS[1,2]

[1]*Centro de Investigación en Medicina Molecular (CIMUS), Universidade de Santiago de Compostela, Instituto de Investigaciones Sanitarias (IDIS), Santiago de Compostela, Spain.*

[2]*Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC, Madrid, Spain*

[3]*Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany*

[4]*Ubiquitylation and Cancer Molecular Biology laboratory, Inbiomed, San Sebastián-Donostia, Spain*

Ebola virus (EBOV) causes a severe and often fatal febrile syndrome in humans. The EBOV genome encodes seven genes, the most abundantly expressed of which is viral protein 40 (VP40), the major viral matrix protein. VP40 associates with cellular membranes and coordinates numerous functions in the viral life cycle of EBOV, including regulation of viral transcription, morphogenesis, packaging and budding of mature virions. In addition, expression of VP40 is sufficient to generate virus-like particles (VLPs) that have similar characteristics to the actual infectious virus. VP40 has been shown to interact with host cell factors such as the endosomal sorting complex required for transport (ESCRT) machinery, COPII proteins, and actin, which have been implicated in the budding, transport, and movement of VP40, respectively. Moreover, release of Ebola VLPs seems to require the tyrosine phosphorylation of VP40 by the c-Abl1 tyrosine kinase. Here we evaluated the regulation of VP40 by SUMO and analyzed the effect of VP40-SUMO interaction on the VP40 egress, VLPs formation, and interaction with other viral components. Our results reveal that SUMO plays an important role on VP40 functions.

(PO 118)

PRESENCE OF PATHOGENIC ENTERIC VIRUSES IN ILLEGALLY IMPORTED MEAT AND MEAT PRODUCTS TO EU BY INTERNATIONAL AIR TRAVELERS

D. RODRÍGUEZ-LÁZARO[1,2],M. DIEZ-VALCARCE[3], R. MONTES-BRIONES[3], D. GALLEGO[4], M. HERNÁNDEZ[1], J. ROVIRA[3]

[1]*Instituto Tecnológico Agrario de Castilla y León, ITACyL, Valladolid, Spain*

[2]*Área de Microibología, Facultad de Ciencias, Universidad de Burgos, Burgos, Spain,*

[3]*Departamento de Biotecnología y Ciencia de los Alimentos, Facultad de Ciencias, Universidad de Burgos, Burgos, Spain,*

[4] *Dependencia de Sanidad de Vizcaya, Delegación del Gobierno en el País Vasco, Bilbao, Spain*

One hundred and twenty two meat samples confiscated from passengers on flights from non-European countries at the International Airport of Bilbao (Spain) were tested for the presence of the main viral pathogens (human norovirusesgenogroups I and II, hepatitis A and E viruses) during 2012 and 2013. A sample process control virus, murine norovirus, was used along the whole process to evaluate the correct performance of the method. Overall, 67 samples were positive for at least one

enteric viruses, being 65 positive for hepatitis E virus(53.3%), 3 for human norovirus genogroup I (2.5%) and 1 for human norovirus genogroup II (0.8%), whereas hepatitis A virus wasnot detected in any sample. The type of positive meat samples was diverse, but mainly was pork meat products (64.2%). The geographical origin of the positive samples was wide and diverse; samples from 15 out 19 countries tested were positive for at least one virus. However, the estimated virus load was low, ranging from 55 to 9.0×10^4 PDU per gram of product. The results obtained showed the potential introduction of viral agents in travelers' luggage, which constitute a neglected route of introduction and transmission.

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DECIPHERING THE PARTNERS OF GEMIN5 IMPACTING ON TRANSLATION CONTROL

R. FRANCISCO-VELILLA, J. RAMAJO, E. MARTÍNEZ-SALAS

Centro de Biología Molecular Severo Ochoa, CSIC, Madrid, Spain

Initiation of translation of several RNA virus genomes is governed by internal ribosome entry sites (IRES) elements. IRES function depends on the interaction with eukaryotic initiation factors (eIFs) and cellular RNA-binding proteins termed IRES transacting factors (ITAFs). Mass spectrometry analysis of factors interacting with two viral IRES (foot-and-mouth disease virus (FMDV) and hepatis C (HCV)) allowed the identification of proteins interacting with specific RNA domains. One of these factors is Gemin5, the RNA-binding factor of the survival of motor neurons (SMN) complex that assembles Sm proteins in splicesomal snRNPs. We have shown that, beyond its role in snRNPs biogenesis, Gemin5 acts as a down-regulator of translation (1), competing out PTB from its binding site on the FMDV IRES (2). The minimal region of Gemin5 being able to repress internal initiation of translation in cells depleted of the endogenous protein was mapped to the most C-terminal domain ($G5_{1383\text{-}1508}$) (3). However, deciphering the potential partners of this factor influencing translation control is a challenging unresolved question. To determine the mechanistic basis of the role of Gemin5 on translation control we have undertaken the analysis of a potential relation of Gemin5 with the ribosomal particles. In addition, we carried out tandem affinity purification (TAP) using different regions of the protein followed by mass spectrometry analysis to identify factors bound to Gemin5. To get information about factors linked to Gemin5 by RNA bridges, we conducted the TAP purification after exhaustive RNase A treatment during. Candidates were chosen based on both high score and biological function, aimed at discovering new regulatory pathways impacting on translation control. Promising candidates have been produced as GST-fusions and used in pull-down assays with different regions of Gemin5 to verify whether the interaction was direct. Results on the most promising candidates influencing the role of Gemin5 on translation control will be presented.

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(PO 120)

RNA POLYMERASE SLIPPAGE AS A MECHANISM FOR THE PRODUCTION OF FRAMESHIFT GENE PRODUCTS IN PLANT VIRUSES OF THE POTYVIRIDAE FAMILY

DAVID SAN LEÓN *[1], ADRIAN VALLI *[2], ARES MINGOT *[3], DAVID BAULCOMBE [2], JUAN J. LÓPEZ-MOYA [3], JUAN A. GARCÍA [1], BERNARDO RODAMILANS *[1]

* These authors equally contributed to this work

[1] *Centro Nacional de Biotecnología CNB, CSIC, Darwin 3, 28049-Madrid, Spain*

[2] *Department of Plant Sciences, University of Cambridge, Downing street, Cambridge CB2 3EA, United Kingdom*

[3]*Centre for Research in Agricultural Genomics CRAG, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, 08193¬Barcelona, Spain*

Viruses can use polymerase slippage to generate newly synthesized RNAs with nucleotide insertions or deletions, and thus produce alternative proteins relevant for infection in overlapping open reading frames. This phenomenon, that is well described for some animal viruses (EBOV, MARV, HCV), has not been observed in any plant-infecting virus. The present work uses high-throughput sequencing to study RNA polymerase slippage during natural infections of viruses from the Potyviridae family, the largest and economically most important group of plant RNA viruses, which is included in the Picorna¬like super group of viruses. The detection of modified RNA molecules suggests that the alternative frameshift product P3N+PIPO is produced, at least partially, through this mechanism. Besides, we describe the production of RNA molecules with an extra A inside the P1 coding sequence, which would be originated in a similar polymerase slippage event and yield a novel ORF product, P1N+PISPO in the infection of diverse sweet potato¬infecting potyviruses. Altogether, these findings suggest that slippage might be a general property of viral RNA polymerases that can be exploited by viruses of different kingdoms to expand the coding capacity of their small genomes contributing to viral adaptation and evolution.

(PO 121)

FREQUENCY OF HEPATITIS C VIRUS GENOTYPE 1A NS3, NS5A AND NS5B MUTATIONS ASSOCIATED TO ANTIVIRAL RESISTANCE

A. AVELLÓN CALVO, A. ZAPATA, M.C. GARCÍA GALERA, G. RODRÍGUEZ RODRÍGUEZ

Unidad de Hepatitis. Centro Nacional de Microbiología. Instituto de Salud Carlos III. Madrid

Introduction:Routine resistance associated variants (RAVs) analysis in hepatitis C virus (HCV) infection is currently limited to 80K NS3 mutation pretreatment in genotype 1a, according to clinical guidelines. However recent reports indicate that some other NS3 or NS5A substitutions may predict the virologic failure (VF).

Objective:To describe the frequency of RAVs in NS3, NS5A and NS5B in HCV genotype 1a infected patients, candidates for antiviral treatment.

Material and methods:Samples: serum from: 219 patients (analysis of NS3), 62 patients (NS5A) and 55 patients (NS5B); Methods: in house developed nested PCR systems.

Results:

- NS3 RAVs: 80K (5%); 80R (0%); 122R (0.9%); 155K (3.1%); 168AEV (0%); 170T (0%)
- NS3 mutations in scored positions: 122TGNC (10.3%), 170VP (4.5%)
- NS5A RAVs: 30R (2.2%), 30H (2.2%), 30R+28L (1.6%), 31M+30C (1.6%)
- NS5A mutations in scored positions: 28M (1.6%), 58P (6.4%), 58R+28L (1.6%)
- NS5B RAVs: none
- NS5B scored positions: 316NC: 5.4%
- NS3+NS5A RAVs: 3/62 (4.8%)

(NS3:80K+NS5A:58L+28L; NS3:80K+NS5A:28M; NS3:122G+NS5A:30R)

Conclusions:

NS3 RAVs other than 80K are almost as frequent as 80K (4% versus 5%).

NS5A RAVs reached to 7.7%.

NS5B RAVs were not detected in our samples, in agreement with the low RAVs rates previously reported.

Almost a 5% of the studied samples combine both NS3 and NS5A RAVs mutations.

The role of routine testing of RAVs previously to treatment is not defined yet.

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EFFECTS OF VALPROIC ACID ON HSV-1 INFECTIONS

ANTONIO CRESPILLO[1], BEATRIZ PRAENA[1], LUIS ÁNGEL DORADO[1], LAURA LERMA[1], ENRIQUE TABARÉS[1], FRANCISCO SOBRINO[2], RAQUEL BELLO-MORALES[1], JOSÉ ANTONIO LÓPEZ GUERRERO[1].

1: Universidad Autónoma de Madrid, Madrid, Spain.

2: Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

Valproic acid (VPA) is a small fatty acid used as drug in different neurologic diseases such as epilepsy, migraines or bipolar disorders. VPA acts by inhibiting histone deacetylases (HDACs) in the cell nucleus, GABA pathways, Na^+ channel in cellular membranes, glycogen synthase kinase 3 (GSK3), protein kinase A (PKA) and lipid metabolism. On the other hand, many studies aim at the feasible role of VPA in demyelinating diseases (e.g. multiple sclerosis) and its effect on the susceptibility of several cell types to the infection of HIV, EBV and others viruses.

Taken these data into account and the fact that HSV-1 has been involved in some neuropathies, we have characterized the effect of VPA on this herpesvirus infection of the human oligodendrocyte cell line HOG. First of all, the role of this compound in virus entry was tackled. Incubation with VPA induced a slight but reproducible inhibition in the virus particles uptake. In addition, transcription and expression of viral proteins were significantly downregulated in the presence of VPA as well. Last but not least, the viral production was assessed with or without the inhibitor of HDACs, measured by

means of TCID50 assay. Surprisingly, and in comparison with the results shown above, VPA dramatically blocked the detection of infectious HSV-1 particles.

In conclusion, VPA, a clinical compound currently used in convulsant and others neurologic disorders, could be considered in a future as a therapeutic alternative against viral infections such as several herpesvirus members.

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PHOSPHORYLATION OF CORONAVIRUS NUCLEOCAPSID PROTEIN MODULATES VIRUS-HOST INTERACTIONS

S. ZUÑIGA, M. BECARES, A. PASCUAL-IGLESIAS, I. SOLA, AND L. ENJUANES.

Department of Molecular and Cell Biology.National Center of Biotechnology (CNB-CSIC). Madrid. Spain.

Phosphorylation-based networks are essential for cell proper function and phosphorylate viral proteins leading to a fine-tuning of virus-host interaction. Coronavirus (CoV) nucleocapsid (N) protein is a multifunctional phosphoprotein with key functions for both CoV life cycle and CoV-host interaction, as this protein affects multiple pathways in infected cells. N protein phosphorylated residues have been identified in several CoV members from all genera. N protein phosphorylation during CoV infection was analyzed using transmissible gastroenteritis virus (TGEV) as a model. A combination of OffGel and Western-blot techniques was used. N protein phosphorylation pattern changed during virus replication cycle. This protein was hypophosphorylated in the viral particle, as determined using mass-spectrometry analysis. In contrast, CoV N protein was hyperphosphorylated at early times post-infection, suggesting that phosphorylation may modulate early events during viral infection. To study the role of CoV N protein phosphorylation during viral infection six recombinant TGEVs, each one including sets of grouped sequential phosphorylable residues mutated to alanine were constructed. Overall, these residues accounted for 33 TGEV N protein predicted phosphorylation sites. Mutant viruses were recovered with peak titers similar to those of the wild type virus, suggesting that N protein phospho-mutants did not significantly affect virus behavior in cell cultures, with the exception of two mutants showing a delayed viral growth and reduced genomic RNA accumulation at early times post infection. Different patterns of interaction between phospho-mutant N proteins and host-cell proteins were observed. According to these patterns we proposed that N protein phosphorylation extent affects its interaction with host cell pathways, modulating CoV virulence.

(PO 124)

IDENTIFICATION OF A GAIT-LIKE RNA MOTIF AT THE 3' END OF THE TRANSMISSIBLE GASTROENTERITIS CORONAVIRUS GENOME MODULATING INNATE IMMUNE RESPONSE

S. MÁRQUEZ-JURADO[1], S. ZÚÑIGA[1], L. ENJUANES[1], F. ALMAZÁN[1]

[1]. Department of Molecular and Cell Biology, Centro Nacional de Biotecnología (CNB-CSIC), Campus Universidad Autónoma de Madrid, Madrid, Spain

Coronavirus (CoV) replication and transcription are complex processes that

require the specific recognition of RNA *cis*-acting elements located at the ends of the viral genome and are mediated by a huge protein complex encoded by the viral replicase gene together with cellular proteins. In previous studies using transmissible gastroenteritis CoV (TGEV) as a model, nine cellular proteins interacting with the 3' end of the genome were identified, from which a functional role on CoV RNA synthesis was demonstrated for heterogeneous nuclear ribonucleoprotein (hnRNP) Q, glutamyl-prolyl-tRNA synthetase (EPRS), arginyl-tRNA synthetase (RRS), and poly(A)-binding protein. In this work, the RNA motifs interacting with these proteins were further analyzed to study their mechanisms of action. A 32-nt RNA motif located at 410 nt from the 3' end of the TGEV genome was found to specifically interact with aminoacil tRNA synthetases EPRS and RRS. This RNA motif has high homology in sequence and secondary structure with the gamma interferon activated inhibitor of translation (GAIT) element, which is located at the 3' end of several mRNAs encoding proinflammatory proteins. The GAIT element is involved in the translation silencing of these mRNAs through its interaction with the GAIT complex (EPRS, hnRNP Q, ribosomal protein L13a, and glyceraldehyde 3-phosphate dehydrogenase) to favor the resolution of inflammation. Similarly to the cellular GAIT element, the viral RNA motif bound the GAIT complex and inhibited the *in vitro* translation of a chimeric mRNA containing this RNA motif, suggesting that the viral RNA motif could constitute the first GAIT-like motif described in a positive RNA virus. To test the functional role of the GAIT-like motif during TGEV infection, a recombinant virus harboring mutations in this motif was engineered and characterized. Mutation of the GAIT-like motif did not affect virus growth in cell cultures, indicating that the GAIT-like motif was dispensable for TGEV replication in cell culture. However, an exacerbated innate immune response, mediated by the melanoma differentiation-associated gene 5 pathway, was observed in cells infected with the mutant virus compared with the parental virus. Furthermore, the mutant virus was more sensitive to interferon beta than the parental virus. Altogether, these data strongly suggest that the viral GAIT-like RNA motif modulates the host innate immune response.

(PO 125)

RELEVANCE OF GENUS-α CORONAVIRUS NSP14 DOMAINS IN VIRUS VIABILITY

M. BECARES, A. PASCUAL-IGLESIAS, I. SOLA, L. ENJUANES, AND S. ZUÑIGA.

Department of Molecular and Cell Biology.National Center of Biotechnology (CNB-CSIC). Campus Universidad Autónoma de Madrid. Darwin 3. Madrid, Spain.

The replication and maintenance of the largest RNA genome known is a hallmark of coronaviruses (CoVs). As a consequence, these viruses encode a unique set of RNA modifying enzymes in the replicase gene. One of them is non-structural protein 14 (nsp14) that is part of the CoV core replication-transcription complex, providing proofreading activity during CoV RNA synthesis. Nsp14 is a bifunctional enzyme with 3'-5' exoribonuclease (ExoN) and guanine-N7-methyltranferase (N7-

MTase) activities. ExoN hydrolyzes single and double-stranded RNAs and is part of a mismatch repair system responsible for the high fidelity of CoVs replication. Betacoronavirus mutants lacking ExoN activity exhibit a mutator phenotype, with a 20 fold increase in the mutation frequency compared to wild-type viruses, and enhanced sensitivity to RNA mutagens such as 5-fluorouracil. ExoN mutant viruses exhibit decreased virulence in mouse models. Nsp14 N7-MTase activity is critical for viral mRNAs capping. The cap structure allows efficient viral mRNA translation and avoids their recognition as "non-self" by the host cell.

To elucidate the role of the different nsp14 domains in RNA synthesis and virus-host interaction, a set of mutants covering different motifs of the transmissible gastroenteritis virus (TGEV) nsp14 protein was engineered. The sequence changes include mutations in: (i) the ExoN active site that, according to published information, abolish ExoN activity, (ii) the zinc finger motif, which mediates nsp14 binding to RNA, (iii) the N7-MTase activity, and (iv) highly conserved regions in the N7-MTase domain. The effect of these mutations in CoV replication and transcription was analyzed using replicons. Six out of the ten mutants showed severe defects in RNA synthesis, with a moderate decrease of the replication levels and a strong to total reduction of transcription levels. This result confirmed the essential role of nsp14 protein during CoV RNA synthesis. Full-length infectious cDNA clones of those mutants showing efficient RNA synthesis were generated. In contrast to betacoronaviruses, recombinant TGEV viruses lacking ExoN activity were not recovered, despite their competence in viral RNA and protein synthesis after transfection. The mutant lacking N7-MTase activity was recovered, though it was highly prone to reversion. One of the mutants in the zinc finger domain was rescued and characterized. Further analyses are in progress in order to clarify the interaction of the mutant viruses with the host cell.

(PO 126)

EXPRESSION OF PSEUDORABIES VIRUS IE180 PROTEIN UNDER THE CONTROL OF HUMAN TUMOR-SPECIFIC PROMOTERS (hTERT AND CEA): II.- EFFECT OF IE180 ON APOPTOSIS INDUCTION

L. LERMA, S. ALCALÁ, B. MARTÍN, B. SAINZ JR., E. TABARÉS.

Department of Preventive Medicine, Public Health and Microbiology.School of Medicine.Autónoma University of Madrid. Spain.

Pseudorabies virus (PRV) belongs to the viral subfamily *Alphaherpesvirinae*. During productive infection, genes are temporally expressed in three ordered phases: immediate early (IE), early (E) and late (L) genes. IE genes are essentially transcription factors that induce E and L genes expression. PRV has a single IE gene, encoding the protein IE180, which has a negative self-regulating function on its own promoter, repressing its mRNA transcription. Like other herpes immediate early proteins, the role of PRV IE180 as a potent transactivator has been well studied and it has been shown that IE180 can activate the transcription of various human cellular and viral promoters. Recently, it has been demonstrated that

IE180 expression in transgenic mice produces neurological symptoms, suggesting a possible implication in the induction of apoptosis.

In this study, we produced recombinant PRV viruses that express IE180 protein under the control of the human tumor promoters: PRV-TER (human telomerase reverse transcriptase promoter, hTERT) and PRV-CEA (carcinoembryonic antigen, CEA) to better study the role of IE180 expression in vitro. The levels of IE180 mRNAs expressed under the control of tumor-specific promoters in the recombinant viruses constructed was measured by qPCR and compared to the parental virus vBecker2. PRV-TER-mediated IE180 mRNA expression levels were two-fold lower at 12 hpi but equal at 24 hpi compared to vBecker2, while PRV-CEA-mediated IE180 mRNA expression levels were 16- and 54-fold higher at 12 and 24 hpi, respectively. These results are consistent with those obtained previously in our laboratory by plasmid co-transfection, where we showed that IE180 reduced the activity of the hTERT promoter while an opposite activating effect was seen with the CEA promoter. The over-expression of IE180 in U2OS cells infected with PRV-CEA produced a higher cytopathic effect resulting in apoptosis induction, which was confirmed by Annexin V staining. The percentage of late apoptotic cells after PRV-CEA virus infection reached 3.47% compared to 0.31% in mock-noninfected cells and 0.52% and 0.46% in cells infected with vBecker2 or PRV-TER, respectively. In addition, the pro-apoptotic role of IE180 was additionally confirmed in PK15-IE180 cells. Thus, if sufficiently expressed, IE180 can induce apoptosis, a characteristic that could be beneficial for targeting specific cell types, such as cancer cells.

(PO 127)

CELLULAR SENESCENCE LIMITS VIRAL REPLICATION

M. BAZ-MARTÍNEZ[1,2], C.F. DE LA CRUZ-HERRERA[3], S. DA SILVA-ÁLVAREZ[2], A. FERREIRÓS[2], A. ELMOTIAM[1,2], M. COLLADO[2], C. RIVAS[1,3].

[1] *Centro de Investigación en Medicina Molecular (CIMUS), Universidade de Santiago de Compostela, Instituto de Investigaciones Sanitarias de Santiago de Compostela (IDIS), E15706 Santiago de Compostela, Spain*

[2] *Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), SERGAS, E15706 Santiago de Compostela, Spain*

[3] *Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC, Darwin 3, E28049 Madrid, Spain*

Cellular senescence is a stable proliferation arrest triggered in the cell as a response to stressful conditions that could endanger cell integrity. Although initially identified for cells after prolonged *in vitro* culture, the description of oncogene-induced senescence was paradigmatic in defining this process as a tumor suppressor mechanism, and in general as a stress response. Indeed, many other situations such as oxidative stress, fibrosis, DNA damage, etc, also result in senescence as a protective response. Surprisingly though, little is known regarding the relationship between cellular senescence and viruses. Here, we evaluated cellular senescence as a response triggered by viral infection and addressed the putative protective effect it

can have limiting viral replication. Our results demonstrate that whereas oncogenic viruses inhibit senescence, oncolytic viruses can stimulate senescence. Furthermore, we show that cellular senescence limits viral replication and evaluate the different mechanisms and pathways that may account for this antiviral activity.

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IDENTIFICATION OF FACTORS INTERACTING DIFFERENTIALLY WITH STRUCTURAL RNA MOTIFS

J. FERNÁNDEZ-CHAMORRO, E. MARTÍNEZ SALAS

Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain

Internal Ribosome Entry Site (IRES) elements are highly structured RNA that control initiation of translation in picornaviruses, among other RNA viruses. The IRES element of foot-and mouth disease virus (FMDV) is organized in five domains (1-5). Domains 2, 4, and 5 provide binding sites for eukaryotic initiation factors (eIFs) and RNA-binding proteins, designated IRES trans-acting factors (ITAFs). However, little is known about proteins interacting with domain 3. This domain (nts 86-299) is self-folding RNA that harbors conserved RNA motifs, essential for IRES activity. The apical region (nts 151-227) consist of a cruciform structure, of which the stem-loop 3a (nts 159-194) harbors the conserved GNRA motif. This motif has been proposed to participate in long-range interactions with the C-rich loop (located in transcript 137-249). To identify proteins able to recognize RNA structure-mediated patterns, we generated four transcripts (the entire domain 3, and subdomains C-rich, apical region and stem-loop 3a, encompassing nts 86-299, 137-249, 151-227 and 159-194, respectively). RNAs were expressed using tRNA scaffold vectors, which preserve their native RNA structure. Besides, the expressed RNAs are tagged with streptavidin aptamers that facilitate the capture of ribonucleoprotein complexes by using streptavidin-coated magnetic beads. Cellular factors associated to these RNAs were purified, and later identified by mass-spectrometry. Silver staining of SDS-PAGE gels loaded with the RNA-interacting proteins revealed different pattern of factors depending on the subdomain exposed to the cell lysate. Various groups of proteins, including eIFs, ITAFs, nucleic acid-binding proteins, trafficking factors, cytoskeleton, signalling factors and metabolic enzymes were unequivocally identified by mass spectrometry. The identified proteins were differentially purified with the RNA transcripts, disclosing factors that bind preferentially to certain RNA structures. Proteins belonging to different groups, ARF5, Rab1b, CELF-1 and RPS25, previously unknown to interact with the FMDV IRES, were selected to validate its ability to bind directly with the RNA structural subdomains by using *in vitro* approaches (UV-crosslink and RNA mobility assay). Our data show that these factors behave as IRES-binding proteins.

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REVERSE GENETICS FOR AVIAN REOVIRUS

Z. AFONSO PUERTA, L. K. BUSCH, C. DÍAZ JULLIEN, F. J. BENAVENTE MARTÍNEZ, J. M. MARTÍNEZ COSTAS

Departamento de Bioquímica y Biología Molecular, Centro Singular de Química Biológica y Materiales Moleculares (CIQUS), Universidad de Santiago de Compostela, 15782-Santiago de Compostela, Spain.

Avian reovirus (ARV) are the agents responsible for avian arthritis (infectious tenosynovitis) and malabsorption syndrome among other avian diseases. Molecular virologists have devoted much more attention to the study of mammalian reovirus (MRV), considered the prototype of genus Orthoreovirus (1). Recently, significant differences between the two groups of viruses were made evident. More significantly, ARV but not MRV are able to induce the formation of syncitia in the infected cells due to the presence of small FAST proteins (Fusion-Associated Transmembrane Protein) that cause membrane fusion. This characteristic lead to the division of the genus Orthoreovirus into two differentiated groups: non-fusogenic reoviruses, being MRV their prototype, and the fusogenic reoviruses, whose prototype virus is ARV. The most powerful technique in modern virology is reverse-genetics. Different systems have been developed for most virus families to produce infectious viruses from plasmid constructs. Reverse-genetics systems allow the researcher to develop viruses carrying designed mutations to make functional studies, with high reproducibility due to the absence of accumulated mutations because of serial virus passage. Reverse genetics also allows manipulating viruses to convert them into effective tools for gene-deliver, epitope exposure, etc. However, such a system could not be develop for any of the Reoviridae members until only 2007 (2). The inherent difficulty to develop reverse-genetics for reoviruses can be partly explained by the presence of a segmented genome with an exquisite selection method that picks up one (and only one) copy of each segment into every viral particle, together with the presence of precisely defined sequence ends in each genomic segment. In the last few years, several different methods have been described for the different members ot the Reoviridae (2, 3). ARV present an aditional difficulty when developing a method for reverse genetics, that is the need of using primary chicken embryo fibroblasts (CEF) for their in vitro culture. Here we describe the design and optimization of constructs and methods aiming to develop a general reverse genetics protocol for ARV.

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(PO 130)

STRONG REPLICATIVE SELECTIVE ADVANTAGE OF A MULTIPLY PASSAGED HEPATITIS C VIRUS IN CELL CULTURE

G. BLANCO[1], E. MORENO[1], N. BEACH[1], I. GALLEGO[1], A.I. DE ÁVILA[1], E. DOMINGO[1,2] AND C. PERALES[1,3]

[1]*Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Campus de Cantoblanco, 28049, Madrid, Spain*

[2]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain*

[3]*Liver Unit, Internal Medicine, Laboratory of Malalties Hepàtiques, Vall d'Hebron Institut de Recerca-Hospital Universitari Vall d'Hebron, (VHIR-HUVH), Universitat Autònoma de Barcelona, Barcelona, Spain*

The internal interactions within viral quasispecies can dictate the behavior of mutant ensembles. The relevance of these interactions is being increasingly recognized in natural infections in which distinct mutant clouds converge in the same environment. For example in the competition between two hepatitis C virus (HCV) populations following liver transplantation. Two major types of interaction have been observed among viral populations in general: complementation among mutants of the same mutant spectrum, and interference of replication of standard virus by mutated mutant spectra.

Previous results obtained with vesicular stomatitis virus showed that replication of a high fitness was suppressed by a majority of a low fitness cloud of mutants (de la Torre and Holland, J. Virol. 64: 6278-6281, 1990). We aimed at extending this observation to HCV using two viral populations which differ about 2.2-fold in fitness, and also in resistance to antiviral agents in particular to telaprevir and ribavirin (Sheldon et al., J. Virol. 88:12098-12111, 2014). The low fitness HCV is HCV p0, and the high fitness HCV is HCV p100 (fitness gain due to 100 serial passage in human hepatoma cells). We reconstructed 5 different quasispecies containing a majority of low fitness HCV p0 and decreasing proportions of the high fitness HCV p100. Infectivity in the course of 5 passages in human hepatoma cells in the absence or presence of ribavirin and telaprevir was measured. Three independent experiments in the absence of drug confirmed a similar infectivity level for all the reconstructed quasispecies, suggesting the lack of suppression of HCV p100 by excess HCV p0. A strain-selective qPCR confirmed the increasing dominance of HCV p100 throughout the 5 passages. In the presence of telaprevir all the reconstructed quasispecies which contained HCV p100 showed the characteristic antiviral resistance phenotype expected of HCV p100. Thus, a minority of high fitness HCV can drive an entire population to behave as the minority component, without evidence of suppressive effect by the majority population. The underlying molecular mechanisms are under investigation.

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INSERTIONS AND/OR DELETIONS IN THE MAIN REGULATORY REGION OF HEPATITIS B VIRUS SUGGEST MULTICODING OF THE X PROTEIN

A. CABALLERO GARRALDA[1], J. GREGORI FONT[2], M. BUTI FERRET[3,4], D. TABERNERO CAELLAS[1,3,5], J. QUER SIVILA[2,3], M. BLASI FORNAGUERA[1,3,5], F. RODRIGUEZ-ALGARRA[6], R. CASILLAS GOMEZ[2], C. GONZÁLEZ FERNÁNDEZ[1], I. BELMONTE MULA[1], L. NIETO APONTE[1], R. ESTEBAN MUR[3,4], M. HOMS RIBA[1,3,5], F. RODRIGUEZ-FRIAS[1,3,5]

[1]. Servicio de Bioquímica, Hospital Vall d'Hebron, Barcelona, España

[2]. Laboratorio Enfermedades Hepáticas, Institut de Recerca Vall d'Hebron, Barcelona, España

[3]. CIBEREHD, Instituto de Salud Carlos III, España

[4]. Departamento de Hepatología, Hospital Vall d'Hebron, Barcelona, España

[5]. Servicio de Microbiología, Hospital Vall d'Hebron, Barcelona, España

[6]. Departamento de Telematica, Universidad Politécnica de Cataluña, Barcelona, España

Background: The basic core promoter (BCP, nt 1743-1849) of hepatitis B virus (HBV) overlaps with the 3'-end of the X ORF (nt 1374-1838), the 5'-end of the preCore region (nt 1814-2548) and the enhancer II (ENHII). The ENH II regulates viral replication, encodes the preCore region and the C-terminal region of the multifunctional transactivating protein X (HBx), which most well-characterized binding partner is the protein DDB1.

Naturally occurring sequence variation can be detected along the whole HBV genome, especially relevant in the regions that regulate the replication. In this regard nucleotide insertions and deletions (Ins-Del) in ENHII that modify HBx are associated with the severity of HBV infection.

Aim: Evaluate the presence of insertions and/or deletions in ENHII and the possible effect of truncation or elongation of HBx on the HBV quasispecies in untreated chronic hepatitis B patients.

Patients and methods

Fifty samples from 50 antiviral-untreated patients with chronic active hepatitis were analyzed by ultra-deep pyrosequencing (454, Roche).

The region analyzed clustered positions 1596-1912, encompassing the 3'-end of X ORF, the complete preCore and the 5'-end of the Core gene, including the BCP and ENII.

Insertion, deletion and insertion+deletion (Ins-Del) were studied, as well as their proportion in the total of sequences and the different variants (haplotypes).

Results:A total of 960921 sequences, median 16734 sequences /patient (range 1905-57993) and 1039 haplotypes, median 17 haplotypes/patient (range 4-55) were analyzed.

Overall, 128 different Ins-Del were detected in 7.1% of sequences and 27.5% of haplotypes. Ins-Del were observed in 47/50 samples (94%), a median of the 3.4% sequences per patient (range, 0-74.5%) showed insertions and/or deletions.

All these insertions and deletions changed the standard HBx stop codon (position 155), leading to 49 altered codons and resulting in a premature (truncated HBx) or late (elongated HBx) stop codon. This occurred in 7.6% of sequences and 29.2% of haplotypes.

Main Ins-Del included: (1) 8 nucleotide deletions between positions 1754-1777 causing truncated HBx, (2) duplications at nt 1644-1670, that modified the HBx interaction motif with DDB1 (3) one insertion or (4) deletion of a T at position 1825, both encoding elongated HBx.

Conclusions: The significant presence of genomes that code for truncated or elongated HBx suggest a multicoding HBV mechanism with different HBx versions with possible different functional roles. The systematic presence of deletions and/or insertions could potentially affect the transcriptional activity of HBV ENHII and the clinical relevance should be evaluated in further studies.

(PO 132)

NON-COVALENT INTERACTION WITH SUMO IS NECESSARY FOR FULL ACTIVATION OF PKR

C. F. DE LA CRUZ-HERRERA[1], M. BAZ-MARTÍNEZ[2], A. VIDAL[2], V LANG[3], A. E. MOTIAM[2], M. S. RODRÍGUEZ[3], M. ESTEBAN[1], C. RIVAS[1, 2]

[1] *Departamento de Biología Molecular y Celular, Centro Nacional de Biotecnología-CSIC, Madrid, Spain*

[2] *Centro de Investigación en Medicina Molecular (CIMUS), Universidade de Santiago de Compostela, Instituto de Investigaciones Sanitarias (IDIS), Santiago de Compostela, Spain*

[3] *Ubiquitylation and Cancer Molecular Biology laboratory, Inbiomed, San Sebastian-Donostia, Gipuzkoa, Spain*

The double-stranded RNA (dsRNA)-dependent serine/threonine kinase (PKR) is induced by type I interferon (α/β) and is activated in response to stress signals such as the presence of dsRNA, cytotoxic cytokines, growth factor deprivation, oxidative stress, and DNA damage. PKR can phosphorylate the alpha subunit of the eukaryotic initiation factor (eIF)-2 complex that results in inhibition of general translation, plays a major role in the activation and/or regulation of several transcription factors such as nuclear factor (NF)-kB and p53, and promotes apoptosis in response to viral infection. PKR is activated by binding to dsRNA, which causes the homodimerization and autophosphorylation of the kinase. In addition, PKR can be activated by binding to cellular proteins ISG15 or PACT/RAX. Recently, our group demonstrated that covalent attachment of small ubiquitin-like modifiers (SUMO) to PKR protein promoted the activation of the kinase, potentiated the control of protein synthesis by PKR and contributed to its antiviral activity. Here we demonstrate that PKR also interacts with SUMO in a non-covalent manner via SUMO interaction motifs (SIM). We also show that non-covalent interaction of PKR with SUMO is required for full activation of PKR, inhibition of protein synthesis in response to dsRNA, induction of apoptosis in response to viral infection, and control of viral replication. In summary, these data highlight the relevance of SUMO in regulating the activity of this antiviral protein.

(PO 133)

INFLUENCE OF CARGO ON P22 CAGE STABILITY

J.R. CASTÓN[1], A. LLAURÓ[2], D. LUQUE[1], B.L. TRUS[3], E. EDWARDS[4], J. AVERA[4], T. DOUGLAS[4], P.J. DE PABLO[2]

[1]Department of Structure of Macromolecules, Centro Nacional de Biotecnología/CSIC, 28049 Madrid, Spain; [2]Department of Physics of Condensed Matter, Universidad Autónoma de Madrid, 28049 Madrid, Spain; [3]Imaging Sciences Laboratory, Center for Information Technology/NIH, Bethesda, MD 20892, USA; [4]Department of Chemistry, Indiana University, Bloomington, IN 47405 USA

Virus capsids are used as protein cages to incorporate various types of materials at inner and/or outer capsid surfaces, or as nanocontainers to encapsulate proteins or other biomolecules with potential application in nanomedicine and nanobiotechnology. Virus-like particles (VLP) derived from the *Salmonella typhimurium* bacteriophage P22 have been used to encapsulate heterologous cargos, green fluorescence protein (GFP) or -glucosidase (CelB) from the hyperthermophile *Pyrococcus furiosus*. The P22 capsid is built of 420 copies of a coat protein (CP), which assemble into a T=7 icosahedral lattice with the aid of 100-300 copies of a scaffolding protein (SP); SP C-terminal residues interact with CP.

P22 VLP undergoes a series of defined structural transitions after heating that emulate bacteriophage P22 maturation. The P22 procapsid/capsid transition involves an increase in the internal volume, as well as thinning and greater porosity of the capsid shell. Heterologous expression of CP and N-terminal truncated SP fused to other gene product results in self-assembly of the procapsid (PC, 58 nm diameter, 58,000 nm^3); heating at 65ºC for 10 min causes irreversible expansion to yield the expanded capsid (EX, 64 nm, 113,000 nm^3). By heating PC or EX at 75ºC for 20 min, pentamers are released, and EX with 10 nm holes at 5-fold vertices are know as wiffleball (WB) particles. We used the SP fusion strategy to incorporate GFP or CelB into the VLP interior when expressed in *E. coli* together with CP; self-assembled chimeric PC mature into EX and WB.

We used 3D cryo-electron microscopy (cryo-EM) and atomic force microscopy (AFM) to study how the nature of the cargo and its interplay with the capsid inner surface might influence the properties of these nanocontainers. Cargo-loaded P22 PC showed less deformation after adsorption to a substrate than empty cages, regardless its nature. The rigidity increased for both GFP- and CelB-PC. There are nevertheless fewer SP-mediated CelB-PC connections to cargo than for GFP-PC, and CelB-PC were more fragilethan GFP-PC or empty PC. CelB-EX and CelB-WB particles were more rigid than empty-EX and -WB particles, although the cargo was detached from the shell, as shown by 3D cryo-EM. Rigidity is probably increased by an electrostatic repulsion effect (electrostatic potential surfaces of both inner capsid surface and CelB tetramers are highly negative), although the elastic collisions of the tetramers against the internal wall is also a plausible explanation. The detachment between cargo and capsid prevents an increase in fragility. CelB-EX particles are heterogeneous; there are full capsids (48%), capsids with collapsed cargo (28%), and empty, broken capsids (24%).

Most CelB-WB particles are filled homogeneously (75%) indicating that released pentamers reduced cargo-capsid tension, although the stabilizing cargo-mediated effect is preserved. GFP-EX contain collapsed cargos and the diffusion effect appears to be dominant. Our results show that the mechanical stability of the protein cages depends on cargo-cargo and cargo-capsid interactions. This influence is reciprocal, as P22 cages also modulate rigidity of encapsulated cargo.

(PO 134)

ESTRATEGIAS ADAPTATIVAS EN POBLACIONES VIRALES. HACIA LA IDENTIFICACIÓN DE COMPORTAMIENTOS GENÉRICOS EN EVOLUCIÓN MOLECULAR

S. MANRUBIA

Centro Nacional de Biotecnología (CSIC), Madrid, España

El desarrollo de teorías cuantitativas de la evolución requiere sintetizar principios generales a partir de un gran número de observaciones. La dinámica adaptativa de una población implica muchos niveles distintos de descripción y selección, incluyendo mecanismos que causan variabilidad genómica, los efectos de las mutaciones en el fenotipo, las interacciones entre individuos, la competición inter-grupos o las restricciones impuestas por ambientes que varían en el tiempo. Como resultado, las aproximaciones teóricas solo han alcanzado un éxito parcial hasta el momento. En parte, esto se debe a una limitada comprensión de la relación entre aspectos moleculares y los cambios fenotípicos causados por las mutaciones, ello a pesar de los esfuerzos dedicados a comprender los orígenes de la adaptación y del fácil acceso a nuevas técnicas que permiten una exploración exhaustiva de la diversidad genotípica. Cualquier aproximación teórica a la evolución requiere un conocimiento suficiente de la evidencia empírica a fin de diseñar modelos realistas y falsables. Actualmente, los virus representan el mejor sistema experimental para (i) estudiar la evolución en tiempo real, (ii) explorar estrategias adaptativas alternativas gracias al gran número de mecanismos moleculares que estos organismos despliegan, (iii) unir el nivel molecular al poblacional y (iv) diseñar modelos evolutivos con capacidad predictiva. En esta contribución presentaremos una serie de ejemplos de dinámica de poblaciones virales que indican que parece posible definir lo que en física se denomina *clases de universalidad*. Estas clases dinámicas representan comportamientos genéricos que caracterizarían, en particular, transiciones adaptativas y transiciones a la extinción en poblaciones virales basadas únicamente en estrategias moleculares. Un ingrediente esencial que permitiría una clasificación de este tipo es la independencia de la dinámica poblacional de ciertos detalles (como el tipo de genoma o la tasa de mutación), pero no de la estrategia molecular precisa utilizada en la adaptación (como la existencia de recombinación o el uso de genomas bipartitos).

Este estudio se halla en un estadio incipiente y se completará en el futuro mediante el desarrollo de modelos dedicados a virus específicos, a fin de predecir las respuestas poblacionales y

complementar conocimientos previos, y modelos de aplicabilidad general, capaces de establecer una correspondencia entre mecanismos moleculares y las respuestas adaptativas de las poblaciones. A medio plazo, el objetivo es contribuir al desarrollo de una teoría evolutiva actualizada que incluya la compleja estructura del espacio de genomas y las correspondientes estrategias adaptativas de poblaciones naturales.

(PO 135)

STRUCTURE OF ADENOVIRUS MATURATION INTERMEDIATES CLARIFIES CAPSID ARCHITECTURE AND SHOWS CHANGES RELATED TO SCAFFOLD PROCESSING

G. CONDEZO[1], R. MARABINI[2], M. CHILLÓN[3], J. FLINT[4] AND C. SAN MARTÍN[1].

[1] *Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain.*

[2]*Escuela PolitécnicaSuperior, UAM, Madrid, Spain.*

[3]*Centro de Biotecnología Animal y Terapia Génica (CBATEG). UAB, Bellaterra, Spain.*

[4]*Princeton University, New Jersey, USA.*

Adenovirus (AdV) is one of the most complex icosahedral, non-enveloped viruses. Although its structure was solved by both cryo-EM and X-ray crystallography, the location of minor coat proteins is still controversial *(1, 2)*. Light density AdV particles lack genome, contain the putative scaffold L1 52/55k, and may represent assembly intermediates. L1 52/55k is required for packaging, and cleavages by the maturation protease facilitate its release from the nascent virion *(3)*. Here we present the molecular and structural characterization of two different AdV light particles. We show that they lack core polypeptide V but do not lack the density corresponding to this protein in the X-ray structure, therefore adding support to the adenovirus cryo-EM model. The two types of light particles present different degrees of proteolytic processing, and their structure provides the first glimpse on the organization of L1 52/55k protein inside the capsid shell, and on how this organization changes upon partial maturation.

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(PO 136)

DIRECT VISUALIZATION OF BREATHING MOTIONS IN THE HIV CAPSID LATTICE, AND MODULATION OF ITS EQUILIBRIUM DYNAMICS AND MECHANICAL PROPERTIES

A. VALBUENA, M. G. MATEU

Centro de Biología Molecular Severo Ochoa (Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid), Universidad Autónoma de Madrid, Madrid, Spain.

The emerging area of Mechanical Virology is providing novel insights into properties of viruses such as elasticity, resistance to rupture and susceptibility to material

fatigue, unveiling the structural determinants of these properties, and revealing biological adaptations based on mechanical features of virus particles. In addition, virus particles and virus-derived materials are increasingly being explored as a source of novel biomaterials and nanodevices in Biomedicine and Nanotechnology. However, viral particles and other protein assemblies still present several issues for many intended applications. One basic issue is that they are "soft" materials, and may be too sensitive to degradation or disruption by chemical agents, temperature, and/or mechanical forces during their production, storage and/or use. Thus, it may be critically important to understand the structural basis of virus particle mechanics and dynamics, and develop the know-how to manipulate their dynamic behaviour and mechanical features to improve their suitability for different technological applications.

The protein capsid of the human immunodeficiency virus (HIV) provides an excellent model system to investigate and manipulate the mechanical properties and equilibrium dynamics of viral particles and novel virus-derived biomaterials. In this study we have used atomic force microscopy to directly visualize and quantify the large-scale equilibrium dynamics ("breathing") and determine the mechanical behaviour (elasticity, mechanical strength, and self-healing) of the HIV capsid lattice in close to physiological conditions. We also demonstrate that breathing amplitude and mechanical features of the HIV capsid lattice can be manipulated by binding a small ligand, betaine. The results provide: i) a unique, simple method to directly quantify large breathing motions in a viral capsid; ii) a description of the dynamics and mechanical features of a virus-based bidimensional nanomaterial with applicability as a biological nanocoating; iii) proof-of principle that it is possible to manipulate both breathing amplitude and mechanical strength of viral capsids and derived nanomaterials by binding small molecules.

(PO 137)

MOLECULAR EPIDEMIOLOGY OF MEASLES VIRUS IN SPAIN.GENOTYPE D4.

A.GÓMEZ-VECINO[1], J.E.ECHEVARRIA[1,3], M.M.MOSQUERA[1,3], A.CASTELLANOS[1,3], C.HOYAS[1,3], F.DE ORY[1,3], M.BANGERT[1], J.MASA[2,3], N.LÓPEZ-PEREA[2,3], A.FERNÁNDEZ-GARCÍA[1,3].

1. Centro Nacional de Microbiología. Instituto de Salud Carlos III. Majadahonda. Madrid. Spain. 2. Centro Nacional de Epidemiología. Instituto de Salud Carlos III. Madrid. Spain.

3. CIBER de Epidemiología y Salud Pública (CIBERESP). Madrid. Spain.

Introduction: Measles virus (MeV) causes a highly contagious disease that is currently targeted for a National Plan Elimination in our country since 2001. The MV genotyping and phylogenetic analysis are an essential part to trace the chains of transmission and to determine the elimination of the virus. The genotype D4 caused massive outbreaks in Europe (2010-2012). This study is focused on updating the molecular epidemiology of this genotype in Spain from 2008 to 2012.

Methods: The phylogenetic analysis was based on the450 nucleotides encoding the

C-terminal end of the viral nucleoprotein (N-450). A total of 1640 sequences obtained from GenBank and MeaNS databases were analyzed, 680 from Spain. They were aligned (BioEdit v.7.0.5), haplotypes were described (DNAsp v.5) and their genetic distance analyzed (MEGA v.6). The phylogenetic trees were constructed by maximum likelihood (RaxML v.7 y PhyML v.3).

Results: We found 41 Spanish haplotypes, grouped in 18 phylogenetic clusters, belonged to prevalent European variants during these years: 11 haplotypes (182 sequences) related to MVs/Enfield.GBR/14.07/-variant (D4-Enfield); 2 haplotypes (16 sequences) related to the sub-variant MVs/Hamburg.DEU/03.09/-variant (D4-Hamburg) and 14 haplotypes (251 sequences) related to the sub-variant MVs/Manchester.GBR/10.09/-variant (D4-Manchester). Furthermore there were 2 sub-variants from D4-Machester (Mvs/Marmande.FRA/43.11/2/ and MVs/Maramures.ROU/3.11/) in minority-way and we described a new one: MVs/Madrid.ESP/46.10-variant (D4-Madrid) with 10 different haplotypes (126 sequences). We denoted 2 strains older than the reference strains of the D4-Enfield (Mv/Raichur.IND/38.06/1) and D4-Manchester (MVs/Lisieux.FRA/27.07/1) variants. In 2008 the variant D4-Enfield was detected in Spain in a low incidence rate as in the next year 2009. During 2010 the measles incidence increased and the variants D4-Hamburg and D4-Manchester replaced D4-Enfield. The highest incidence of measles was in 2011, mainly due to the D4-Manchester variant and sub-variants related to this, which were also detected during the 2012 but with lower number of cases.

Discussion: The pattern of measles genotype D4 circulation in Spain during the period of studywas the same that in the rest of Europe. Our results suggest the existence of different events of importation instead of continuous circulation. In addition we described a new variant D4-Madrid that had a wide distribution in our country which caused local outbreaks during years 2011 and 2012 and was further detected in Rumania and United Kingdom. The existence of 2 identical strains older than the reference strains D4-Enfield and D4-Manchester determine an older origin of the variants in India and France, respectively.

(PO 138)

OCCURRENCE OF INTRA-OUBREAK VARIABILITY IN SEMI-CLOSED INSTITUTIONS

A. SABRIÀ, R. M. PINTÓ[1], A. BOSCH, N. TORNER[2,3], A. MARTÍNEZ[2,3], A. DOMÍNGUEZ[3, 4], S. GUIX[1] and the Catalan Viral Gastroenteritis Study Group of the Project PS09/02516.

[1] *Enteric Virus Group, Department of Microbiology, University of Barcelona, Barcelona, Spain.*

[2] *Department of Health, Generalitat of Catalonia, Barcelona, Spain.*

[3] *CIBER Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain.*

[4] *Department of Public Health, University of Barcelona, Barcelona, Spain.*

Human noroviruses (NoV) are the most common cause of acute nonbacterial gastroenteritis in humans, causing large outbreaks worldwide. They are a highly

diverse group of viruses with a single-stranded RNA genome encoding 3 major ORFs. ORF1 encodes the nonstructural proteins, while ORF2 and 3 encode the capsid proteins. The major structural protein (VP1) has a hypervariable domain (P2 domain) which is the most exposed part of the virion. Classification is established according to nucleotide diversity in the full length VP1-encoding ORF2 gene, but genotyping is often performed based on sequence information in the ORF1/2 junction region (region C). Genotype GII.4 is responsible for the majority of infections in healthcare settings.

The aim of this work was to assess the occurrence and characteristics of genetic variability within 13 acute gastroenteritis outbreaks caused by GII.4 NoV (New Orleans 2009 strain) in semi-closed institutions during a 2-year study period in Catalonia. Transmission route for all 13 studied outbreaks was person-to-person, except for 2 outbreaks, which were foodborne and then were transmitted interpersonally. The study analyzed 37 individuals including patients and samples from asymptomatic caregivers or food handlers related to the outbreaks. Region C sequences for most of the outbreaks were 100% identical, but nucleotide variations were found in 5 person-to-person outbreaks (38.5%). The degree of nucleotide diversity within region C ranged between 0.35%-1.53%. To further investigate whether variability was due to the co-circulation of multiple strains in the same setting, or whether nucleotide variations were due to the quasiespecies distribution within infected individuals, sequence of the most variable P2 domain was also analyzed. Sequence analysis within the P2 domain showed a higher degree of genetic diversity (0.14%-2.90%) and allowed us to confirm that more than one strain co-circulated in 4 out of the 5 outbreaks which showed variability within region C.

In conclusion, sequence of the P2 domain proved useful for multi-strain tracking during outbreak investigations and results indicated that multi-strain outbreaks, probably of a nosocomial origin, are very common within semi-closed institutions. If made promptly available, P2 sequence analysis may help to make decisions regarding control measures which are especially important in healthcare settings where nosocomial infections are common.

(PO 139)

EPIDEMIOLOGIC STUDY OF INFLUENZA VIRUSES IN THE 2009 POST-PANDEMIC PERIOD (2010-2015)

I. SANZ MUÑOZ[1, 2], S. ROJO RELLO[1, 2], A. RODRÍGUEZ FERNÁNDEZ[2], R. ORTIZ DE LEJARAZU LEONARDO[1, 2]

1. *Valladolid National Influenza Centre, Valladolid, Spain*
2. *Microbiology and Immunology Service, Clinic University Hospital of Valladolid, Valladolid, Spain*

Introduction: Although influenza viruses have been widely studied and described, it has been done a high number of epidemiological and pathogenic scientific approaches regarding their circulation since 2009 influenza pandemic. One of the most relevant conclusions is the rate of hospitalizations that occur in the different age groups, depending on the influenza

type and subtype that circulate mainly. The aim of this study is to analyze the demographic and epidemiological characteristics of the last 5 influenza seasons after 2009 pandemic influenza emergence.

Material and methods: An observational retrospective study was conducted including results from 1,356 respiratory samples from hospitalized patients from Castilla y León Hospital Network (Spain) and out-patients from Sentinel Surveillance Network of Castilla y León (RCSCyL-Spain). These samples were obtained from 2010-11(n=151),2011-12(n=337),2012-13(n=120),2013-14(n=320) and 2014-15(n=428) influenza seasons. Identification of influenza A and B viruses and their subtypes (A/H1N1pdm09; A/H3) was performed using different molecular diagnostic techniques and platforms (*Luminex 200-XTAG RVPFAST-Luminex; ABi7500Fast-CDC real time InfluenzaVirus subtyping Panel-Applied Biosystems; LightCycler 2.0-Influenza A/H1N1 Detection Set Subtyping -Roche; Clondiag Array Mate-Influenza A Genotyping Panel-Alere*), and also by cellular culture using MDCK and MDCK/Siat1 cells. Demographic and epidemiological characteristics were analyzed in influenza seasons included.

Results: Influenza A were the most detected viruses during 2010-11(80.8%-A/H1N1pdm09), 2011-12(94.4%-A/H3), 2013-14(62.2%-A/H1N1pmd09;32.5-A/H3) y 2014-15(70.3%-A/H3) influenza seasons, while influenza B were the most detected virus only in 2012-13 (61.7%) influenza season. The proportion of hospitalized-out patients remained stable during the seasons in which mostly circulated influenza A viruses (65-35% respectively). However, out-patient increased till 60% during 2012-13 influenza season. Average age of hospitalized patients increased constantly from 20.2 years in 2010-11 influenza season till 56.1 years in 2014-15 influenza season. In the case of out-patients, increasing of average age was lower, from 22.1 years in 2010-11 influenza seasons till 28.3 years in 2014-15 influenza season.

Conclusions: Epidemics with higher circulation of influenza A viruses (H1N1pdm or H3) have been characterized by a higher proportion of hospitalized cases. However, in the epidemic with higher circulation of influenza B virus has been detected a higher proportion of out-patients. Similarly, influenza A epidemics have produced a much greater absolute number of cases in hospitalized and also in out-patients. The average age of hospitalized patients has grown steadily due to a complex phenomenon linked to “seasonal adjustment” of a pandemic virus after their emergence.

(PO 140)

IN SILICO EVIDENCES OF ANCIENT ENDOGENOUS PARVOVIRUS SEQUENCES IN THE GENOME OF LIVING PRIMATES: A MOLECULAR AND PHYLOGENETIC ANALYSIS

NOOSHIN BAYAT, CARLOS GALLEGO, AROA TATO, VIRGINIA SANDONÍS, DAVID ABIA*, AND JOSÉ M. ALMENDRAL*

*Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), 28049 Cantoblanco, Madrid, Spain. *Corresponding authors*

The genome of multiple host species has been evolutionary colonized by viruses, mainly by RNA viruses as Retroviruses and Bornaviruses. Recently though, sequences and full genes of some virus members of the ssDNA Parvoviridae have been recognized as evolutionary inserted (endogenized) in a wide range of animal genomes, including mammals. In searching novel endogenous parvoviruses (EPAV), we have performed an extensive in silico analysis of Parvovirus sequences in the genome of currently living primate species, for which assembled genomes are becoming available in databases. Our preliminary obtained data, focused in stretches of amino acids homologous to the type species of the nowdays circulating genera of the Parvoviridae, suggest that EPAV sequences are more common in Primates than previously suspected. Genetic analyses of the putative EPAV indicate intricate and diverse genetic configurations, which may include part or full non-structural (NS) and structural (VP) parvovirus genes. Molecular analyses of these putative EPAV are underway aiming at confirming their actual endogenous nature and genetic features. A wide survey of our findings will be presented, and some possible biological implications will be discussed.

(PO 141)

LACKING OF RELATION BETWEEN HHV-6 IMMUNE RESPONSE AND BRAIN DAMAGE IN ALZHEIMER'S DISEASE PATIENTS

S. AGOSTINI[1], R. MANCUSO[1], A. HERNIS[1], F. BAGLIO[2], M. CABINIO[2], E. CALABRESE[3], M. CLERICI[1,4]

[1] *Laboratory of Molecular Medicine and Biotechnologies, Don Carlo Gnocchi Foundation – ONLUS, Milan, Italy.*

[2] *Magnetic Resonance Laboratory, Don Carlo Gnocchi Foundation–ONLUS, Milan, Italy.*

[3] *Department of Neurorehabilitation, Don C. Gnocchi Foundation – ONLUS, Milan, Italy.*

[4] *Department of Physiopathology and Transplantation, University of Milan, Milan, Italy.*

Introduction: Alzheimer's disease (AD) is the most common form of dementia in the world, and clinically is characterized by progressive memory loss, impairment of other cognitive functions as well as inability to performed daily living activities. The amnestic mild cognitive impairment (aMCI) subjects are individuals with impairment in memory but preserved functional abilities, and they often represent a borderline condition between normal aging and dementia. Although the abnormal features and lesions of AD brain are well studied and characterized, the etiopathogenesis of the disease is still unclear. In the last years our group studied the relation between HSV-1 antibodies and cortical grey matter (GM) volumes of AD

and aMCI patients, finding in patients a positive correlation between high antibody levels anti-HSV-1, and the volumes of brain regions typically affected in disease. The next important step is to understand if this relation is specific for HSV-1 or it is typical of also other herpesvirus, like the HHV-6, a neurotropic virus involved in other neurodegenerative disease and suspected that could related also with AD.

Aims: HHV-6 humoral immune responses were analyzed in patients with a diagnosis of either AD, aMCI and in healthy controls (HC), to verify possible correlations between titers and avidity of HHV-6-specific IgG and cortical grey matter volumes analyzed by MRI.

Material and Methods: 59 early AD, 60 aMCI and 61 age-matched HC were enrolled in the study. Patients underwent Mini Mental State Evaluation (MMSE). Serum HHV-6 IgG Ab levels and avidity index were tested by ELISA. Three randomly selected subgroups of 44 AD, 23 aMCI and 22 HC HHV-6 seropositive patients underwent brain Magnetic Resonance Imaging (MRI) by 1,5 T scanner.

Results: HHV-6 seroprevalence was similar in the 3 groups (AD: 97.6 %; aMCI: 78.3 %; HC: 75.4 %) and also the HHV-6 Ab titers were not different in AD (2.27±1.48 Positivity Index(PI) compared to aMCI (2.36±2.44 PI) as well as to HC (2.22±1.59 PI). No differences were found even for Ab avidity (median AD: 93.2%; aMCI: 88.6%; HC: 93.8%) and no associations were seen between Ab titers, avidity and MMSE. Finally, no correlation was found between Ab titers/avidity and brain damage, neither regarding the region typically affected by disease.

Conclusions: the lacking of any relation between humoral immune response against HHV-6 (Ab titers and avidity) and AD reinforces our hypothesis about a pivotal role of HSV-1, and not of other herpesviruses, in the pathogenesis of the disease.

(PO 142)

POLIOVIRUS REINTRODUCTION MONITORING THROUGH THE ACUTE FLACCID PARALYSIS AND ENVIRONMENTAL SURVEILLANCE: RESULT OF THESE ACTIVITIES IN LOMBARDY, 2014.

L. PELLEGRINELLI[1], L. FIORE[2], V.PRIMACHE[2], L. BUBBA[1], S.FIORE[2] AND S. BINDA[1]

[1].Department of Biomedical Sciences for Health; University of Milan; Milan, Italy.

[2]. National Center for Immunobiologicals Research and Evaluation; Istituto Superiore di Sanità; Rome, Italy.

Introduction. The WHO Strategic Plan of the Global Polio Eradication Initiative indicates the Acute Flaccid Paralysis (AFP) surveillance and the Environmental Surveillance (ES) as a crucial activities in order to detect eventual poliovirus (PV) reintroduction in polio-free countries and to achieve PV worldwide eradication. Nowadays, AFP surveillance is the gold-tandard but ES is able to detect PV reintroduction without polio clinical manifestation. This study aimed at describing the results of the AFP surveillance and ES in Lombardy, Northern-Italy, in 2014.

Methods: The surveillance activities were carried out according to WHO guidelines (WHO/IVB/04.10;WHO/V&B/03.03). All children < 15-years-old who fulfilled the

WHO definition for AFP and placed in Lombardy were included in the study and their stool were collected. During ES, wastewater sample was collected regularly twice a month at the intel of 3 wastewater treatment plants located in Milan. AFP stool and wastewater sample were investigated to detect PV and non-polio Enterovirus (NPEV).

Result. 13 AFP cases were reported with a incidence rate of 1/100 000 children < 15 years of age. The sensitivity of the surveillance system was good. The major clinical diagnoses associated with AFP were Guillain-Barré Syndrome (GBS, 53.8%). According to the virological results, none AFP case was caused by PV infections and NPEV was detected in one patient. During ES, 70 wastewater sample were collected and no PV was isolated; in the other hand, an high rate of NPEV was detected (49/70; 70%). AFP surveillance and ES achieved the WHO performance indicator.

Conclusion. AFP surveillance and ES must be maintained until global PV eradication will be declared. Although AFP surveillance remains the gold-standard, ES is a powerfully tool to detect PV in the absence of *polio* cases; for this reason, ES in Lombardy, as well as in other Italian Region, needs to be improve.

(PO 143)

DISSEMINATION OF VIRAL PATHOGENS IN A MEDITERRANEAN CLIMATE REGION

M. RUSIÑOL[1], X. FERNANDEZ-CASSI[1], N. TIMONEDA[1,2], J. ABRIL[2], S. BOFILL-MAS[1], R. GIRONES[1].

[1.] Laboratory of Virus Contaminants of Water and Food (Vircont), Microbiology Department, Biology faculty, Barcelona University, Catalonia, Spain.

[2.] Computational Genomics Lab (Compgen), Genetics Department, Biology faculty, Barcelona University, Catalonia, Spain.

Fecal contamination of water is closely related to human health. Microorganisms from intestinal tract may arrive to water through sewerage overflows, wastewater treatment plant effluents, surface runoff and direct discharges into the receiving waters. Understanding the environmental fate of pathogens is useful for minimizing the present risks to humans and also evaluate the future trends in relation to climate change.

As part of the EU-FP7-funded VIROCLIME project, the present study developed a surveillance program centered on a typically Mediterranean climate region: the Llobregat River basin (Catalonia, northeast of Spain) in order to evaluate the dissemination of viruses in water. Sample matrices included river water, untreated and treated wastewater from a wastewater treatment plant within the catchment area, and seawater from potentially impacted bathing water. Five viruses were analyzed in the study: a) human adenovirus (HAdV) and b) JC polyomavirus (JCPyV) were analysed as indicators of human faecal contamination of human pathogens and were reported in

urban wastewater (mean values of 10^6 and 10^5 GC/L, respectively), river water (10^3 and 10^2 GC/L) and seawater (10^2 and 10^1 GC/L), indicating that wastewater plays an important role in the transmission of viral pathogens in water; c) Merkel Cell polyomavirus (MCPyV), which is associated with Merkel Cell carcinoma an aggressive skin cancer, was detected in 75% of the raw wastewater samples (31/37), 29% of river water and 18% seawater samples, and quantified by a newly developed quantitative polymerase chain reaction (qPCR) assay (wastewater mean values 10^4 GC/L). Seasonality was only observed for d) norovirus genogroup II (NoV GGII), which was more abundant in cold months with levels up to 10^4 GC/L in river water and 10^6 GC/L in untreated wastewater; and e) human hepatitis E virus (HEV) was detected in 13.5% of the wastewater samples when analysed by nested PCR (nPCR). Secondary biological treatment (i.e., activated sludge) and tertiary treatment using disinfection with chlorination, flocculation and UV radiation removed between 2.22 and 4.52 $\log_{10}$ of the viral concentrations.

Climate projections for the Mediterranean climate areas and the selected river catchment suggest general warming and changes in precipitation distribution. Persistent decreases in precipitation during summer can lead to a higher presence of human viruses because river and sea water present the highest viral concentrations during warmer months. In a global context, wastewater management will be the key to preventing environmental dispersion of human fecal pathogens in future climate change scenarios.

(PO 144)

ANALYSIS OF THE INTERACTION BETWEEN microRNA miR-122 AND THE HCV IRES ELEMENT BY ATOMIC FORCE MICROSCOPY AND BIOCHEMICAL TECHNIQUES

M. MORENO[1], L. VÁZQUEZ[2], A. ARIZA-MATEOS[3,4], A. GARCÍA-SACRISTÁN[1,3], R.M. JÁUDENES[1], J.Á. MARTÍN-GAGO[1,2], J. GÓMEZ[3,4], C. BRIONES[1,3].

[1]. Department of Molecular Evolution, Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Madrid, Spain.

[2]. Instituto de Ciencia de Materiales de Madrid (CSIC), Cantoblanco, Madrid, Spain.

[3]. Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas. (CIBERehd), Spain.

[4]. Laboratory of RNA Archaeology, Instituto de Parasitología y Biomedicina "López-Neyra" (CSIC), Granada, Spain.

Both 5´ and 3´ untranslatable regions (UTR) of the single-stranded RNA genome of hepatitis C virus (HCV) are highly structured and include regulatory elements necessary for viral replication and translation. In particular, the 5´UTR contains an internal ribosome entry site (IRES) element responsible for the cap-independent translation initiation (1). The ion-dependent tertiary fold of the minimal HCV IRES element (containing domains II to IV) has been investigated (2), and significant progress has been made in determining the three-dimensional structure of individual IRES domains and subdomains at high resolution (3). Nevertheless, little information is still available on the tertiary structure of the whole functional HCV IRES element.

Atomic force microscopy (AFM) is a powerful, nanotechnology-based tool for the structural analysis of a wide range of biological entities. It provides a 3D surface profile of the imaged sample without requiring any staining or coating, and its nanometer resolution is optimal for the visualization of RNA and RNA-protein complexes (4). Recently, we have used AFM to investigate the magnesium-dependent folding of the HCV IRES in a sequence context that includes its structured, functionally relevant flanking regions (domains I, V and VI) (5). In the 574 nt-long HCV genomic RNA molecule analysed, a sharp structural switch has been monitored when Mg^{2+} concentration increases from 2 to 4 mM. This effect has been confirmed by classical techniques for RNA structural characterization such as gel-shift analysis and partial RNase T1 cleavage.

Such a Mg^{2+}-induced conformational rearrangement is partially similar to that caused by the liver-specific microRNA miR-122 that, as previously shown (6), interacts with the I-II spacer region of the HCV IRES and induces switching between 'open' and 'closed' conformers. Herein, we present the AFM analysis of IRES-574/miR-122 complexes in buffers containing 100 mM Na^{+} supplemented with 0 to 10 mM Mg^{2+}. Our results, supported by gel-shift assays, show that the Mg^{2+}-induced open/closed switch in IRES-574 is hindered by the interaction of miR-122. The competing effects of Mg^{2+} and mir122 reinforce the previously suggested structural and functional continuity among domains I-VI of HCV IRES in its natural sequence context.

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(PO 145)

PHYLOGENETIC ANALYSIS OF AN EPIDEMIC OUTBREAK OF ACUTE HEPATITIS C IN HIV-INFECTED PATIENTS BY MASSIVE SEQUENCING

N. CARO PÉREZ[1], M. MARTINEZ-REBOLLAR[2], J. GREGORI[3], [4], J.QUER[3], P. GONZÁLEZ[1], M. GAMBATO[1], H.VISSER[2], J. I. ESTEBAN[3], J. MALLOLAS[2], X. FORNS[1], M. LAGUNO[2] S. PÉREZ-DEL-PULGAR[1].

[1]*Liver Unit, Hospital Clínic, IDIBAPS, CIBERehd, Barcelona, Spain.*

[2]*Infectious Diseases Unit, Hospital Clínic, IDIBAPS, Barcelona, Spain.*

[3]*Liver Unit, Vall d'Hebron Institut de Recerca-Hospital Universitari Vall d'Hebron, CIBERehd, Barcelona, Spain.*

[4] *Roche Diagnostics SL. Sant Cugat del Vallès. Barcelona, Spain.*

Background and aims: The incidence of acute hepatitis C among HIV-infected men who have sex with men (MSM) has significantly increased in recent years. This increase may be due to factors such as high HCV viral load in blood and semen, sex with risk of mucosal damage, a higher number of sexual partners, presence of concomitant ulcerative sexually transmitted diseases and the use of recreational drugs. The aim of our study was to investigate the dynamics of HCV

transmission in an outbreak of acute hepatitis C in HIV-infected MSM in Barcelona.

Methods: Between 2008 and 2013, 113 cases of acute hepatitis C in HIV-infected MSM were diagnosed in the Infectious Diseases Unit, Hospital Clínic, Barcelona. Phylogenetic analysis of the HCV NS5B gene was performed in a total of 73 patients. Viral RNA was extracted from serum samples collected from each patient at the time of diagnosis. Massive sequencing was performed using the Roche 454 GS Junior platform. To define possible transmission networks, phylogenetic trees and multidimensional scaling maps were constructed from genetic distance matrices (Da).

Results: At the time of diagnosis of acute hepatitis C, 53 of the 73 (73%) patients included in the study were receiving antiretroviral therapy. HIV viral load was undetectable in 48 patients (66%) and the mean CD4 cell count was 923 cells /ul. HCV viral load was 6.37 log IU/mL (range 3.73-6.99). Thirty-five of 53 (66 %) patients treated with pegIFN and ribavirin achieved a sustained virological response. The prevalence of HCV genotypes was: 4d 48% (n= 35), 1a 44% (n= 32), 1b 7% (n= 5) and 3a 1% (n= 1). Phylogenetic analysis showed the existence of at least 14 monophyletic groups: 5 of genotype 1a, 1 of genotype 1b and 8 genotype 4d. Molecular analysis showed that the genetic distances between genotype 4d viruses were significantly lower than those of the subtypes 1a ($p< 2.2x10$ e-16) and 1b ($p<0.039$) . This result may suggest the existence of a single source of infection for genotype 4d and different sources for subtypes 1a and 1b.

Conclusions: HCV infection spreads rapidly among HIV-infected MSM through a local network in Barcelona. The implementation of public health campaigns and preventive measures, as well as treatment interventions with the new direct-acting antivirals will allow the development of strategies to reduce the HCV transmission of HCV within these high-risk groups.

(PO 146)

ULTRASTRUCTURAL ALTERATIONS INDUCED BY HCV REPLICATION REVEALED BY CRYO SOFT X-RAY TOMOGRAPHY.

PEREZ-BERNA AJ[a]*, RODRÍGUEZ MJ[b]*, CHICHON FJ[b], FRIESLAND M[b], SORRENTINO A[a], CARRASCOSA JL[b], PEREIRO E[a] AND **GASTAMINZA P[b]**

[a]*ALBA Synchrotron Light Source, MISTRAL Beamline – Experiments Division, 08290 Cerdanyola del Vallès, Barcelona, Spain*

[b]*Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (CNB-CSIC), Campus Cantoblanco, 28049 Madrid, Spain*

Chronic hepatitis C virus (HCV) infection causes severe liver disease and hepatocellular carcinoma. The detailed mechanisms underlying HCV pathogenesis are largely unknown, although immune response-mediated events are major players of the liver damage. In addition, HCV replication and protein expression causes alterations of the host cell homeostasis by, among other mechanisms, causing endoplasmic reticulum (ER) and oxidative stress. This is reflected at the ultrastructural level by a dilation of the ER cisternae and mitochondrial abnormalities previously reported in liver biopsies from infected patients.

In this study we have performed full-field cryo soft X-ray tomography (cryo-SXT) in the water window photon energy range to investigate in whole, unstained cells, the ultrastructural alterations induced by HCV replication under conditions that are close to the living physiological state. Using this technology and guided by parallel analyses of the samples by immunolabeling, confocal and transmission electron microscopy, we haveobtained the first native tridimensional mapsofcellular modifications caused by a stable subgenomic HCV replicon in cell culture.

In contrast with control cells, which display continuous ER cisternae of relatively constant diameter, HCV replicating cells show enlarged blind-ended tubules with prominent pseudospherical extrusions. As expected, these ER alterations are reverted to normal after elimination of the viral RNA and proteins from the replicon cells by treatment with DAAs. We have also observed a profound alteration of the mitochondrial morphology that correlated with the extent with which the ER was modified, both in replicons and in a surrogate model of HCV infection. The 3D maps indicate a topological relationship between altered, possibly dysfunctional mitochondria, and highly modified ER tubules. Within the same infected cell, structurally sound mitochondria are observed in areas where ER is indistinguishable from that of control cells, while mitochondria within or juxtaposed to modified ER are clearly altered, indicating a short-range influence of the viral machinery on the mitochondrial viability. Given the strong relevance that has been given to HCV-induced mitochondrial dysfunction on viral pathogenesis we arecurrently investigating the nature of the ER-mitochondria contacts in HCV-replicating cells using cryo-SXT to shed light on the mechanisms by which HCV inflicts mitochondrial damage locally.

(PO 147)

ULTRADEEP PYROSEQUENCING REVEALS POLYMORPHYSMS IN THE PROBE-BINDING SITE OF HCV-3 ASSOCIATED WITH INDETERMINATE RESULTS BY A COMMERCIAL GENOTYPING ASSAY

V. SALUDES MONTORO[1, 2], J. QUER SIVILA[3, 4, 5], JOSEP GREGORI[3,4,6], E. BASCUÑANA PRIETO[1], D. GARCÍA CEHIC[3, 4], J.I. ESTEBAN MUR[3, 4, 5], V. AUSINA RUIZ[1, 5, 7], E. MARTRÓ CATALÀ[* 1, 2]

[1]. Servicio de Microbiología, Hospital Universitari Germans Trias i Pujol (HUGTIP), Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol (IGTP), Badalona, Spain

[2]. Centro de Investigación Biomédica en Red (CIBER) de Epidemiología y Salud

Pública (CIBERESP) del Instituto de Salud Carlos III, Madrid, Spain

[3]. Liver Unit, Internal Medicine, Lab. Malalties Hepàtiques, Vall d'Hebron Insititute of Research (VHIR-HUVH), Barcelona, Spain

[4]. Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Hepáticas y Digestivas (CIBERehd) del Instituto de Salud Carlos III, Madrid, Spain

[5]. Universitat Autònoma de Barcelona, Barcelona, Spain

[6]. Roche Diagnostics SL, Sant Cugat del Vallès, Spain

[7]. Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Respiratorias (CIBERES) del Instituto de Salud Carlos III, Madrid, Spain

Background:HCV genotyping is required in clinical practice in order to determine the type and duration of antiviral therapy. A commercial genotyping assay (Real-Time

HCV Genotype II, Abbott) is used at HUGTIP for this purpose. From 2009 to 2014, an indeterminate genotype result has been obtained for 26 out of 1338 patients (1.94%). The reference genotyping method, based on Sanger sequencing and phylogenetic analysis of the NS5B region, classified 24 cases as genotype 3 (20 as 3a and 4 as 3k), representing 7.95% of all HCV-3 detected cases (N=302). One patient was from Belarus, 15 (62.5%) from Pakistan, and 8 (33.3%) from Spain. HCV-3 is the second most prevalent genotype worldwide (30%) and in Spain (19.6%), it has been associated with a higher risk for liver disease progression, and has shown lower response rates to the latest antivirals.

Aim: To characterize the genetic diversity of the HCV 5'UTR region by ultradeep pyrosequencing (UDPS) in these indeterminate cases, in order to find out whether these results were due to the presence of mutations in the binding site of the HCV-3-specific probe of the commercial assay.

Methods: For the 24 indeterminate samples the 5'UTR region was amplified and subjected to UDPS with the 454/GS-Junior platform (Roche) following a recently published methodology. For all identified haplotypes within each sample, the genotype/subtype was assigned by phylogenetic analysis. For comparison, three additional samples that were correctly identified as HCV-3 by the real-time assay (3a by the reference method) were also analyzed.

Results:A median UDPS coverage of 591× (IQR,141-1830) was obtained per patient. For the highly conserved 5'UTR region only one major sequence was identified in 11 patients. In the rest of patients, 1-3 additional minor sequences were found (representing <6.5% of all sequences). HCV genotyping based on 5'UTR UDPS was in agreement with NS5B Sanger sequencing in all cases, confirming the absence of mixed infections and recombination events between different genotypes/subtypes. The alignment of the 5'UTR sequences evidenced the presence of 1-3 polymorphisms at the probe-binding site differentiating indeterminate from correctly genotyped HCV-3 samples.

Conclusions: The sequences generated in this study could help to improve the ability to detect HCV-3 by this commercial assay. This improvement requires a product change, but would be relevant in Spain and in many other countries where HCV-3 is highly prevalent (eg. inSoutheast Asia, South Asia, and Eastern Europe)or receive significant immigration from these areas.

(PO 148)

LONG NONCODING RNAs WITH PROVIRAL OR ANTIVIRAL PROPERTIES

M. BARRIOCANAL*[1], E. CARNERO*[1], C. PRIOR, N. RAZQUIN, V. SEGURA[2] AND P. FORTES[1]

[1]Department of gene therapy and hepatology.[2]Bioinformatic unit.

Centro de investigación medica aplicada (CIMA/UNAV). Pamplona. Spain.

Few studies have analyzed the antiviral role of long noncoding RNAs (lncRNAs). Therefore, we used HuH7 cells infected with Hepatitis C virus (HCV; JFH-1 strain) or control cells, and we treated them or not with interferon (IFN). Their transcriptome

was analyzed by microarray and RNASeq. The results show that most of the validated candidates are induced after IFN treatment or HCV infection.

ISRs (IFN-stimulated RNAs) are also induced after infection with mutant viruses that do not block the IFN pathway and in cultured cells or patients infected with HCV. Induction results from direct activation of the JAK/STAT pathway or by the effect of NFKB or downstream effectors of the IFN response. Genome-wide guilt-by-association studies predict that ISRs may function in the antiviral response. In fact, the best ISRs are located in the genome close to IFN stimulated genes (ISGs) with well-known antiviral properties, such as GBPs, IL6, IRF1, ISG15 or BST2. Further, ISRs expression levels correlate significantly with the expression level of their neighbouring gene in cultured cells and patients. Inhibition experiments show that they regulate positively or negatively the expression of their neighbouring gene or other ISGs, indicating that they may have proviral or antiviral properties.

Similarly, CSRs (HCV-stimulated RNAs) may have proviral or antiviral potential as they are altered in cells selected to replicate HCV efficiently. Further, inhibition of some of the candidates, leads to decreased viral replication. Interestingly, some CSRs are induced after infection with other viruses, (indicating that they may have a wider pro- or antiviral effect with therapeutic potential); but also in the liver of HCV-infected patients or in patients with liver cirrhosis or hepatocellular carcinoma, suggesting that some CSRs may be involved in the development of the liver disease that follows a chronic infection with HCV.

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KEY ROLE OF RNA STRUCTURAL CONTEXT AND ITS DYNAMIC STRUCTURAL TRANSFORMATIONS PROMOTED BY miR-122 IN THE HEPATITIS C VIRUS IRES-40S PREINITIATION COMPLEX.

A. ARIZA-MATEOS[1,2] AND J. GÓMEZ [1,2]

(1) Laboratory of RNA Archeology, Instituto de Parasitología y Biomedicina 'López-Neyra', CSIC, Armilla, 18100 Granada, Spain. (2) Ciberehd.

The hepatitis C virus (HCV) internal ribosome entry site (IRES) (domains II to IV) exists as a part of a much larger RNA structure which expands from domains I to VI and presents two alternative conformations: closed, “C”, and open, “O”, by miR-122 binding to domain I. An additional miR-122 binding site, recently described *in vitro*, together with one found in this work, form an unexplored tandem binding site at the 3’ end of the IRES. While the HCV IRES binds 40S ribosomal subunits and seems not to be affected by miR-122, it is unknown whether the higher-order structure surrounding the IRES and its dynamic structural transformation outlined above, participate in and modulate 40S-binding.

We found that RNA I-VI was more efficient in 40S binding than shortened forms lacking either or both flanks. In addition, we found that these RNAs formed two pre-initiation complexes that differentially migrate in native gel electrophoresis: the fast and slow forms, corresponding to the closed and open states of RNA I-VI, respectively. Only the slow form of RNA I-

VI, but not shortened RNAs, was progressively stimulated by miR-122, indicating that IRES in its natural context has a regulation level lacking in the shortened forms. Mechanistically, we have demonstrated that both IRES flanks contribute to the tRNA-like structure which includes the AUG start triplet, and that miR-122 alters it either by quick and local destabilization of domain IV, in the case of tandem binding to 3'-IRES sites, or through slow and long-distance effects in the case of tandem binding to the 5'site.

(PO 150)

HEPATITIS C VIRUS GENOMIC RNA DOMAINS. SEARCHING FOR THE SWITCH TRANSLATION-REPLICATION

C. ROMERO-LÓPEZ[1], A. BARROSO-DELJESUS[2], P. RÍOS-MARCO[1], A. BERZAL-HERRANZ[1]

[1]. Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, PTS Granada, Avda. del Conocimiento s/n, 18016, Armilla, Granada, Spain.

[2]. Unidad de Genómica, IPBLN-CSIC.

Hepatitis C virus (HCV) genome is a positive ssRNA molecule encoding for a single open reading frame, which is flanked by untranslated regions (UTRs). These regions bear structurally conserved elements that play essential roles for the consecution of the viral cycle. In the early stages of the infection, viral polyprotein synthesis is a preferential event that is governed by an IRES element (internal ribosome entry site), mainly located at the 5'UTR and spanning a short stretch of the coding sequence. IRES-dependent translation is also regulated by additional RNA elements placed in the 3' end of the viral genome. These include the 3'X-tail and the 5BSL3.2 domain of the CRE (*cis*-acting replicating element) region. Interestingly, the same elements that control HCV protein synthesis are responsible for promoting an enhanced replicative state in the advanced infection. Besides their role as binding platforms for protein factors, functional RNA domains may act as organizing centers for the establishment of complex networks involving intramolecular RNA-RNA interactions. Thus, the apical loop of the 5BSL3.2 domain is complementary to the apical loop of the 3'SLII within the 3'X-tail, while the 8-nts bulge may establish two different contacts: one with the apical loop of the subdomain IIId of the IRES region; the second with the so-called Alt motif, placed upstream of the CRE region. RNA elements are also involved in the establishment of intermolecular contacts by directing the genomic dimerization process, which is initiated at the 3'X-tail and relies on the exposition of the DLS (dimer linkage sequence) motif in the dimerizable isoform. We have demonstrated that the acquisition of this structural isoform in the 3'X-tail depends on both the IRES and the CRE regions. In this model, the CRE would act as an enhancer element of the dimer formation, whereas the IRES could play an inhibitory role. This interference with the genomic dimerization could be feasible even in the presence of the CRE, thus pointing to a yet unknown CRE-independent mechanism for controlling HCV RNA dimer formation. By using a high-throughput structural mapping strategy, this work identifies the residues and specific domains involved in this regulatory event. The presented data validate the existence of a complex, long-

range RNA-RNA interaction network that operates as regulatory partner in multiple viral processes and controls transitions between different steps of the viral cycle.

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PLASMOCYTOID DENDRITIC CELLS CONTROL HCV INFECTION *IN VITRO*

B. DOMÍNGUEZ-MOLINA[1], C. PERALES[2,4], K. MACHMACH[3], J.SHELDON[4], M. LEAL[1], E. DOMINGO[4], E. RUÍZ-MATEOS[1]

[1] *Instituto de Biomedicina de Sevilla/Hospital Universitario Virgen del Rocío. Seville. Spain.*

[2] *Liver Unit, Internal Medicine Hospital Universitari Vall d'Hebron,Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain.*

[3] *Institut de Reserches Cliniques de Montréal. Montréal. Canada.*

[4] *Centro de Biología Molecular "Severo Ochoa" (CSIC/UAM), Madrid, Spain.*

Introduction. Plasmacytoid dendritic cells (pDCs) are the main IFN-α producing cells of the immune system, therefore they are key players against antiviral infections. It has been shown that pDCs inhibit RNA virus replication *in vitro*, such as HIV and Influenza virus. However, the anti-HCV activity of pDCs and the mechanisms involved in this processes remain unclear.

Material and Methods. Human pDCs were isolated from four healthy donors. pDCs (with or without CpG previous stimulation) were cocultured with HCV infected Huh7.5 cells. For infections, the wild type virus (JCI), a virus after 100 serial passages in presence of IFN-α, IFN resistant (IR) and a virus after 100 serial passages (P100), were used. Extracellular-RNA virus was measured by qPCR and HCV infectivity, expressed as TCID50/mL, by immunocytochemistry with anti-NS5a antibody. Huh7.5 apoptosis (AnnexinV/ToproIII) and the expression of the death receptors, DR4/DR5, were analyzed by flow cytometry.

Results. JCI infectivity was inhibited near 70% by unstimulated pDCs, while in this condition, only 42% reduction of the infectivity was observed for IR and P100, which is consistent with previous data of IFN-α resistance of these viruses. However, after CpG pre-stimulation of pDCs, all virus infectivities were completely inhibited, probably due to the high amount of IFN-α released (>5 log pg/ml). Regarding HCV RNA release, unstimulated pDCs inhibited the 58% of JCI, pointing out that not all the produced virus were infective. Specific infectivity (TCID50/mL/HCV-RNA copies/mL) of JCI was reduced a 49.4% by unstimulated pDCs while IR and P100 were reduced 13.8% and 7.4% respectively. Again, CpG pre-stimulation of pDCs induced a strong inhibition of the three viruses (>90%). There was not apoptosis induction by pDCs (with or without CpG previous stimulation) in JCI Huh7.5 infected cells, suggesting that the decrease of JCI infectivity was mainly due to IFN-α production. However, in IR Huh7.5 infected cells, pDCs induced cell apoptosis. Unstimulated and CpG pre-stimulated pDCs induced two- and five-fold apoptosis compare to control, respectively. No apoptosis was induced in P100 infected cells by unstimulated pDCs, however, 2.6 fold increase apoptosis was observed in CpG pre-stimulated pDCs. No apoptosis induction in JCI infected cells by CpG-pre-stimulated pDCs were not due to differences in Huh7.5 TRAIL receptors expression since DR5 were upregulated

three-fold in all CpG-pre-stimulated conditions.

Conclusions.pDCs inhibited HCV replication and infectivity *in vitro*. IFN-mediated infectivity inhibition was the main mechanism in JCI virus while TRAIL-mediated apoptosis was the predominant mechanism in IR HCV inhibition.

(PO 152)

THE PREVALENCE OF HEPATITIS C VIRUS NS3 Q80K SIMEPREVIR RESISTANCE MUTATION IN SPANISH POPULATION ANALYZED BY NEW REAL TIME TECHNOLOGY IS A USEFUL TOOL FOR PERSONALIZED TREATMENT

Q. CHEN[a], F. RODRÍGUEZ-FRIAS[b,c,e], I. BELMONTE MULA[e], M. BUTI FERRET[a,b,c], L. NIETO APONTE[e], D. GARCIA CEHIC[a,b], J. GREGORI I FONT[a,b,d], R. ESTEBAN MUR[a,b,c], J. IGNACIO ESTEBAN MUR[a,b,c], J. QUER SIVILA[a,b,c].

Liver Unit, Internal Medicine, Lab. Malalties Hepàtiques, Vall d'Hebron Institut Recerca—Hospital Universitari Vall d'Hebron (VHIR-HUVH), Barcelona, Spain[a]; Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Hepáticas y Digestivas (CIBERehd) del Instituto de Salud Carlos III, Madrid, Spain[b]; Universitat Autònoma de Barcelona, Barcelona, Spain[c]; Roche Diagnostics SL, Barcelona, Spain[d]; Biochemistry Unit, Virology Unit /Microbiology Department, HUVH, Barcelona, Spain[e].

Background: Hepatitis C virus (HCV) polymorphism Q80K is associated with resistance to Simeprevir, a NS3 protease inhibitor. This direct-acting antiviral (DAA), approved in 2014, in combination with pegylated-interferon and ribavirin as a triple therapy, achieve a sustained virological response (SVR) rates of 85% in naïve patients with chronic HCV genotype 1 infection in clinical trials. However, the presence of substitution Q80K causes a reduction of SVR rates to 58%, similar to patients treated with pegylated-interferon plus ribavirin. Therefore, detection of Q80K before starting therapy with Simeprevir is needed to avoid treatment-failure.

Methods: 368 samples from genotype 1 HCV chronic infected patients were provided by Hospital Universitari Vall d'Hebron, Barcelona. A real time PCR based technique was developed to identify the Q/K variants at NS3 80 position using a FRET technology and melting curves analysis despitethe significant variants surrounding the specifically tested position.

Results: Specific nucleotide probes were able to differentiate between Q and K variants showing a complete concordance with Sanger direct sequencing in all compared samples (n=11). To test the specificity and sensitivity of the new technique, HCV strands carrying Q and K variants were cloned and mixed in different proportions. The prevalence of Q80K resistance mutation in G1 population was 6.5% (24/368). Moreover, among 128 high resolution HCV subtyped samples, the prevalence was 17.5% in G1a patients (n=63) but no cases were detected in G1b samples (n=65).

Conclusion: A new diagnostic tool based on real time technology using specific probe has been developed to detect single Q80K resistant mutation in a highly variable background. As previously reported, the frequency and outcome of Q80K resistant mutation varied between HVC subtypes and its presence is closely

related to the treatment effectiveness. Therefore, optimal treatment regimen will be achieved by performing HVC subtyping and Q80K detection before starting any Simeprevir based treatment.

(PO 153)

PREVALENCE OF THE HEPATITIS C VIRUS (HCV) POLYMORPHISM Q80K IN HCV INFECTED PATIENTS WITH GENOTYPE 1A IN SPAIN

SONIA VÁZQUEZ MORÓN, Mª ÁNGELES JIMENEZ SOUSA, MÓNICA GARCÍA ÁLVAREZ, MÓNICA GUTIÉRREZ RIVAS, SARA GÓMEZ ROBLES, LAURA LÁZARO PÉREZ, AMPARO ALVAREZ FERRERO, SALVADOR RESINO GARCÍA

Unidad de Infección Viral e Inmunidad; Centro Nacional de Microbiología, Instituto de Salud Carlos III; Carretera Majadahonda- Pozuelo, Km 2,2; 28220 Majadahonda (Madrid).

Background and aim: The Q80K polymorphism is a naturally occurring variation in the NS3/4A protease of hepatitis C virus (HCV) which substantially reduces the efficacy of triple therapy with simeprevir, interferon alpha, and ribavirin. The prevalence of Q80K polymorphism variesamongdifferent regionsor countries.The aim of this study was to evaluate the prevalence of Q80K polymorphism in HCV infected patients with genotype 1a in Spain.

Methods: We evaluated the sequence of HCV NS3 protease gene in 1690 samples collected from HCV infected patients with genotype 1a in 108 hospitals distributed geographically across Spain,between October 2014 and April 2015. HCV-RNA was extracted from plasma by using the QIAamp MinElute Virus Spin Kit (QIAGEN). NS3 gene amplification was carried out by reverse transcription PCR (QIAGEN) and nested PCR (Roche). Next, NS3 gene was sequenced by Sanger-based technology.

Results: In total, 471 out of 1690samples analyzedcorresponded toHIV/HCVco-infected patients and 1033 to HCVmonoinfectedpatients. Overall, 179samples hadQ80K polymorphism (10.59%).The prevalence ofQ80K polymorphism in HIV/HCV coinfectedpatients was12.31% (58/471) and9.48% (98/1033) inHCVmonoinfected patients.The higher prevalence ofQ80K polymorphism was found inthe regions ofCeuta (33%), Canary Islands (20.83%), Aragon (20%) and Madrid (19.6%). The Autonomous Communitieswith the lowest prevalencewereCastillaLa Mancha(0%), Valencia (6.09%), Andalucía(6.42%) and Cantabria (6.96%).

Conclusion: The global prevalence ofQ80K polymorphism was similarto that found inother European countries (France, Italy, Germany);however the prevalence of Q80K polymorphism in HIV/HCV coinfected patients was slightly higher.

(PO 154)

STUDY OF THE ROLE OF PHOSPHATIDATE PHOSPHATASES LPIN1 AND LPIN2 IN HEPATITIS C VIRUS INFECTION

L. MINGORANCE[1,2] , M.F.FRIESLAND[1], V. CASTRO[1] AND P.GASTAMINZA[1].

[1]*Laboratorio de Infección por el Virus de la Hepatitis C, Centro Nacional de Biotecnología, Madrid, Spain.*

[2]*Liver Unit, Hospital Clinic, IDIBAPS, CIBERehd, Barcelona, Spain.*

Hepatitis C virus (HCV) is a major causative agent of acute and chronic liver disease worldwide. Compelling evidence indicates that HCV infection relies on cell lipid metabolism and causes profound changes in lipid and lipoprotein homeostasis. Thus, host factors involved in lipid metabolism are interesting targets for antiviral intervention. In accordance with this, we focused our attention on lipins, a phosphatidate phosphatase (PAP) enzyme family that plays a key role in glycerolipid biosynthesis. These phosphatases mediate the conversion of phosphatidate to diacylglycerol, the immediate precursor of triacylglycerol (TAG), which is a major component of Lipid Droplets (LD) and Very Low Density Lipoproteins (VLDL), both required for HCV assembly. Under certain metabolic situations, LPIN1 and LPIN2 may also translocate to the nucleus to act as transcriptional regulators of lipo- and adipogenic genes. Interestingly, we have observed that LPIN1 and LPIN-2 mRNA abundance is modulated by acute HCV infection.

In order to study the role of LPIN family members, we first tested the susceptibility of human hepatoma cell lines (Huh-7) deficient in LPIN1 and determined that cellular LPIN1 expression levels are rate-limiting for HCV infection. This dependence appears to be specific for HCV, as infection of the same cell lines with human coronavirus (CoV-229E) resulted in normal infection efficiency regardless on the levels of LPIN1 expression. Subsequently, we dissected the HCV life cycle in order to determine which step/steps is/are dependent on LPIN1 function, using different surrogate models. While viral entry or primary translation are not affected by LPIN1 downregulation, a step leading to accumulation of intracellular HCV RNA is strongly dependent on this cellular factor. In contrast, LPIN1 does not appear to be rate-limiting for persistent RNA replication nor for infectious virus production. Altogether, these results suggest that LPIN1 is rate-limiting for the establishment of HCV RNA replication in an early state of infection, but not once infection has been established. We are currently conducting similar silencing experiments with a second member of the lipin family (LPIN2), which is expressed in the liver to higher levels than LPIN1 and mRNA expression of which is also regulated during HCV infection. We will present the results describing the impact of silencing LPIN2 alone or simultaneously with LPIN1 on different aspects of HCV infection. Furthermore, we will discuss the consequences that activation of these genes and subsequent unbalance of important intracellular lipid messengers by HCV infection may have on the pathogenesis of this virus.

(PO 155)

THE DEFENSIVE PATHWAY MEDIATED BY SALYCILIC ACID MAY BE INVOLVED IN RESISTANCE OF SOUR ORANGE TO CITRUS TRISTEZA VIRUS

N. GÓMEZ-MUÑOZ, K. VELÁZQUEZ, J. AGÜERO, S. RUIZ-RUIZ, MC.VIVES, P. MORENO, J. GUERRI*

Centro de protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra Moncada-Náquera KM 4,5 Moncada 46113 Valencia, España.

Citrus tristeza virus (CTV) induces decline and death of different citrus varieties grafted on sour orange, one of the most devastating citrus diseases that has killed more than 85 million trees worldwide, 40 of them in Spain. Citrus plants propagated on sour orange and infected by CTV show necrosis in the sieve tubes resulting in the decline and death of the tree. A very attractive hypothesis to explain the necrosis observed in the grafted sour orange trees infected with CTV is the induction in this rootstock of a hypersensitivity reaction (HR) triggering programmed cell death (PCD) that stops virus invasion.Even if the precise mechanism by which HR is triggered remains unknown, it appears to be related to the routeofsalicylic acid. On the other hand, the finding that CTV accumulates at lower levels in seedlings of sour orange in comparison with seedlings of susceptible citrus hosts, like Mexican lime and sweet orange, suggests the existence of certain resistance to CTV in the former. To study if this partial resistance is related with the defense pathway mediated by salicylic acid, two genes involved in this pathway (NPR1 and RdRp 1) have been silenced using a vector derived from citrus leaf blotch virus (CLBV). In sour orange seedlings where these genes were silenced, CTV accumulated to higher levels and displayed a better distribution with regards to non-silenced controls, with this effect being particularly noticeable in RdRp1-silenced plants.

(PO 156)

DEVELOPMENT OF A METHOD TO INCREASE EFFICIENCY OF VIRUS-INDUCED GENE SILENCING IN CITRUS

N. GÓMEZ-MUÑOZ, K. VELÁZQUEZ, J. AGÜERO, S. RUIZ-RUIZ, MC.VIVES, P. MORENO, J. GUERRI*

Centro de protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra Moncada-Náquera KM 4,5 Moncada 46113 Valencia, España.

Virus induced gene silencing (VIGS) is a helpful tool to evaluate

plant gene function by reverse genetics. This technology has advantages with respect to traditional approaches like mutagenesis and genetic transformation, because it allows to study the function of genes in a short time. However, VIGS is affected in many plants by the position, length and orientation of the insert designed to silence a specific gene, and depends also on the accumulation in target tissues of the viral vector at a level sufficient to trigger silencing, which otherwise is incomplete. A standardization of the VIGS protocol is required for each plant species. Recently, several vectors based on citrus leaf blotch virus (CLBV) have been developed for VIGS in citrus. CLBV induces a symptomless infection and

is not phloem-restricted. In this work we have analyzed the silencing of RNA-dependent RNA polymerase 1 (RdRp1), which is involved in antiviral defense in plants, including restricting the accumulation of citrus tristeza virus (CTV) in citrus. For this aim two CLBV-derived vectors were prepared containing: i) a 281-nt fragment of RdRp1, and ii) based on transitive RNA silencing, a 159-nt fragment of the sulfur gene (Su) coding for a subunit of the magnesium chelatase, which is highly expressed in all plant tissues, fused to a 148-nt RdRp1 fragment. The vectors were subsequently agroinfiltratred in leaves of *Nicotiana benthamiana* and semipurified virion preparations were then mechanically inoculated into rough lemon and from this intermediate citrus host by grafting to sour orange, in which CTV accumulation is restricted. Once CLBV infection was established in sour orange, plants were re-inoculated with a CTV-GFP clone expressing the green fluorescent protein. The levels of RdRp1 mRNA and CTV accumulation were estimated by real time RT-PCR and microscopy. Our results show that in plants carrying CLBV-Su-RdRp1 the levels of RdRp1 were decreased twice and CTV accumulation were increased four times regard to CLBV-RdRp1 inoculated plants. These results suggest that constructions with two genes in tandem increase the silencing efficacies through the mechanism of transitive RNA silencing.

(PO 157)

EVALUATION OF POTENTIAL SOURCES OF BIAS IN VIRAL METAGENOME PROTOCOLS

M. PARRAS-MOLTÓ[1], A. RODRÍGUEZ-GALET[1], P. SUÁREZ-RODRÍGUEZ[1], A. LÓPEZ-BUENO[1].

[1] *Centro de Biología Molecular Severo Ochoa (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), Madrid, Spain.*

Metagenomic surveys of viruses are subjected to a number of biases derived from particle purification, genome extraction, random DNA amplification and library preparation for next generation sequencing. However, these viral enrichment steps are required to increase the sensitivity of detection for viruses in metagenomics. Only a few methodological studies have assessed the impact of different protocols in the preservation of community composition during metagenomic approaches. Although these studies have identified ClCs gradient, chloroform treatment and multiple displacement amplification (MDA) as major bias sources, an optimal standard protocol is not yet available.

Here we performed a quantitative approach to analyze biases introduced in every step of a simple purification protocol using an artificial viral community. This community consists of a balanced mixture of seven DNA viruses with different genome and structure. The protocol included two consecutive low-speed centrifugation steps, filtration through 0.22 µm or 0.45 µm filters, iodixanol cushion and nuclease treatment. Extracted genetic material was alternatively amplified by two commonly used procedures: MDA and

sequence-independent, single-primer amplification(SISPA). Viral community composition was analyzed by quantitative real-time PCR (qPCR), in triplicates. The relative proportion of a large virus such as poxvirus was affected when 0.22 µm filters and low-force centrifugation were used. These two steps are normally employed in viral metagenome preparation. Furthermore, a recent report has shown important bias introduced by CsCl gradients, another frequently used technique. On the contrary, viral community remained invariable after 0.45 um filtration and iodixanol cushion. Regarding to random amplification, we found that MDA results in an over-representation of small circular ssDNA viruses (M13 or PCV2a), as previously reported, and the consistent under-representation of linear ssDNA viruses (MVMp). Interestingly, SISPA amplification preserves the ratio of the original viral mixture better than MDA. In addition, we purified viruses from a natural complex sample derived from the oral cavity and amplified their genomes by MDA or SISPA before Miseq-Illumina sequencing (~2 million paired-end reads). Consistently with the qPCR results described above, some of the most abundant viral contigs from these two viromes exhibit notable differences in coverage.

This research will contribute to establish a standard-gold protocol for preparation of viromes.

(PO 158)

HCV RNA-DEPENDENT RNA POLYMERASE INTERACTS WITH AKT/PKB INDUCING ITS SUBCELLULAR RELOCALIZATION

ROSARIO SABARIEGOS[1,4],MARÍA LLANOS-VALERO[1], FRANCISCO CIMAS[2], CELIA PERALES[5,6], ESTEBAN DOMINGO[5,6,7], RICARDO SÁNCHEZ-PRIETO[2,7,8], AND ANTONIO MAS[1,3,7,*]

[1] Laboratorio de Virología Molecular and [2] Laboratorio de Oncología Molecular, Centro Regional de Investigaciones Biomédicas (CRIB), Universidad de Castilla-La Mancha, Albacete 02008-Spain.

[3]Facultad de Farmacia and [4]Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete 02008-Spain.

[5]Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Cantoblanco 28049-Madrid, Spain.

[6]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain.

[7] Unidad de Biomedicina UCLM-CSIC. [8] Parque Científico y Tecnológico de Albacete (PCyTA), Albacete 02008-Spain.

HCV interacts with cellular components and modulates their activities for its own benefit. The cellular kinase Akt/PKB must be activated to increase the effectiveness of HCV entry, but is rapidly inactivated as the viral replication cycle progresses. Viral components have been postulated as responsible of Akt/PKB inactivation but the underlying mechanism remained elusive. In this study we demonstrate that HCV polymerase (NS5B) interacts with, is a substrate of, and changes the subcellular localization of Akt/PKB. Recombinant Akt/PKB can phosphorylate HCV NS5B *in vitro*. The specific Akt/PKB inhibitor MK-2206 prevents NS5B phosphorylation *in vitro*, and delays the cell culture

propagation of HCV infectious particles, both in a dose-dependent manner. HCV NS5B expressed either ectopically or from a replicating viral RNA co-immunoprecipitates with Akt/PKB. Moreover, Akt/PKB in the presence of transiently expressed NS5B or in replicon- or virus-infected cells modifies its cellular localization from cytoplasmic into perinuclear region where HCV replication complexes are located. The NS5B-Akt/PKB interaction represents a new regulatory step in the HCV infection cycle, opening new therapeutic options.

(PO 159)

ISG15 MODULATE VACCINIA VIRUS INDUCED MACROPHAGE POLARIZATION

MERCEDES FERNÁNDEZ ESCOBAR, BEATRIZ MARTÍN MORENO, SARA BALDANTA AND SUSANA GUERRA.

Laboratorio D-9, Dto. De Medicina Preventiva, Salud Pública y Microbiología, Facultad de Medicina, Universidad Autónoma de Madrid, Spain

Macrophage polarized to M1 or to M2 phenotypes in response to environmental signals. M1 macrophages characterize a proinflammatory phenotype, exhibiting increased phagocytic and antigen processing activity as well as increased production of proinflammatory to promote host defence and removal of damaged tissue. In contrast, M2 macrophage represents a phenotype that is potentially important in the promotion of wound healing and tissue remodelling as well as the resolution of inflammation. Recently we have demonstrated that the interferon stimulated gen 15 (ISG15) governs the phagocytosis capacity of peritoneal macrophages. In this study, we examined the polarization of bone marrow derived macrophage following the infection with vaccinia virus (VACV) infection and the role of ISG15 in macrophage polarization after viral infection.

The major aim of this proposal is to dissect the antiviral role and function of the ISG15-conjugation machinery in poxviruses infection. Several reports have demonstrated that the antiviral effects of ISG15 are attributed to ISG15-modification of viral proteins. Our preliminary data show, for the first time, ISG15-modification of virion particules from a pannel of completely different virus. Particularly when whe analyzed the virion of poxvirus we observed ISG15 residius in particles oft wild type Vaccinia virus (VACV) but not of the NYVAC mutant virions, indicating that the ISGylated viral proteins candidates are encoded by genes that are present in the wild type virus genome but absent in the mutant virus NYVAC genome (a total of 18 protein candidates) we will perform a complementary but different approach. If our hypothesis that viral proteins are also targeted for ISGylation and that this modification impacts virulence, we want to study the biological characteristics acquired by a virus that propagated exclusively in ISG15-/- cells. A VACV stock will be amplified and purified using ISG15-/- cells and as a control we will perform the same purification in ISG15+/+ cells (Fig. 8). We will be compare the viral properties of both stocks, primarily the ISGylation levels in both purified virion stocks will be analyzed by western blot as previously shown. Also we will compare the in vitro infection of mouse fibroblasts ISG15 +/+ and ISG15-/- with both viral stocks.

*Flash presentations

(PO 160)

THE MOVEMENT PROTEINS (NSm) OF DISTINCT TOSPOVIRUSES PERIPHERALLY ASSOCIATE WITH CELLULAR MEMBRANES AND INTERACT WITH HOMOLOGOUS AND HETEROLOGOUS NSm AND NUCLEOCAPSID PROTEINS

M. O. LEASTRO[1], V. PALLÁS[2], R. O. RESENDE[1], J. A. SÁNCHEZ-NAVARRO[2]

[1]*Departamento de Biologia Celular, Universidade de Brasília, 70910-900 Brasília, Brasil;*

[2] *Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-CSIC, E-46022 Valencia, Spain.*

Tospovirus is the only genus containing virus species which infect plants in the *Bunyaviridae* family. In the present work we have analyzed the *in vivo* membrane association of the movement protein (NSm) of the tospovirus species *Bean necrotic mosaic virus* (BeNMV), *Chrysanthemum stem necrosis virus* (CSNV), *Tomato chlorotic spot virus* (TCSV) and *Tomato spotted wilt virus* (TSWV) and the homologous and heterologous interactions among NSm and nucleocapsid protein (N). The results obtained by bimolecular fluorescence complementation (BiFC) assay and chemical treatments after membrane fractionation, revealed that the four NSm proteins are associated with the biological membranes with the N- and C-termini oriented to the cytoplasm. Similar membrane-associated pattern has been reported for other members of the 30K family, including the movement protein of *Prunus necrotic ringsport virus* (Martínez-Gil *et al*., 2009. J Virology, 83: 5535) and, more recently, the movement protein of *Tobacco mosaic virus* (Peiró *et al*., 2014.J Virology, 88: 3016), the type member of the 30K family, suggesting that the membrane-associated topology could be a general property for all members of the 30K family.BiFC analysis for protein-protein interactions showed: i), dimer formation for all NSm and N proteins; ii), interaction between NSm and the cognate N and iii), heterologous interactions between the NSm and N proteins. However, the heterodimers formed between the NSm proteins revealed compatible interaction only among TSWV, CSNV and TCSV. In contrast, BeNMV was unable to interact with the other heterologous NSm proteins. Interesting, TSWV, CSNV and TCSV have been grouped in the same clade into 'New World' tospoviruses, meanwhile BeNMV belong to a complete new branch of the American species. This observation supports the idea that compatible NSm interaction occurs only amongst viruses that are phylogenetically related. In contrast, the NSm proteins are able to interact with the three heterologous N proteins assayed, indicating that the NSm does not discriminate between virus particles, including members of the 'New world' (TSWV, CSNV, TCSV) grouping in one clade and BeNMV placed in a complete new branch. This observation differs significantly from that reported for other movement proteins of the 30K family, in which the viral movement proteins interact with the cognate CP but not with the heterologous CPs. These results raise the question whether the capacity of the NSm to interact with heterologous N proteins is a peculiarity of the Tospovirus genus or could be extended to other members of

the 30K family and, by other hand, shed light on the potential interactions in mixed infections.

(PO 161)

COMPARISON OF THE IMMUNOGENICITY OF THE *THEILERIA PARVA* CTL ANTIGEN Tp1, WITH OR WITHOUT A LEADER SEQUENCE, USING HAd5 AND MVA VACCINE VECTORS

N. SVITEK[1], A. LACASTA[1], R. SAYA[1], E. AWINO[1], R. PELLÉ[1], S. GILBERT[2], V. NENE[1], AND L. STEINAA[1]

[1]*International Livestock Research Institute, Nairobi, Kenya.*

[2]*The Jenner Institute, Oxford, UK.*

Theileria parva is a tick-borne parasite able to transform bovine T lymphocytes resulting in a lethal lymphoproliferative disorder in cattle. This pathogen claims the life of approximately one million cattle each year and results in economic losses of more than 300 million US dollars per year. Immune animals develop a lifelong immunity based on a cytotoxic T cell (CTL) response against homologous strains, with a strong immunodominance restricted by the major histocompatibility complex (MHC) class I molecules. Human adenovirus serotype 5 (HAd5) and Modified Vaccinia virus Ankara (MVA) are promising antigen delivery systems able to induce CTL responses against several intracellular pathogens. In this study, we aimed at inducing CTL responses in cattle against the *T. parva* BoLA-1*01301/01302-restricted CTL antigen Tp1 by using a heterologous HAd5 prime – MVA boost vaccination regimen. We compared the immunogenicity of a Tp1 construct harbouring the tissue plasminogen activator (tPA) signal peptide with a Tp1 construct without a signal peptide. Ten BoLA-1*01302-positive animals were inoculated intramuscularly with HAd5 and MVA vectors expressing either of the Tp1 constructs, and cell-mediated immunity is currently being monitored by ELISpot, proliferation and cytotoxicity assays, as well as by flow cytometry using newly generated bovine peptide-MHC class I tetramers. Initial findings indicate that HAd5/MVA viral vectors containing the Tp1 antigen without a signal peptide induce a stronger Tp1-specific CD8 response than the vectors expressing the Tp1 antigen harbouring the tPA leader sequence. This information will be valuable in our design of a next-generation vaccine for the control of *T. parva.*

(PO 162)

MECHANISM OF ACTION OF 4-DEOXYPHORBOL ON HIV-1 INFECTION. A NEW MEMBER OF ANTI-LATENCY DRUGS

HE DE LA TORRE[1], M BELTRÁN [1], LF NOTHIAS [2], J PAOLINI [3], LM BEDOYA [1], M LITAUDON [2], J ALCAMÍ [1]

[1] *AIDS Immunopathogenesis Unit, National Centre of Microbiology, Instituto de Salud Carlos III, Madrid, Spain*

[2] *Gif Research Center, Institute of Chemistry of Natural Substances (ICSN), CNRS, Labex CEBA, Gif sur Yvette, France*

[3] *Laboratoire de Chimie de Produits Naturels, UMR CNRS SPE 6134, University of Corsica, Corte, France*

Introduction: Antiretroviral therapy (ART) cannot eliminate HIV infection mainly due to the persistence of HIV in latently infected cells in the blood and organs of

infected patients. Viral reactivation has been proposed as ART adjuvant therapy to eradicate viral reservoirs. In order to do that, new drugs with different mechanisms of action and less toxicity are needed.

Material and Methods: Anti-HIV-1 and anti-latency effects of a 4-deoxyphorbol isolated from *Euphorbia amygdaloides*were evaluated *in vitro* in MT-2 cells and freshly isolated human PBMCs using recombinant HIV carrying luciferase-Renilla reporter genes. Receptor expression was evaluated by single-, double- or three-color immunophenotyping and performed with a FACScalibur flow cytometer. Transcriptional activity was performed by HIV plasmid DNA cell transfection using an Easyject plus Electroporator.

Results: 4-deoxyphorbol showed antiviral activity with IC_{50}s of 3nM in MT-2 cells and 0.3nM in PBMCs, infected with recombinant HIV (NL4.3-Ren). Specificity index of the compound is >10000, and no long-term toxicity was observed in PBMCs. Moreover, 4-deoxyphorbol induced the internalization of the lymphocyte receptors CD4, CXCR4 and/or CCR5 in MT-2 cells and IL-2 preactivated PBLs. 4-deoxyphorbol was able to reactivate viral transcription in HIV-1 transfected MT-2 cells and resting human PBMCs at concentrations as low as 10nM. Finally, 4-deoxyphorbol increase transcriptional activity of LTR and NF-kB regions in resting PBMCs.

Conclusion: 4-deoxyphorbol represents a new member of anti-latency HIV agents that could be further developed as ART adjuvants to eradicate latent HIV reservoirs or achieve a functional cure.

(PO 163)

EXPRESSION OF ARTIFICIAL MIRNAS: AN ANTIVIRAL STRATEGY IN PLANT BIOTECHNOLOGY

F. MESEL-CASANOVA[1], M. ZHAO[1], J.A. GARCÍA[1], C. SIMÓN-MATEO[1]

[1]. Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología-CSIC, Madrid, Spain

MiRNAs are important regulators of gene expression in both plants and animals. They are short single-stranded RNAs generated from longer premiRNA precursors and recruited to RISC effector complexes, which, in a sequence-specific manner, down regulate target mRNAs. Sharka disease, caused by *Plum pox virus* (PPV), is a persistent threat to the production of stone fruit trees of the *Prunus* genus, and novel approaches for protection are needed. Trying to explore new strategies to develop virus resistance and understand how viruses can evolve to escape from antiviral pressure, we have developed *Nicotiana benthamiana* transgenic plants expressing artificial miRNAs (amiRNAs) amiR-C and amiR-D, targeting NIb and CP PPV RNA regions, respectively. Several transgenic lines expressing either one of these amiRNAs or both (amiR-CD) showed complete protection against PPV-R. Other transgenic lines were only partially resistant and a few plants were infected. A large diversity of virus variants with different mutations in the amiRNA targets emerged in the infected plants. Several species, frequently with more than one mutation, used to accumulate in single plants. Sequence analysis of different escaping mutants

suggests that targeting of the genomic RNA by the mature amiRNA and of the complementary viral RNA by the amiRNA star strand contribute to the viral resistance. Viral progeny from some infected amiR-D plants was passaged in transgenic amiR-D lines with different levels of virus resistance and in wild type plants. Mutations selected in the partially resistant plants allowed the virus to infect the highly resistant plants and were stable in wild type plants. However, additional mutations appeared to be necessary to facilitate PPV infection under strong antiviral pressure. Broadness of the antiviral infection was assessed by inoculating amiR-C, amiR-D and amiR-CD lines with PPV isolates of the strains M and C, which differ in 1 to 3 nt from PPV-R in the C and D targets. Whereas one mismatch located in the seed of the star strand of amiR-D does not prevent antiviral activity, mismatches in the seeds of both the mature and star strands of amiR-C facilitated infection of transgenic plants. Interestingly, the amiRNA target sequence of some PPV-R escaping mutants selected under amiRNA pressure, mimicked the sequence of the natural PPV-PS (strain M) and PPV-SwCM (strain C) isolates, which could suggest the paths by which viruses evolve, by drift or under different selective pressures, are limited.

(PO 164)

CLEAVAGE OF THE ADENOVIRUS PACKAGING PROTEIN L1 52/55K BY THE VIRAL PROTEASE: IMPLICATIONS FOR VIRUS ASSEMBLY

A. J. PÉREZ-BERNÁ[1], G. N CONDEZO[1], R. MARABINI[2], W. F. MANGEL[3], J. FLINT[4], M. CHILLÓN[5], C. SAN MARTÍN[1]

[1]Centro Nacional de Biotecnología, CNB-CSIC, Madrid, Spain; [2]Escuela Politécnica Superior, UAM, Madrid, Spain; [3]Brookhaven National Laboratory, NY, USA; [4]Princeton University, NJ, USA; [5]CBATEG-UAB, Barcelona, Spain.

Adenoviruses are among the most complex non-enveloped icosahedral viruses, with a 95 nm icosahedral capsid composed of 9 different proteins, plus a 35 kbp dsDNA genome condensed by multiple copies of 3 core proteins *(1)*. Adenovirus maturation consists in proteolytic cleavage of several capsid and core proteins by the adenovirus protease (AVP) *(2)*. Immature particles lack infectivity because of their inability to uncoat. We have previously shown how adenovirus maturation modulates virion stability, and therefore its ability to uncoat correctly for a successful infection *(3-5)*.

In this communication we focus on the relationship between maturation and genome encapsidation in adenovirus, by characterizing the proteolytic processing of the packaging protein L1 52/55k by AVP. By treating immature particles with recombinant AVP we prove that L1 52/55k is a substrate for the maturation protease, and reveal multiple non-consensus cleavage sites. Proteolytic processing of L1 52/55k disrupts its interactions with other capsid and core proteins, providing a mechanism for its removal during viral maturation *(6)*. Cryo-electron microscopy

of two maturation intermediates shows the location of L1 52/55k in genome-lacking capsids and how this changes upon maturation. Immature, full length L1 52/55k is poised beneath the vertices to engage the viral genome. Upon proteolytic processing, L1 52/55k disengages from the vertex region, liberating it for the initial steps of sequential uncoating.

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(PO 165)

A VERSATILE ADENO-ASSOCIATED VIRUS VECTOR TO MONITOR THE INDUCTION OF TYPE I INTERFERON SIGNATURES *IN VIVO*

E. NISTAL-VILLAN*, Y. POUTOU[2], E. RODRÍGUEZ-GARCIA[2], J. PRIETO[2], R. HERNANDEZ-ALCOCEBA[2], E. LARREA[1], G. GONZÁLEZ-ASEGUINOLAZA[2]

[1]*Instituto de Salud Tropical, University of Navarra, Pamplona, Spain*

[2]*Gene Therapy and Regulation of Gene Expression Program. Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain*

Development of reporter systems to monitor type I interferon (IFN-I) induction in vivo is of great interest to characterize viral infections. We show here the generation of a type I IFN induction sensitive system that can be triggered both by the IFN-β induction and by the type I IFN signaling pathways. With the use of adeno-associated virus vectors (AAV), we have delivered this type I IFN sensitive element into the liver and lung of mice. Specific expression of a transgene like luciferase can be induced by different stimuli like Poly I:C, CpG DNA, Imiquimod or recombinant IFN-β. Intravenous injection of Newcastle disease virus (NDV) can induce luciferase in the liver numerous times, despite the generation of NDV neutralizing Ab. Intranasal instillation of the AAV vector allows upper and lower respiratory tract allows a continuous monitorization of type-I IFN signature after intranasal infection with Newcastle disease virus or influenza virus. The vector presented here can be accommodated to study both the strength and the kinetics of type I IFN signature in different animal organs in response to viral infections.

*Flash presentations **(PO 166)**

IN VIVO DELIVERY OF IFN-B INDUCTION PATHWAY ACTIVATING ELEMENTS USING ADENO ASSOCIATED VIRUS VECTORS TO GENERATE AN ANTIVIRAL STATE

[1]E. NISTAL-VILLÁN, [1]E. RODRÍGUEZ GARCÍA, [1]M. DI SCALA, [1]Á. VALES, [1]R. FERRERO LABORDA, [2]E. LARREA, [1]J. PRIETO, [1]G. GONZÁLEZ-ASEGUINOLAZA

[1] *Gene Therapy and Regulation of Gene Expression Program. Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain.*

[2]*Instituto de Salud Tropical, University of Navarra, Pamplona, Spain.*

RIG-I like receptors (RLRs) are cellular sensor proteins that detect certain RNA species produced during viral infections. RLRs activate a signaling cascade that results in the production of interferon-beta (IFN-β) as well as several other cytokines

with antiviral and proinflammatory activities. The potential of different constructs based on RLRs to induce the IFN-β pathway and create an antiviral state in type I IFN-unresponsive models was analyzed. A chimeric construct composed of RIG-I 2CARD and the first 200 amino acids of MAVS (2CARD-MAVS200) showed an enhanced ability to induce IFN-β as compared to other stimulatory constructs. Furthermore, this human chimeric construct showed a superior ability to activate IFN-β expression in cells from various species. This construct was found to overcome the restrictions of blocking IFN-β induction or signaling by a number of viral antagonist proteins. Additionally, the antiviral activity of this chimera was demonstrated in influenza virus and HBV infection mouse models using adeno-associated viral (AAV) vectors as a delivery vehicle. We propose that AAV vectors expressing 2CARD-MAVS200 chimeric protein can reconstitute IFN-β induction and recover a partial antiviral state in different models that do not respond to recombinant IFN-β treatment.

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