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Technical Note

An easy assay to detect autoantibodies neutralizing cytokines in subjects with critical infections

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ABSTRACT

Autoantibodies against type I interferon (IFN) are associated with a worse outcome in COVID-19. The measurement of cytokine-neutralizing autoantibodies has been limited, hindering understanding of their role in clinical practice. We showed that an easy and reliable assay can be reproduced and validated to measure the neutralizing potency of autoantibodies directed to type I or type II IFN. Identifying of anti-cytokine autoantibodies might reflect on early treatments for subsequent infections, such as with antivirals or virus-neutralizing monoclonal antibodies.

1. Introduction

The role of cytokine-neutralizing autoantibodies in severe infections has recently been brought to the forefront by the finding that autoantibodies against type I interferon (IFN) are associated with a worse outcome in COVID-19 (Bastard et al., 2020). Indeed, the knowledge that some subjects can develop autoantibodies neutralizing specific cytokines dates back to forty years (Pozzetto et al., 1984).

The impact of anti-IL-17 autoantibodies in chronic mucocutaneous candidiasis in subjects with Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED) disease, an inherited immunodeficiency associated with multiple autoimmune features since childhood, made it clear how detrimental these autoantibodies are for the susceptibility to particular infections, such as mucocutaneous candidiasis (Puel et al., 2010). Since then, it has been shown that various anti-cytokine autoantibodies can cause phenocopies of primary immunodeficiencies with susceptibility to specific infections (Browne and Holland, 2010). For example, while autoantibodies against type I IFN were associated with the worst outcome from viral infections, autoantibodies against type II IFN were found to underpin diffuse mycobacterial disorders (Browne and Holland, 2010). However, cytokine-neutralizing autoantibodies have been measured only in a few specialized centers, hindering a complete understanding of their potential role in clinical practice. Low anti-cytokine autoantibody levels may be a physiological part of a built-in negative feedback system that controls excessive cytokine release (Ross et al., 1990).

Therefore, it needs to be clarified what levels favor the development of severe infections and can be considered clinically meaningful. For this reason, by exploiting the availability of commercial cytokine-reporter cell lines, we developed an easy assay to measure the neutralizing potency of autoantibodies directed to type I or type II IFN, which could easily be adapted to other cytokines.

2. Material and methods

2.1. Development of the assay

We aimed to measure the functional inhibition of IFN action on target cells by autoimmune sera. Therefore, we relied on commercial cell lines with a built in reporter system that can be exploited to measure the response to added cytokines and the inhibition by patients' sera.

2.2. Culture of IFN-reporter cells

To assess the IFN-neutralizing potency of candidate sera, we used reporter-cell systems responding either to type I IFNs (HEK-Blue IFNα/β) or to type II IFNs (HEK-Blue IFNγ cells), both from InvivoGen (San Diego, California, USA). In these genetically modified cell lines, the two

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IFN receptors (type I or type II) are coupled with a reporter system, leading to the secretion of embryonic alkaline phosphatase (SEAP). Both cellular lines were cultured according to the manufacturer's instruction in a medium composed of Dulbecco's Modified Eagle Medium (DMEM), 4.5 g/L glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), Pen-Strep (100 U/mL - 100 µg/mL) all from EuroClone (Milan, Italy), and supplemented with 100 µg/mL Normocin (InvivoGen). After the second passage, 30 µg/mL of Blasticidin and 100 µg/mL of Zeocin (both from InvivoGen) were added to the culture medium.

For the assay, 5×10^4 HEK-Blue cells (either IFNα/β, or IFNγ specific) were added to each well of a 96-well plate, together with the proper dilution of the heat-inactivated serum sample in the presence of either recombinant human IFNα2a or IFNγ (Miltenyi Biotec, Bergisch Gladbach, Germany) as stimulus (to a final volume of 200 µL per well). Heat-inactivated human AB serum was used in all the experiments as the negative control. The plate was then incubated for 24 h at 37 °C in humidified air containing 5% CO_2.

The secreted SEAP activity, which is proportional to the amount of active cytokine stimulating the cell receptor, was measured according to the manufacturer's instructions. In a new 96-well plate, 180 µL of QUANTI-Blue™ detection solution (InvivoGen) and 20 µL of cell supernatant were allotted in each well and incubated for 1 h at 37 °C. The colorimetric reaction was assessed by reading the optical density at 600 nm. A reduced response to a given amount of added cytokine reflects the inhibitory potency of the serum sample.

2.3. Serum samples

The study was developed in three steps: 1) setting up of the method by using healthy controls (mean age 37 ± 9, 47 male and 43 females) and a positive control (a sera from a patient with APECED with high titer autoantibodies to multiple cytokines, Table A1); 2) double blind validation of the assay by analyzing 24 positive or negative serum samples that had already been studied for the presence of anti-IFNα and anti-IFNγ autoantibodies at the NIH laboratories provided by prof. Steven Holland's research group at the National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA); 3) analysis of IFNα neutralizing (mean age 31 ± 18, 8 male and 16 females, Table A2) antibodies in two groups of adults below 60 years of age who had experienced either a mild or a severe COVID-19 during 2020, before the availability of SARS-CoV-2 vaccines. The first group comprised of medical residents contracting SARS-Cov-2 infection with mild or negligible symptoms (age range 25–31 years, 4 male and 4 females). The second group included of subjects admitted to the Intensive Care Unit of the Azienda Sanitaria Universitaria Giuliana (ASUGI) for COVID-19, (mean age 52 ± 7, 4 male and 4 females, Table A3). All subjects survived the infection and underwent a blood draw for serum sampling one month after recovery.

Serum samples were collected at the IRCCS Burlo Garofolo Institute for Maternal and Child Health (Trieste, Italy), within the IRB-approved research project #30/22. Samples were taken after receiving the patient's or donor's informed consent according to the study protocol. All sera were stored at −80 °C until use.

2.4. Data analysis

The *intra*-assay coefficient of variation (CV) has been calculated for the first 2 plates, each containing 45 sera analyzed in duplicate. The *inter*-assay CV has been calculated on 10 independent measurements of 2 samples (one "highly neutralizing" serum and one "not-neutralizing" serum), in comparison with the positive control (IFNα2a in the same plate in the absence of patient serum). For the "highly neutralizing" serum, the CV was calculated on the percentage of inhibition of the positive control; for the "not-neutralizing serum", the CV was calculated as the percentage of the positive control.

Data analysis was performed using Prism GRaphPad8 software; differences between groups were evaluated with the Mann-Whitney test (p values <0.05 were considered statistically significant).

IC50 was calculated with the online tool "Very Simple IC50 Tool Kit" (http://ic50.tk/, accessed on 18 August 2023).

3. Results

3.1. Optimization of a functional assay to detect anti-interferon activity in human sera

We first determined the maximum concentration of human serum that can be added to the cultures without affecting the growth and responsivity of reporter cells. Indeed, human serum contains various molecules and protein factors that might interfere with a functional assay based on living cells.

The response of HEK-Blue IFNα/β reporter cells to human recombinant IFNα2a (at the concentration of 100 or 1000 U/mL) was reduced when high concentrations of human AB serum were added to the system. It could be due to a nonspecific effect of some serum components on the reporter system, and lower nutrient availability in AB serum compared to culture media. We thus decided to settle on a maximum volume of serum samples of 10% of the total volume for further experiments.

We then evaluated the inhibitory action of a positive control serum on the reporter cell response to increasing concentrations of IFNα2a. Since the reporter cells were based on the response to stimulation of the common Type I IFN (IFNAR), the assay could also be optimized to study autoantibodies against other type I IFN family cytokines. However, in this study we only used IFNα2a stimulation. As a positive control, we employed a serum sample obtained from a patient with APECED, which was previously evaluated at another center for type I IFN inhibitory autoantibodies. The effect of the positive serum was compared with a healthy-donor's serum and a commercial AB serum sample. The higher dilution of the APECED serum (0.1% of total volume) led to almost complete inhibition of the response to IFNα2a except for at the highest concentration of the cytokine (Fig. 1A).

Given the high inhibitory potency observed for the positive control, we searched for the lowest concentration of IFNα2a that could induce a linearly predictable response in the reporter cell lines in order to be able to use serum samples at sufficiently high dilutions.

Based on the dose-response curve of the reporter system as described by the producer and verified at our laboratories, we chose 50 U/mL of IFNα2a at the ideal concentration for our experiments. This concentration should have a medium intensity stimulation within the linearity range of the dose-response curve but with a target Optical Density (OD) averaging 1 (Fig. 2). To test the system, we analyzed a commercial antibody neutralizing IFNα which showed an IC50 concentration of 1/453757 +/− 3995 (data not shown).

We thus measured the response of the reporter cells to a fixed concentration of 50 U/mL of IFNα2a in the presence of increasing dilutions of the positive control serum (Fig. 1B). The figure shows that the positive serum maintained an almost complete inhibitory action at a dilution in the range of 1:8000, with 60% of inhibition at a concentration of 1:16000, which was comparable with the findings obtained with other methods in patients with IFN neutralizing autoantibodies (Bastard et al., 2020).

Based on these results, we hypothesized that a dilution of 1:100 of human serum samples could be suitable for the screening of neutralizing autoantibodies with subsequent confirmation at higher dilutions (1:1000 or higher), even if we were still not able to determine the clinical significance of these thresholds. To evaluate if this dilution could be responsible for non-specific neutralizing results in healthy donors, we analyzed 90 serum samples from healthy volunteers. Only 1 sample at the 1:100 dilution led to a considerable inhibition of the response to IFNα2a (13.8%). Based on the average values plus three standard deviations of the inhibition percentages measured in the 90 donors' samples, we could consider a 30% of inhibition as the threshold of normality

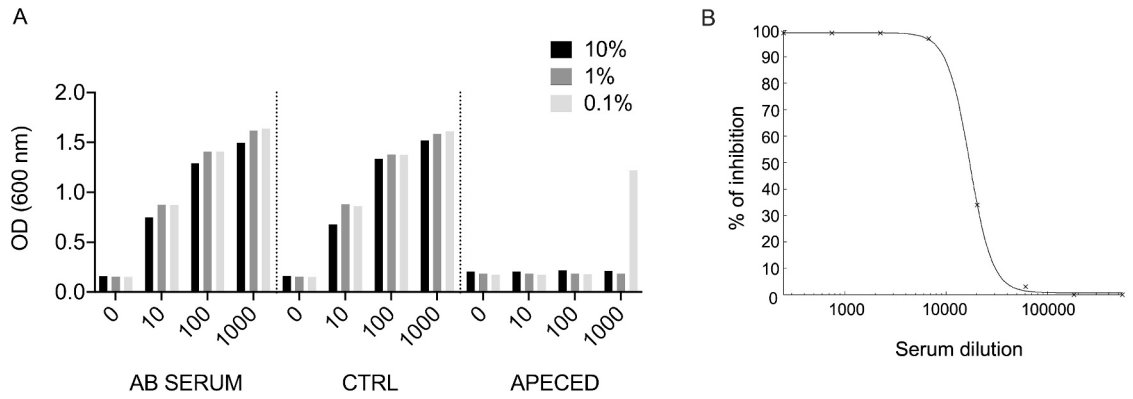

Fig. 1. A) Response to IFNα2a as revealed by secreted alkaline phosphatase activity (OD = optical density after colorimetric reaction) in HEK-Blue IFNα/β reporter cells in the presence of various concentrations of commercial AB human serum, healthy donor serum (CTRL) and serum from a patient with APECED. B) Inhibition of the response to IFNα2a by the serum of a subject with APECED at increasing dilutions (IC50 = 1/17064 +/− 355). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

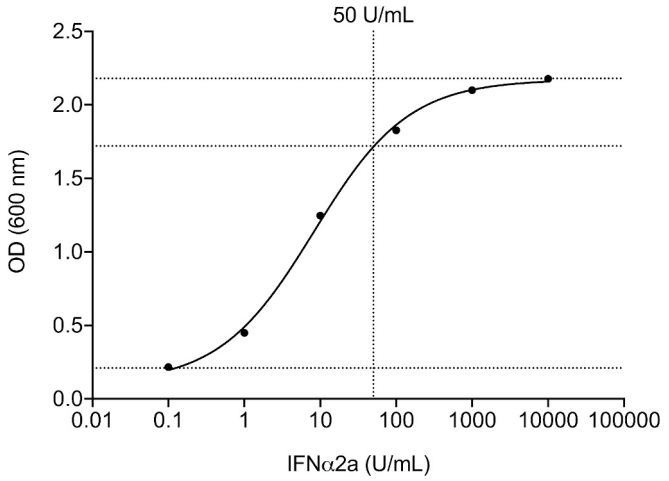

Fig. 2. The concentration of 50 U/mL of IFNα2a was chosen as it was sufficient to stimulate >80% of the maximum response, still falling in the linear zone of the dose-response curve (OD = optical density after colorimetric reaction).

threshold for sera diluted 1:100 (specifically, 28.8% rounded to 30%). Samples that resulted in an inhibitory action more outstanding than 30% should be re-evaluated at a higher dilution.

3.2. Double blind validation of the assay on positive and negative sera with known anti-interferon action

To evaluate the potential clinical relevance of our assay, we decided to analyze samples already evaluated for clinical reasons (susceptibility to severe viral or mycobacterial infections) with the finding of neutralizing autoantibodies against either or both of type I and type II IFN. The samples had been previously analyzed at the NIH laboratories with a Multiplex particle-based flow cytometry assay (Bastard et al., 2020; Ding et al., 2012). We thus double blinded analyzed a set of 24 serum samples kindly given to us by Dr. Steven Holland and his research group (Table 1). We repeated the test keeping the same conditions as before (samples dilution of 1:100; IFNα2a concentration of 50 U/mL). The bar plot in Fig. 3A shows that among the 24 samples tested, 13 were negative for autoantibodies against type I IFNs (percentage of inhibition below 30%). At the same time, 11 were positive, all inhibiting at least 95% of the reporter signal. These results were then checked and confirmed.

We further evaluated the average inhibition potency in the positive sera, and proved that almost all maintained a high inhibitory potency at dilution above 1:1000 (Fig. 3B).

Table 1
Anti-IFNs autoantibodies titers of 24 samples provided by NIH and used for double blind validation of the assay.

Cod.	Positive for:	anti-IFNα FI	anti-IFNγ FI
S1	IFNα	11,261	800
S2	IFNα	12,610	98
S4	IFNα	15,730	551
S7	IFNα	10,611	298
S8	IFNα	10,881	982
S10	IFNα	6859	89
S13	IFNα	10,503	108
S14	IFNα	10,058	80
S17	IFNα	10,190	163
S21	IFNα	5959	572
S23	IFNα	12,095	109
S16	IFNγ	153	11,768
S19	IFNγ	1284	13,892
S20	IFNγ	647	10,819
S24	IFNγ	162	11,047
S3	–	163	469
S5	–	133	56
S6	–	14	39
S9	–	104	188
S11	–	76	555
S12	–	237	198
S15	–	51	126
S18	–	254	328
S22	–	49	289

Fluorescence Intensity (FI) is a function of autoantibody titer; cut-off for positivity above 1500 FI.

Since several reporter cell lines were commercially available to measure the response to specific cytokines, we evaluated if our system was easily scalable to other cytokines. We tested whether the same protocol, developed for type I IFN, could be also suitable for type II IFN, using InvivoGen's HEK-Blue IFNγ cells (for this purpose, we used the same cut-off developed for Type I IFN). The assay was validated by a double-blinded measure of IFNγ neutralizing autoantibodies on the 24 NIH sera. We confirmed the presence of neutralizing autoantibodies in the same 4 samples that resulted positive at the NIH (S16, S19, S20, S24, Fig. 3C). Negative results were obtained not only for the other 20 sera of the blind test but also for the serum samples derived from the patients affected by APECED and COVID-19 (data not shown). Also for IFNγ, we demonstrated that almost all positive sera maintained high inhibitory

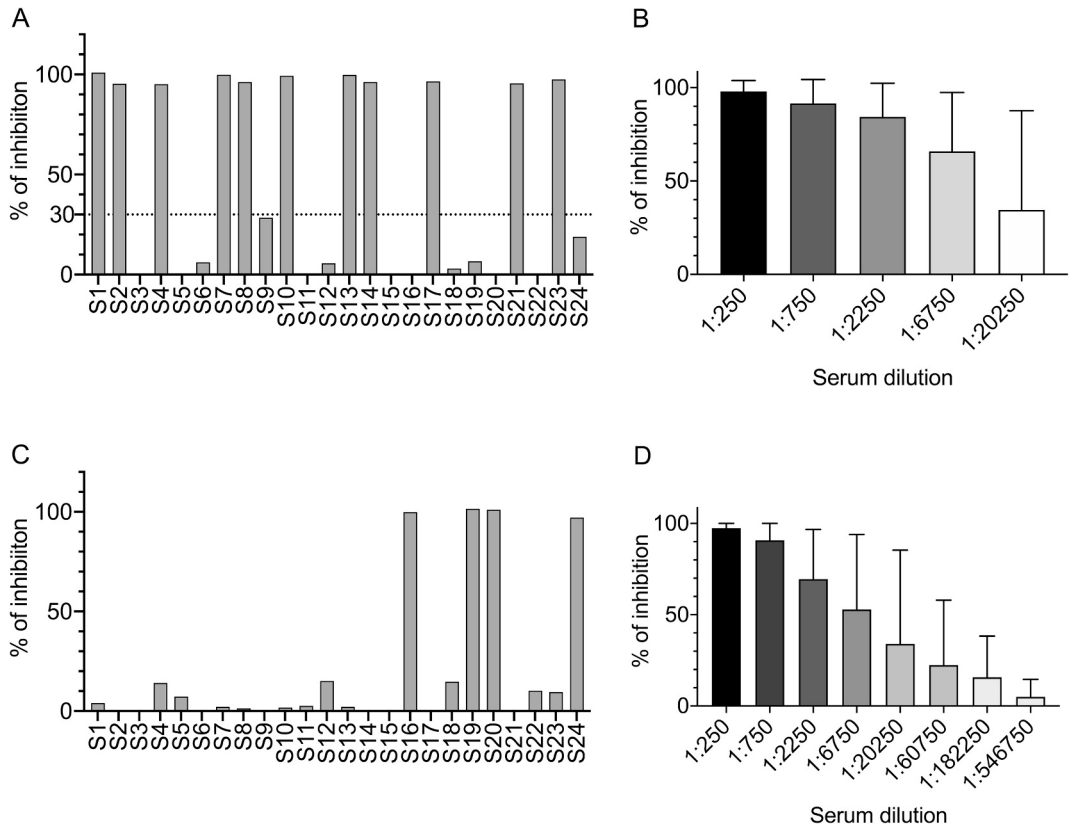

Fig. 3. A) Inhibition of the response to IFNα2a by 24 serum samples provided by the NIH. The same samples (S1, S2, S4, S7, S8, S10, S13, S14, S17, S21, S23) resulted positive at both laboratories (the dashed line represents the 30% inhibition cut-off value). B) Mean (+ range) response percentage to IFNα2a in positive samples at increasing serum dilutions. C) Inhibition of the response to IFNɣ by 24 serum samples provided by the NIH. The same samples (S16, S19, S20 and S24) resulted positive at both laboratories. D) Mean (+ range) response percentage to IFNɣ in positive samples at increasing serum dilutions.

potency at dilutions above 1:1000. (Fig. 3D).

3.3. Analysis of IFNα neutralizing autoantibodies in two groups of young unvaccinated adults who had experienced either a mild or a severe COVID-19

We had a further opportunity to train our assay by analyzing two sets of sera obtained from young unvaccinated adults one month after recovery from either mild or severe COVID-19.

As shown in Fig. 4A, only 1 out of 8 subjects with severe COVID-19 displayed autoantibodies neutralizing type I IFN versus none of the mild. However, when compared in the two groups, subjects with severe COVID-19 displayed a significantly higher percentage of inhibition (median 4.93% in group C vs 9.65% in group S, $p = 0.0062$), even if still in the negative range (below 30% of inhibition, Fig. 4B). The difference remained significant also without considering the highly positive S8 sample (median 4.93% in group C, vs 9.22% in group S, $p = 0.0121$, Fig. 4C). Fig. 4D showed data distribution of patients with mild and severe COVID-19 compared with our cohort of 90 healthy donors. The test was performed with a sample dilution of 1:100 total volume and with a final concentration of stimulus of 50 U/mL.

As a final step of test validation, we have calculated the *intra* and *inter* assay coefficients of variation (CV), both of which were found to be <10%. The *intra*-assay CV ranged between ~5% e ~ 6%. The average *inter*-assay CV calculated on both a neutralizing and on a not-neutralizing serum, was 3.94% (average inhibition of the "neutralizing serum" = 99.92%, Standard Deviation, SD = 1.65, CV = 1.65%; average inhibition of the "not-neutralizing serum" = 0.99%, SD = 0.062, CV = 6.23%).

4. Discussion

We developed an easy and reliable assay to detect anti-cytokine autoantibodies, which can be reproduced at any biomedical laboratory with minimal expertise in cell culture and colorimetric assays. The assay measured the inhibitory effect of human sera on the biological action of specific cytokines rather than the concentration of inhibitory autoantibodies. This approach was meaningful on a clinical grounds, as it could reflect the immune deficiency due autoantibodies and the related risk of severe infections (Browne and Holland, 2010). Breivik et al. previously proposed a similar approach to detect anti-IFN autoantibodies in subjects with APECED found in almost all the patients (Breivik et al., 2014). Our results confirmed those of Breivik et al. in another setting and the suitability of the assay to detect IFN-neutralizing autoantibodies in subjects with critical course of specific infections.

Although there are several publications on the role of anti-IFN autoantibodies in severe COVID-19, they came from a limited number of multicenter collaborative studies and are based on a few prevalently homemade immune assays. Multiplex-particle-based assays or enzyme-linked immunosorbent assays are usually used to screen for cytokine autoantibodies (Bastard et al., 2020). Various functional assays can be proposed to assess biological relevance of such autoantibodies. The first is based on the measure of phosphorylation of STAT1 in peripheral blood cells from healthy donors after stimulation with IFN in the presence or absence of the anti-cytokine serum, and it can be difficult to standardize (Kisand et al., 2008). Another test is based on engineered reporter cells whose response to IFN can be tracked by various methods like enzymes (e.g. luciferase, secreted alkaline phosphatase) whose activity is detected as the readout of the assay (Bastard et al., 2020). We demonstrated that this kind of assay can be quickly developed through commercial cell

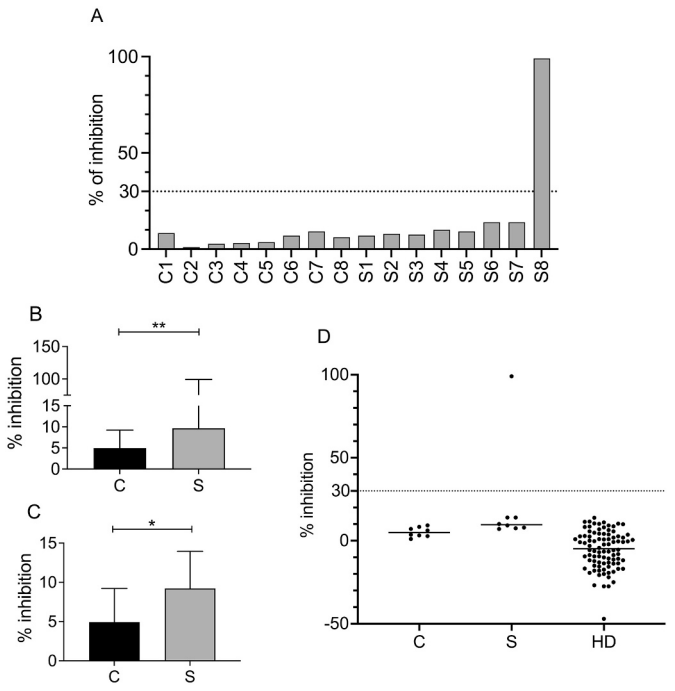

Fig. 4. (A) Inhibition of response to IFNα2a in 8 subject with mild SARS-CoV-2 infection (C1–8) and in 8 patients critically ill for COVID-19 (S1–8). Only one patient showed high titer anti-IFN activity. The median inhibition (+SD) was significantly higher in group S compared with group C (B) and the difference remained significant after excluding patient S8 (C). (D) Data distribution of patients with mild (C) and severe (S) COVID-19 compared with 90 healthy donors (HD). The dashed line represents the 30% inhibition cut-off value.

lines making results better reproducible and comparable among laboratories. Indeed, although our work focuses on relatively small numbers, it shows that our results are comparable with those obtained with other methods at an international reference center. Furthermore, the method developed at our laboratories allows us to propose a functional assay as the screening for neutralizing autoantibodies, which can be advantageous for clinical purposes.

The detection of autoantibodies anti-Type-I interferons can be of particular interest in the fight against novel coronaviruses since a genetic or acquired defect in the IFN cascade has been associated with a severe course of the infection, even in young people (Bastard et al., 2020). The knowledge of anti-IFN autoantibodies is not only a medical curiosity. Unfortunately, the search for neutralizing autoantibodies is only made in subjects undergoing severe viral infection and is unlikely to influence the course of the disease. Indeed, the role of type I IFNs in coronavirus disease is in the early phase of infection and late administration of pharmaceutical IFNs have displayed limited success, if any (Channappanavar et al., 2019). However, subjects recovering from critical viral infections and positive for anti-IFN autoantibodies should be thoroughly investigated for possible defects in immunity and autoimmune conditions. It occurs primarily in young people, while autoantibodies may be a para-physiological phenomenon in elderly population. Identifying anti-cytokine autoantibodies in subjects recovering from critical infection might reflect on early treatments in the event of subsequent infections, for example, with antivirals or viral-specific monoclonal antibodies. Anti-B-lymphocyte treatments might also be considered in particular cases, especially if other signs of autoimmunity are present. Even if there is no recommendation, clinical trials are ongoing to evaluate the possible benefits of this treatment in subjects with neutralizing autoantibodies to type II IFNs, IL-17 and GM-CSF (NCT01842386).

We validated our assay by analysis of reference sera gently provided by Steven Holland's NIH group and then tested it on two groups of sera collected during the first waves of COVID-19 from young people with a mild or severe infection. Even if the two groups were relatively small, we could detect one subject with high titer neutralizing autoantibodies to type I IFN, which is in line with experience on larger groups (Arrestier et al., 2022). The patient was a 59 years-old man with over-weight (BMI of 36), who required a 27-days stay in Intensive Care Unit with non-invasive ventilation and supportive care.

Of note, patients with severe COVID-19 displayed higher titers of neutralizing autoantibodies than subjects with mild disease, even if not clinically relevant based on the range defined in healthy controls. Since the sera were obtained for all the subjects one month after recovery, we could hypothesize that the observed titers of type I IFN-neutralizing autoantibodies just reflected an homeostatic response to the infection as previously described (Ross et al., 1990). Actually there is no evidence that anti-IFN antibodies develop after infections, although this is an intriguing hypothesis proposed by some authors. Some studies, for example, support the possible role of viral infections in the genesis of autoantibodies directed towards IFNs and other inflammatory molecules, and argue that levels of these antibodies may decline within a few months, although, in some likely predisposed cases, they may persist for several years (Feng et al., 2023; Wang et al., 2021; Shaw et al., 2022; Steels et al., 2022),

However, this finding might open the way for further studies to understand the clinical significance of low titer anti-IFN autoantibodies and the potential role of viral infections in their genesis.

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CRediT authorship contribution statement

Nicola Donadel: Writing – original draft, Methodology, Formal analysis, Data curation. **Alessandra Tesser:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Erica Valencic:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization. **Eleonora De Martino:** Software, Methodology, Formal analysis. **Valentina Boz:** Writing – review & editing, Validation. **Alessia Pin:** Methodology, Validation. **Francesca Zorat:** Writing – review & editing, Investigation. **Gabriele Pozzato:** Resources, Investigation, Data curation. **Alberto Tommasini:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2024.113696.

References

Arrestier, R., Bastard, P., Belmondo, T., Voiriot, G., Urbina, T., Luyt, C.E., et al., 2022. Auto-antibodies against type I IFNs in > 10% of critically ill COVID-19 patients: a prospective multicentre study. Ann. Intensive Care 12 (1), 121.

Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.H., Zhang, Y., et al., 2020. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 370 (6515).

Breivik, L., Oftedal, B.E., Bøe Wolff, A.S., Bratland, E., Orlova, E.M., Husebye, E.S., 2014. A novel cell-based assay for measuring neutralizing autoantibodies against type I interferons in patients with autoimmune polyendocrine syndrome type 1. Clin. Immunol. 153 (1), 220–227.

Browne, S.K., Holland, S.M., 2010. Immunodeficiency secondary to anticytokine autoantibodies. Curr. Opin. Allergy Clin. Immunol. 10 (6), 534–541.

Channappanavar, R., Fehr, A.R., Zheng, J., Wohlford-Lenane, C., Abrahante, J.E., Mack, M., et al., 2019. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. J. Clin. Invest. 129 (9), 3625–3639.

Ding, L., Mo, A., Jutivorakool, K., Pancholi, M., Holland, S.M., Browne, S.K., 2012. Determination of human anticytokine autoantibody profiles using a particle-based approach. J. Clin. Immunol. 32 (2), 238–245.

Feng, A., Yang, E.Y., Moore, A.R., Dhingra, S., Chang, S.E., Yin, X., et al., 2023. Autoantibodies are highly prevalent in non-SARS-CoV-2 respiratory infections and critical illness. JCI Insight 8 (3).

Kisand, K., Link, M., Wolff, A.S., Meager, A., Tserel, L., Org, T., et al., 2008. Interferon autoantibodies associated with AIRE deficiency decrease the expression of IFN-stimulated genes. Blood 112 (7), 2657–2666.

Pozzetto, B., Mogensen, K.E., Tovey, M.G., Gresser, I., 1984. Characteristics of autoantibodies to human interferon in a patient with varicella-zoster disease. J. Infect. Dis. 150 (5), 707–713.

Puel, A., Döffinger, R., Natividad, A., Chrabieh, M., Barcenas-Morales, G., Picard, C., et al., 2010. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J. Exp. Med. 207 (2), 291–297.

Ross, C., Hansen, M.B., Schyberg, T., Berg, K., 1990. Autoantibodies to crude human leucocyte interferon (IFN), native human IFN, recombinant human IFN-alpha 2b and human IFN-gamma in healthy blood donors. Clin. Exp. Immunol. 82 (1), 57–62.

Shaw, E.R., Rosen, L.B., Cheng, A., Dobbs, K., Delmonte, O.M., Ferré, E.M.N., et al., 2022. Temporal dynamics of anti-type 1 interferon autoantibodies in patients with coronavirus disease 2019. Clin. Infect. Dis. 75 (1), e1192–e1194.

Steels, S., Van Elslande, J., De Munter, P., Bossuyt, X., Group LC-S, 2022. Transient increase of pre-existing anti-IFN-α2 antibodies induced by SARS-CoV-2 infection. J. Clin. Immunol. 42 (4), 742–745.

Wang, E.Y., Mao, T., Klein, J., Dai, Y., Huck, J.D., Jaycox, J.R., et al., 2021. Diverse functional autoantibodies in patients with COVID-19. Nature 595 (7866), 283–288.