



Mo1927 Mucosa-Associated Microbiota and Promoter Methylation Status of Genes Involved in Immune Response in Crohn's Disease Patients

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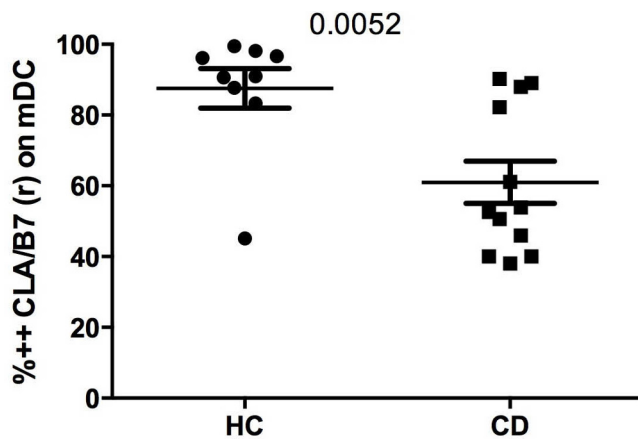


Figure 1: CLA/β7 double positive expression on mDC in Crohn's disease and healthy controls

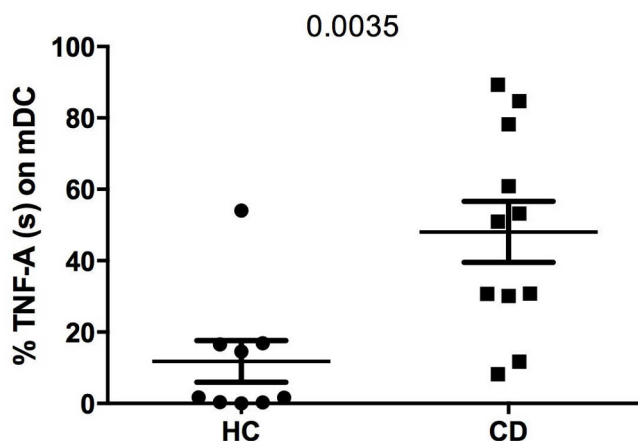


Figure 2: TNF-α on-going production by mDC in Crohn's disease and healthy controls

Mo1926

The Antimicrobial Peptide, LL-37, Is Induced via Toll-Like Receptor 3 in Human Colonic Subepithelial Myofibroblasts

Atsushi Nishida, Hirotugu Imaeda, Akira Andoh

(Backgrounds and aims) Antimicrobial peptides (AMPs) are molecules associated with the innate immune system. The most prominent families of antimicrobial peptides are defensins and cathelicidins. The only endogenous cathelicidin found in humans is hCAP-18/LL-37. Previous reports suggested that LL-37 influences microbial growth and intestinal inflammation. In this study, we investigated the induction and the biological activity of LL-37 in human colonic subepithelial myofibroblasts (SEMFs). (Materials and methods) LL-37 mRNA was analyzed using real-time PCR. The expression of LL-37 protein was evaluated by Immunoblotting and enzyme-linked immunosorbent assay (ELISA). Molecular mechanisms underlying LL-37 induction were evaluated using immunoblotting and small interference RNA (siRNA)-transfected cells. (Results) LL-37 mRNA expression was not enhanced by the stimulation with various kinds of cytokine stimulation. On the other hand, the stimulation with polyriboinosinic:polyribocytidylic acid (Poly(I:C)), toll-like receptor (TLR) 3 ligand, significantly enhanced the expression of LL-37 mRNA and protein. Poly(I:C) induced the mRNA and protein expression of LL-37 in dose- and time-dependent manner. The transfection of siRNAs specific for intracellular adaptor proteins, TRIF, TRAF6, IRAK1, and TAK1, significantly suppressed the expression of LL-37 mRNA induced by Poly(I:C). The inhibitors for ERK1/2 (PD98059 and U0216), a p38 MAPK (SB203580), and a JNK inhibitor significantly suppressed Poly(I:C)-induced LL-37 mRNA expression. In addition, the transfection of siRNAs specific for NF-κBp65 and c-Jun (AP-1) also markedly suppressed the mRNA expression of LL-37. Furthermore, the mRNA expression and protein secretion of IL-6 and IL-8 induced by LPS was significantly reduced in the presence of LL-37 as compared to in the absence of LL-37. (Conclusions) LL-37 is induced by the stimulation with Poly(I:C) via the assembly of intracellular adaptor proteins, TRIF, TRAF6, IRAK1 and TAK1, into a complex, leading to the activation of MAPKs followed by the activation of transcription factors, NF-κB and AP-1 in human colonic SEMFs. LL-37 suppresses the induction of IL-6 and IL-8 by LPS stimulation in human colonic SEMFs. In conclusion, we propose that TLR3 signaling may play an important role in regulating intestinal mucosal barrier by inducing LL-37 and by exhibiting the anti-endotoxin effect.

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Mucosa-Associated Microbiota and Promoter Methylation Status of Genes Involved in Immune Response in Crohn's Disease Patients

Clelia Cicerone, Valentina Totino, Valerio Iebba, Piero Vernia, Stefano Pontone, Barbara Porowska, Maurizio Giovannone, Floriana Santangelo, Antonella Gagliardi, Francesca Guerrieri, Massimo Leviero, Fabrizio Pantanella, Serena Schippa, Enrico Corazzari

Background: Crohn's Disease (CD) results from a complex immune response combined with multiple genetic and environmental factors. Microbiota environment can induce epigenetic changes, such as DNA methylation, in CD inflammation. **Objective.** We aimed to assess in biopsies mucosal samples, 1) mucosa-associated microbiota (MAM) and promoter methylation status (PMS), involved in the inflammatory response; 2) correlations between MAM and PMS. **Patients and Methods.** Twenty-one CD pts, 12 males, 50.5 years $\pm$ 5 SD, with inactive disease (CDAI <150) and twenty seven healthy controls (HC) were recruited. Exclusion criteria were infectious and immune diseases, malabsorption, prior antibiotic therapy. At ileocolonoscopy 3 biopsies were collected from terminal ileum in 14 CD pts and neo-terminal ileum in 7 operated CD pts. Total DNA was extracted from biopsies to evaluate: i) MAM characterization through 16S rDNA metagenomics (MiSeq); ii) PMS of 22 genes involved in inflammatory response through dedicated qPCR arrays (in 12 HC and 12 CD pts). We recorded data on serum concentration of C-reactive protein (PCR), erythrocyte sedimentation rate (ESR). **Results. 1. Characteristics of CD population.** The disease phenotype was inflammatory in 91% of pts and stricturing and penetrating in 9% of pts. Three pts had increased PCR and ESR. At endoscopy, mucosal healing or minimal endoscopic lesions were found in 81%; Rutger score was > 2 , in 2 operated pts. Treatment at time of inclusion was 5-Aminosalicylate, 43%; immunomodulators, 29%; other therapy (anti-TNF) or no therapy in 28%. **2. MAM characterization and PMS evaluation.** RadViz analysis of NGS data on MAM evidenced a significant separation between CD and HC ($P=0.0001$) indicating a dysbiosis status with higher relative abundance of Proteobacteria phylum and Pseudomonas genus in CD. Abundance-based Coverage Estimator, a community richness estimator, showed a reduction of biodiversity in CD ($P=0.033$). A significant decrease in methylation levels of IL13RA1 and InhA promoter's genes, both involved in a pro-inflammatory pathway, was observed in CD. Pearson correlation among bacteria Phyla and Genera level and methylation status of the 22 promoter genes involved in inflammation, showed that bacteria differently affect epigenetic modifications in CD and HC. **Conclusion:** A gut dysbiosis with a reduction of biodiversity in the microbiota ecosystem are still present in CD in remission. The impact of the MAM on the methylation level of promoter genes, related to the immune responses, in CD is markedly different from HC, indicating that, within the limits of a study performed in a small sample of patients, the different mucosa associated environment appear to affect the methylation status. Supported by: PRIN 2012, Protocol 2012WJSX8K, Serena Schippa; University "Sapienza", 2013, Enrico Stefano Corazzari.

Mo1928

Caspase-11 Exacerbates Acute, but Not Chronic, Experimental Colitis in a Microbiota-Dependent Manner

Ting-Jia Fan, Diana Arsene, Sandrine Y. Tchaptchet, Ian Carroll, Jonathan J. Hansen

BACKGROUND: Inflammasome activation is variably associated with human inflammatory bowel diseases (IBDs) and experimental colitis. Caspase-11 (Casp11), a non-canonical activator of the inflammasome that responds to bacterial lipopolysaccharide, has previously been shown to attenuate acute chemical injury mediated colitis. However, the role of intestinal microbiota and Casp11 in more IBD-relevant chronic immune-mediated experimental colitis is limited. We hypothesize that Casp11 reduces chronic intestinal inflammation in mice in a microbe-dependent fashion. **METHODS:** Quantitative real-time PCR was used to measure Casp11 expression in colon tissue from Il10-deficient (Il10^{-/-}) mice (a model of immune-mediated chronic colitis) and dextran sodium sulfate (DSS)-treated wild-type (WT) mice (a model of acute and chronic chemical injury colitis depending on length of treatment). We quantified histological inflammation in colon sections and spontaneous IL-12/23 p40 secretion by colon explants in WT, Il10^{-/-}, Casp11^{-/-}, and Il10^{-/-};Casp11^{-/-} (DKO) mice, as well as in WT and Casp11^{-/-} mice with acute and chronic DSS-induced colitis. Antibiotic treatment and bacterial 16S rRNA Illumina sequencing of fecal microbes were used to assess the role of microbiota in Casp11-mediated effects during acute DSS colitis. **RESULTS:** Casp11 mRNA is increased in colon tissue from Il10^{-/-} mice (5.06 $\pm$ 0.54 vs. 2.29 $\pm$ 1.15, Il10^{-/-} vs. WT; $p<0.05$) and DSS-treated mice with acute and chronic colitis compared with healthy, untreated WT mice (acute 2.08 $\pm$ 0.61; $p<0.001$, chronic 2.74 $\pm$ 0.75; $p<0.005$). Composite histological colon inflammation scores and IL-12/23 p40 secretion by colon explants were similar in DKO vs. Il10^{-/-} mice as well as in Casp11^{-/-} vs. WT mice with chronic DSS colitis (Table1). Contrary to published reports, WT mice in our facility lost more weight and had worse histological colitis compared with Casp11^{-/-} mice during acute DSS colitis (Table2). Antibiotic pretreatment reversed the increased weight loss observed in WT vs. Casp11^{-/-} mice and was associated with reduced bacteria in the phylum Tenericutes. **CONCLUSIONS:** Casp11 is up-regulated during acute and chronic experimental colitis, does not affect severity of chronic colitis, but does exacerbate acute colitis in microbiota-dependent manner. These findings suggest that Casp11 does not play a role in chronic immune-mediated colitis and highlight the importance of the intestinal microbiota in mediating the effects of the inflammasome on acute colitis.