Interferon-α Auto-Antibodies in Convalescent Plasma Therapy for COVID-19

Matthijs Raadsen (m.p.raadsen@erasmusmc.nl)
Erasmus Medical Centre: Erasmus MC https://orcid.org/0000-0002-3774-8165

Arvind Gharbharan
Department of Medical Microbiology and Infectious diseases, Erasmus MC

Carlijn.C.E. Jordans
Department of Medical Microbiology and Infectious diseases, Erasmus MC

Anna Z. Mykytyn
Erasmus Medical Centre: Erasmus MC

Mart M. Lamers
Erasmus Medical Centre: Erasmus MC

Petra B. van den Doel
Erasmus Medical Centre: Erasmus MC

Henrik Endeman
Intensive Care department, Erasmus MC

Johannes P.C. van den Akker
Intensive Care department, Erasmus MC

Corine H. GeurtsvanKessel
Erasmus Medical Centre: Erasmus MC

Marion P.G. Koopmans
Erasmus Medical Centre: Erasmus MC

Casper Rokx
Department of Medical Microbiology and Infectious diseases, Erasmus MC

Marco Goeijenbier
Erasmus Medical Centre: Erasmus MC

Eric C.M. van Gorp
Erasmus Medical Centre: Erasmus MC

Bart J.A. Rijnders
Department of Medical Microbiology and Infectious diseases, Erasmus MC

Bart L. Haagmans
Erasmus Medical Centre: Erasmus MC

Research Article

Keywords: COVID-19, SARS-CoV-2, Interferon alpha, Auto-antibodies, Convalescent plasma.

DOI: https://doi.org/10.21203/rs.3.rs-670949/v1

Abstract

Purpose: To study the effect of Interferon-α auto-antibodies (IFN-α Abs) on clinical and virological outcomes in critically ill COVID-19 patients and the risk of IFN-α Abs transfer during convalescent plasma treatment.

Methods: Sera from cases of COVID-19 and other respiratory illness were tested for IFN-αAbs by ELISA and bioassay. IFN-α Abslevels were compared between critically, severely and moderately ill groups in both acute and convalescent stages. Longitudinal analyses were performed to determine whether IFN-α Abs levels change after convalescent plasma transfusion.

Results: Critically ill COVID-19 caseshad significantly higher IFN-α Abs detection rate and levels compared tonon-COVID-19 controls.Neutralizing IFN-α Abs levels were found in 1 out of 118plasma donors.Plasma from 2 positive donors was administered to 5 patients, with no subsequent elevation of IFN-α Abs levels in the recipients. Neutralizing levels of IFN-α Abswere associated with delayed viral clearance from the respiratory tract.

Conclusions: IFN-α Abs can be detected by ELISA in critical, severe, moderate and mild COVID-19 cases in both the acute and convalescent stages of disease. The presence of neutralizing IFN-α Abs in critically ill COVID-19 is associated with delayed viral clearance. Levels of IFN-α Abs inCOVID-19 convalescent plasma donorsare likely too low to be clinically relevant to the recipients.

Summary

This study compares the presence of interferon-α auto-antibodies in COVID-19 patients in the acute and convalescent phase, across multiple levels of disease severity, and ICU patients with other infectious respiratory diseases. Additionally, the effect of these antibodies on the safety of convalescent plasma treatment is investigated.

Introduction

The production of type I interferons (IFN) is crucial in the engagement of cellular antiviral defenses and occurs when cells sense viral replication through Toll-like (TLR) or RIG-I-like (RLR) receptors (1). IFNs efficiently inhibit infection and replication of SARS coronavirus-2 (SARS-CoV-2) in vitro, but viral infection induces less type I IFNs compared to other respiratory viruses (2, 3). Impaired type I IFN activity in COVID-19 patients has been associated with the persistence of SARS-CoV-2 viral RNA in blood and elevated markers of inflammation (4). Underlying causes of an impaired IFN-response could be genetic, such as rare putative loss-of-function variants of X-chromosomal TLR7 (5). In addition, advanced age also impairs type I IFN production in response to viral infection (6).

Another cause of diminished antiviral activity of IFNs is the presence of circulating auto-antibodies (Abs) to IFNs. Recent evidence indicates that Abs against type I IFNs are present in up to 10% of patients with

severe COVID-19, without previous recombinant IFN treatment, suggesting that IFN-α Abs impair the hosts' ability to control viral replication (7). These Abs were not found in mild cases and were rare in healthy individuals (~ 0.3%). Based on these findings, the authors noted the potential risk posed by convalescent plasma donated by individuals recovering from severe COVID-19 and suggest either excluding these donors or testing them first, but this has not been studied so far (7). Recent findings of interventional and observational studies indicate convalescent plasma treatment in hospitalized patients is safe but ineffective, likely because most COVID-19 patients have already developed virus neutralizing Abs by the time they are admitted to the hospital, rendering those in the donor plasma redundant (8). New trials are underway, administering virus neutralizing convalescent plasma earlier in the course of disease. This has the potential to aid viral clearance, but may also carry the risk of transferring harmful IFN neutralizing Abs to these patients at a time when they are more reliant on their early innate antiviral response.

In this study, we compared detection rate and neutralizing potential of IFN-α Abs in sera from critically ill COVID-19 patients and ICU patients with other infectious respiratory diseases. Using the COVID-19 and non COVID-19 ICU patients as a reference, we determined whether neutralizing IFN-α Abs are present in sera of COVID-19 convalescent plasma donors and whether these are transferred to patients following convalescent plasma therapy. Subsequently, we determined whether the presence of neutralizing IFN-α Abs affected clinical and virological outcomes in COVID-19 patients.

Methods

Human samples

Patients admitted to the ICU at the Erasmus MC, Rotterdam, the Netherlands with severe respiratory disease with suspected infectious etiology, including SARS-CoV-2, were included in a biorepository study. Written informed consent was signed by study subjects or their representatives if they were incapacitated due to severe illness. Serum was obtained on the first day of inclusion into the study and stored at -80 ºC until further analysis. The study protocol was approved by the institutional review board of Erasmus MC, Rotterdam, the Netherlands (MEC-2017-417 and MEC-2020-0222). Serum samples taken during the CONCOVID clinical trial (NCT04342182) were kindly made available by the trial investigators. Sera were heat-inactivated for 30 min at 56°C. Broncho Alveolar Lavage (BAL) samples were taken for routine clinical diagnostic purposes and leftover material was stored at below − 20°C until further analysis.

Clinical data

Clinical data, including age, sex, days of disease duration, vital parameters, medical history, and results of pathogen identification tests were extracted from patient electronic medical files. Public registries were consulted for additional survival data. Three-day average Sequential Organ Failure Assessment (SOFA) scores and Ratios of partial pressure of oxygen in arterial blood to fraction of inspired oxygen (P/F) were

calculated based on the vital parameters available at the the first 3 days of ICU admission at the timepoint closest to 6 a.m. Age and medical history were used to calculate a Charlson Comorbidity Index (CCI) for each ICU patient. All COVID-19 patients were diagnosed by a positive respiratory sample using a real-time quantitative PCR test (RT-qPCR) for SARS-CoV-2. For ICU patients with COVID-19, frequent measurements of RT-qPCR data (Ct values) were available. Clearance of viral RNA from the respiratory tract was defined as a single Ct value above 35, without a decrease in Ct value in subsequent samples. Time to clearance was calculated as the number of days between inclusion into the study and the day of viral clearance.

IFN-α Ab detection

For detection of binding Abs against Human IFN-α, a commercially available ELISA was used (BMS217 from Invitrogen) according to the manufacturers' instructions. The cutoff for positivity was 7 ng/mL, the lower limit of detection specified by the manufacturer.

Serum pools and antibody purification

Sera ($n = 7$) in which the highest concentration of IFN-α Abs were detected, were pooled and a separate pool of negative sera was made as a control. To remove any serum factors that could interfere with the IFN blocking assay, pools were subjected to protein G column affinity chromatography to obtain purified Ig preparations, using a Proteus protein G antibody purification kit (Bio-Rad Laboratories, UK).

rVSV ΔG GFP rescue and production

Replication restricted recombinant Vesicular Stomatitis Virus encoding Green Fluorescent Protein (VSV ΔG GFP) was rescued as described previously (9). Propagation to high titers was achieved by infecting HEK-293T cells transfected with VSV-G 24 hr prior with a multiplicity of infection of 0.1 of rescued virus. Supernatant was collected after 48 and 72 hours, cleared by centrifugation at 2000 x g for 5 minutes and stored at -80°C. Titers were determined by preparing 10-fold serial dilutions in Opti-MEM I (1X) + GlutaMAX (Gibco). Aliquots of each dilution were added to monolayers of 2×10^4 Vero cells in the same medium in a 96-well plate. Plates were incubated at 37°C overnight and then scanned using an Amersham™ Typhoon scanner. Infected cells were quantified using ImageQuant TL software (GE Healthcare Life Sciences). Vero cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% F, 100 µg/ml streptomycin, 100 U/ml penicillin, 20mM Hepes (Lonza) and 1mM sodium pyruvate (Gibco). HEK-293T cells were maintained in DMEM supplemented with 10% FBS, 100 µg/ml streptomycin, 100 U/ml penicillin, 1X non-essential amino acids (Lonza) and 1mM sodium pyruvate (Gibco). All cell lines were maintained at 37°C in a humidified CO_2 incubator.

IFN-Abs neutralization assay

A549 cells where maintained in F12 medium, supplemented with 10% FBS, 100 µg/ml streptomycin and 100 U/ml penicillin. Cells were plated in 96 wells cell culture plates and incubated at 37°C for 24 hours before infection. Purified Ab or serum was pre-incubated for 1 hour at 37°C in twofold serial dilutions ranging from 1:50 to 1:1600 with PEG-IFN-α2a (Roche Pharmaceuticals) at a concentration of 3 ng/mL,

the minimum concentration causing ~ 90% GFP + plaque reduction in optimization experiments.. Confluent monolayers of A549 cells where infected with 500 Plaque Forming Units (PFU) per well of replication-restricted recombinant Vesicular Stomatitis Virus encoding Green Fluorescent Protein (rVSV ΔG GFP) in culture medium containing 2% FBS, after at least 4 hours of incubation with purified Ig or serum, both with and without PEG-IFN-α2a. Infections were performed under Biosafety Level II (BSLII) conditions. GFP positive plaques were quantified 8–24 hours after infection using an Amersham™ Typhoon scanner with ImageQuant TL colony counting software (GE Healthcare Life Sciences). IFN neutralizing activity was determined by calculating the ratio of plaque neutralization in IFN + serum/Ig incubated wells and IFN incubated wells, corrected for low-level VSV neutralization in some sera. All functional IFN blocking experiments were performed in triplicate. A control condition was included using IFN-λ1 (10 ng/mL) on A549 cells.

SARS-CoV-2 serology

Total SARS-CoV-2 Receptor Binding Domain specific Ig was detected using the Wantai SARS-CoV-2 ELISA (Beijing Wantai Biological Pharmacy Enterprise, China) as previously described (10). An in-house plaque-reduction neutralization test (PRNT50) was used to quantify virus-neutralizing Ab as previously described (11).

Statistical analysis

Data analysis was performed using SPSS statistics version 25 (IBM), Prism version 9 (Graphpad), and Excel 2016 (Microsoft). Comparisons of continuous variables between patient groups was performed using Student's t-tests for normally distributed data (e.g. age, days of disease duration and clinical scores) and Mann-Whitney U tests for data following a skewed distribution (e.g. IFN-α Abs and SARS-CoV-2 ELISA results, PRNT 50 titers). IFN-α Ab concentrations determined by ELISA were 10 Log transformed. Geometric Mean Titers (GMT) were calculated from PRNT 50 titers. Comparisons of categorical data was performed using the Chi-squared test. Dose-response curves were plotted using 4-parameter nonlinear regression, least-squares method, with top and bottom constrained to ≥ 0 and ≤ 100 respectively. Survival curves were compared using the logrank (Mantel-Cox) test. Correlation analysis was performed using Pearson's correlation for parametric- and Spearman for nonparametric variables.

Results

Assay validation and determination of neutralization cutoff

Validation of the ELISA assay was performed using COVID-19 ICU patients as cases and ICU patients with other respiratory diseases as controls. Baseline characteristics of COVID-19 and non-COVID-19 ICU patients are listed in Table 1. Most SARS-CoV-2 infections were community-acquired, whereas the non-COVID-19 group represented a more diverse spectrum of severe infectious disease resulting in respiratory failure. Non-COVID-19 ICU patients were more likely to suffer from multi-organ failure and had more

comorbidities. Non-COVID-19 infections included viral, bacterial and fungal pathogens (Supplementary table 1).

Table 1
Baseline characteristics and IFN-α Ab status of COVID-19 and non-COVID-19 affected ICU patients.

	COVID-19 N = 102	Non-COVID-19 N = 47	p value
Age (Mean years ± SD)	62 ± 12	61 ± 12	0.612
Female (N)	16 (16%)	14 (30%)	0.075
Charlson comorbidity index (Mean ± SD)	2.7 ± 1.7	4.2 SD ± 2.1	< 0.0001
Primary diagnosis (N)			
Aspiration pneumonia		9	
COPD Exacerbation		2	
HAP	4	3	
Neutropenic Sepsis		1	
CAP	98	18	
Pneumonia following chest injury		1	
Sepsis		11	
Meningitis		1	
Soft tissue infection		1	
Reason for ICU admission (N)			
Coma		2	
OHCA		1	
Respiratory Failure	102	37	
Postoperative		1	
Shock		6	
SOFA score (Mean ± SD)	6.4 ± 3.0	8.3 ± 3.5	0.001
P/F ratio (Mean ± SD)	209 ± 84	236 ± 112	0.103
Anti IFN-α detectable (N)	32 (31%)	6 (13%)	0.015
Anti IFN-α > 685 ng/mL (N)	7 (7%)	0	0.098

SD = Standard Deviation, COPD = Chronic Obstructive Pulmonary Disease, HAP = Hospital-acquired pneumonia, CAP = Community-acquired pneumonia, OHCA = Out-of Hospital Cardiac Arrest, SOFA = Sequential Organ Failure Assessment, P/F = Ratio of partial pressure of oxygen in arterial blood to fraction of inspired oxygen.

ELISA measured concentrations of IFN-α Abs in positive sera from COVID-19 patients (32/102, 31%) ranged from 7,1 ng/mL to 10,9 mg/mL. Positive sera from non-COVID ICU patients (6/47, 13%) had a concentration range of 7,7–27 ng/mL. Sera were subsequently tested in a IFN-α neutralization assay, using a non-replicating Vescicular Stomatitis Virus (pseudoparticle) based system on a human lung epithelial cell line. A selection of individual serum samples (n = 24) covering the full range of concentrations was tested in dilutions ranging from 1:5 to 1:1280 to quantify IFN-α neutralizing activity. A 4-parameter nonlinear regression model was fitted (Fig. 1B, R^2 = 0.90) and an ELISA measured IFN-α Abs concentration of 685 ng/mL was defined as a cutoff which correlates to significant IFN-α neutralization (Mean IFN-α activity 82.5 %, 95% CI 74.9–90.2%), compared to samples at the ELISA LLOD (Lower Limit of Detection) of 7 ng/mL (Mean 90.2%). Purified antibody from pooled positive (N = 7) sera caused strong functional inhibition of IFN-α2a but not of IFN-λ1 (supplementary Fig. 1).

Comparative IFN-α Abs across disease severity and stage

To determine whether IFN-α Abs were affected by disease severity or disease stage and whether they occur specifically in COVID-19 cases, 4 groups were tested for serum IFN-α Abs. Overall, 10 out of 272 COVID-19 patients tested in this study had neutralizing levels IFN-α Abs, with proportions increasing with disease severity (Fig. 1A).

As shown in Fig. 1A, critically ill COVID-19 cases were significantly more likely to have detectable IFN-α Abs compared to non-COVID-19 cases and positive sera had significantly higher IFN-α Abs concentrations. Neutralizing IFN-α Abs were present in 7/102 COVID-19 ICU patients, but not in non-COVID-19 patients. There was no difference in number of days after symptom onset between COVID-19 ICU patients with detectable IFN-α Abs (15 days, 95% CI 13–17 days) and those who were negative (12 days, 95% CI 9–15 days). There were 21 bronchoalveolar lavage (BAL) samples available from COVID-19 ICU patients, 5 of which had IFN-α Abs concentrations above the detection limit, which showed moderate correlation to their corresponding serum levels (Spearman R = 0.45, P = 0.039).

From the CONCOVID clinical trial, sera were available from 61 hospitalized severe COVID-19 cases, 28 of whom had received convalescent plasma treatment, while the rest received standard of care. Baseline samples were taken on the day of inclusion, which was the same day patients in the treatment arm received convalescent plasma. Follow-up samples were taken approximately 1 week later (median 7 days, range 3–56 days). Of these patients, 11 (18%) were admitted to ICU at baseline, whereas 50 (82%) were receiving care in the general ward, 21 (42%) of whom subsequently required intensive care. The IFN-α Abs levels at baseline are shown in Fig. 1A. Out of the total, 14 (23%) had detectable IFN-α Abs in ELISA, of whom 2 (3,2%) had serum concentrations with neutralizing activity, both of whom deteriorated clinically, resulting in one death. The detection rate before plasma transfusion in this group did not differ significantly from ICU-admitted COVID-19 patients, convalescent plasma donors, or ICU-admitted non-COVID-19 patients.

Sera from blood plasma donors were taken at a mean of 51 (SD ± 14 days) days after COVID-19 disease onset. The donors had mostly had mild disease, with 15 (13%) having been hospitalized. Of 118 plasma donors screened, 26 (22%) had detectable IFN-α Abs, which did not differ significantly from the detection rate in in ICU patients. A single convalescent donor had a serum concentration with significant IFN neutralizing activity (0.8%), which was a significantly lower proportion compared to the ICU-admitted COVID-19 group (p = 0.026). No differences were found between IFN-α Abs positive and negative donors regarding level of care or days post onset of disease.

Seroconversion of COVID-19 patients

To determine whether COVID-19 patients treated with convalescent plasma had an increased risk of seroconversion compared to untreated individuals, we first determined the seroconversion rate of COVID-19 ICU patients that were not included in the CONCOVID trial. A total of 33 patients with undetectable IFN-α Abs at the earliest time point had weekly follow up sera available until discharge or death. Of these patients, 3 seroconverted, with one patients developing IFN-α Abs, with increasing levels for 3 consecutive weeks until the end of follow-up (supplementary Fig. 2).

Next, we examined plasma donors and recipients in de CONCOVID trial. Retrospectively, we found that plasma from 2 donors with low serum levels of IFN-α Abs (8.05 and 7.18 ng/mL), was administered to 5 COVID-19 patients as part of the CONCOVID clinical trial. Out of 5 cases who received plasma from IFN-α Abs positive donors, 3 did not have detectable IFN-α Abs after plasma transfusion, 1 was already positive at baseline with higher IFN-α Abs than the donor and 1 did not have a follow-up sample available. Overall, serum samples taken after convalescent plasma treatment were equally likely to contain IFN-α Abs compared to follow up samples taken from the control group (14% vs 15 %, p = 0.441). All patients with detectable IFN-α Abs at baseline remained positive in their follow-up samples and there were no seroconversions in either the treatment or standard of care arm.

Risk factors for IFN-α Abs and effect on clinical and virological outcomes.

Analysis of all three COVID-19 cohorts combined (*n* = 281) showed no significant differences in age and levels of SARS-CoV-2 binding or neutralizing antibodies between IFN-α Abs positive and negative patients (Table 2). Women were significantly more likely to have detectable IFN-α Abs compared to men. The proportions of patients with neutralizing IFN-α Abs levels did not differ significantly between the sexes. No difference in 60-day survival was found when comparing critical and severe COVID-19 patients with IFN-α Abs serum concentrations below (*n* = 153) versus above (*n* = 9) neutralizing levels in univariate analysis (HR 1.74, 95% CI 0.41–6.56). In ICU COVID-19 patients, no correlations between IFN-α Abs concentration and Severe Organ Failure assessment (SOFA) score or P/F ratio were found (data not shown). COVID-19 patients with neutralizing levels of IFN-α Abs had a longer time to viral clearance from the respiratory tract compared to ICU patients with undetectable IFN-α Abs (Median 21 days vs 8 days, HR 3.4, 95% CI 1.8–6.6, Fig. 1C).

Table 2
Comparisons between IFN-α Abs positive and negative COVID-19 patients.

	IFN-α Abs positive (N = 72)	IFN-α Abs negative (N = 209)	P
Age (Mean years ± SD)	54 ± 15	54 ± 17	0.911
Female (N)	20 (28%)	32 (15%)	0.048
Highest level of care received (N)			
ICU	41 (57%)	94 (45%)	0.251
Ward	8 (11%)	35 (37%)	
Outpatient	23 (32%)	77 (17%)	
Unknown	0	3 (1%)	
Disease duration (Mean days ± SD)	28 ± 22	31 ± 22	0.380
SARS-CoV-2 IgG (Median ratio ± IQR)	14.74 ± 12.79	18.39 ± 9.60	0.206
SARS-CoV-2 PRNT 50 (GMT ± SD)	110 ± 6	131 ± 4	0.774

ICU = Intensive Care Unit, ELISA = Enzyme Linked Immunosorbent Assay, PRNT 50 = 50% Plaque Reduction Neutralization Titer, GMT = Geometric Mean Titer.

Discussion

In this study, we tested the presence of IFN-α Abs in COVID-19 patients and determined their relevance for convalescent plasma treatment and relation to disease outcomes. Using a commercially available ELISA, we found a relatively high detection rate of IFN-α Abs in COVID-19 cases compared to another recent study, including convalescent patients after moderate to mild disease. (7) However, neutralization of IFN-α in a functional assay only occurred in sera with ELISA measured IFN-α Abs well above the LLOD of the commercial assay. Using this neutralization cutoff we found a detection rate that is consistent with that in the aforementioned publication. When measured in the acute phase of COVID-19, this more stringent cutoff identifies patients in whom IFN-α Abs may have contributed significantly to the development of severe disease. Severe COVID-19 is associated with a state of immune hyper activation and the production of a broad spectrum of autoreactive antibodies, which in turn have not been linked to clinically apparent autoimmune disease (12–14). This must be taken into account when interpreting the results of antibody binding assays designed to be performed under more physiologically stable conditions. This includes the ELISA results presented in this study, where a large number of samples were quantified above the ELISA LLOD, without reaching bioactive levels in critical and severe COVID-19 patients. With the aim of controlling for this background, we chose to include a control group of critically ill ICU patients with other infections. Although the rate of IFN-α Abs detection was higher in these patients than is expected from a healthy population, they represent a more diverse group of different types of infections,

with a more heterogeneous clinical phenotype. Nevertheless, we argue that this is a more relevant control group than healthy controls with no active inflammatory disease. Only critical COVID-19 cases had serum IFN-α Abs levels that differed significantly from this control group.

An earlier study in a Hepatitis C patient receiving exogenous recombinant IFN-α treatment, demonstrated that low levels of IFN-α Abs before treatment were boosted 1 week after administration of the drug started, to levels which completely blocked the activity of recombinant IFN-α (15). This capacity of IFN-α Abs to be quickly boosted during increased IFN-α exposure indicates that low levels of IFN-α Abs in convalescent or pre-symptomatic individuals could become relevant during an acute infection. Only a single convalescent sample in this study had neutralizing levels of IFN-α Abs. IFN-α Abs positive donors of whom plasma was administered to COVID-19 patients were close to the ELISA detection limit. Given the fact that a single transfusion of 300 mL plasma was administered, this is not expected to result in neutralizing IFN-α Abs levels in the recipient.

Bastard et al. showed a strong overrepresentation of men among COVID-19 patients with type I IFN Abs, and an X-linked genetic association was proposed (7). In contrast, our data show a higher IFN-α Abs detection rate among women. Notably, the convalescent donor cohort consists mainly of males who had had mild disease. Indeed, during the first months of the pandemic only male donors were recruited for convalescent plasma donation for reasons of urgency, since additional HLA and HNA antibody tests are required in female donors before their plasma can be used (16, 17). Therefore, most female subjects analyzed originate from the higher severity groups, which are more likely to have IFN-α Abs. When convalescent donors were excluded from the analysis, the difference in IFN-α Abs detection rate between genders was no longer statistically significant.

Recently an analysis of a subset of the data presented in the aforementioned paper was published, which correlates neutralizing Abs against type I IFN with a higher mortality rate from COVID-19 induced multi-organ failure (18). We found no association between neutralizing IFN-α Abs and mortality, or between SOFA score and IFN-α Abs serostatus, but studies with larger study sizes would be needed before robust conclusions could be drawn. Interestingly, we were able to demonstrate a delay in viral clearance from the respiratory tract in COVID-19 ICU patients with neutralizing levels of IFN-α Abs.

Our finding that most patients who had detectable IFN-α Abs already tested positive at the earliest available time point, and seroconversion during the course of disease was rare, suggests that these individuals already harbored anti-IFN B cell clones before they were infected. The SARS-CoV-2 infection may have boosted the anti-IFN Abs rather than being causally linked to the induction of autoimmunity. Whether this is unique to this virus, or a more widespread phenomenon among primary infections in later adulthood, remains a subject for further study.

In conclusion, we confirm previous finding that IFN-α Abs can be detected in COVID-19 patients, with neutralizing levels being most common in critically ill COVID-19 patients in the acute stage of disease. In these patients, neutralizing IFN-α Abs were associated with delayed viral clearance from the respiratory tract. In contrast, we did not find neutralizing levels of IFN-α Abs in critically ill ICU patients with

respiratory illness caused by other infectious diseases. COVID-19 convalescent individuals also had detectable IFN-α Abs, but generally below neutralizing levels. We did not detect any cases were convalescent plasma transfusion was associated with an increase in IFN-α Abs in the recipient.

Declarations

Acknowledgements

The authors would like to thank D. Van Duijn, P.M. Ormskerk and A.M. Glasbergen- van Beijeren for their dedicated role in the management of the CIUM observational study.

Author contributions

Conceptualization: Bart L. Haagmans, Matthijs P. Raadsen; Methodology: Anna Z. Mykytyn, Mart M. Lamers, Petra B. van den Doel; Formal analysis and investigation: Matthijs P. Raadsen; Data curation;Arvind Gharbharan, Carlijn C.E. Jordans; Resources:Casper Rokx, Bart J.A. Rijnders, Corine H. GeurtsvanKessel, Henrik Endeman, Johannes P.C. van den Akker Supervision: Bart L. Haagmans, Eric C.M. van Gorp, Marco Goeijenbier, Marion P.G. Koopmans. The first draft of the manuscript was written by Matthijs P. Raadsen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

No funding was received to assist with the preparation of this manuscript.

Conflict of Interest:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material:

All data presented in the current manuscript is available on request to the corresponding author.

Ethics Approval:

All subjects were recruited according to protocols approved by the competent local institutional review boards.

Consent to Participate:

All protocols conformed to local ethics recommendations and informed consent was obtained when required.

Consent for Publication:

Included subjects or their representatives have consented to publication of their data, excluding identifiable details.

References

1. Bourdon M, Manet C, Montagutelli X. Host genetic susceptibility to viral infections: the role of type I interferon induction. Genes and immunity. 2020:1-15.
2. Lokugamage KG, Hage A, de Vries M, Valero-Jimenez AM, Schindewolf C, Dittmann M, et al. Type I Interferon Susceptibility Distinguishes SARS-CoV-2 from SARS-CoV. Journal of virology. 2020;94(23).
3. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 2020;181(5):1036-45.e9.
4. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science (New York, NY). 2020;369(6504):718-24.
5. van der Made CI, Simons A, Schuurs-Hoeijmakers J, van den Heuvel G, Mantere T, Kersten S, et al. Presence of Genetic Variants Among Young Men With Severe COVID-19. Jama. 2020;324(7):1-11.
6. Molony RD, Nguyen JT, Kong Y, Montgomery RR, Shaw AC, Iwasaki A. Aging impairs both primary and secondary RIG-I signaling for interferon induction in human monocytes. Science signaling. 2017;10(509).
7. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science (New York, NY). 2020;370(6515).
8. Gharbharan A, Jordans CCE, GeurtsvanKessel C, den Hollander JG, Karim F, Mollema FPN, et al. Convalescent Plasma for COVID-19. A randomized clinical trial. medRxiv. 2020:2020.07.01.20139857.
9. Mykytyn AZ, Breugem TI, Riesebosch S, Schipper D, van den Doel PB, Rottier RJ, et al. SARS-CoV-2 entry into human airway organoids is serine protease-mediated and facilitated by the multibasic cleavage site. Elife. 2021;10.
10. GeurtsvanKessel CH, Okba NMA, Igloi Z, Bogers S, Embregts CWE, Laksono BM, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. Nature communications. 2020;11(1):3436.

11. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. Emerging infectious diseases. 2020;26(7):1478-88.
12. Zhou Y, Han T, Chen J, Hou C, Hua L, He S, et al. Clinical and Autoimmune Characteristics of Severe and Critical Cases of COVID-19. Clin Transl Sci. 2020;13(6):1077-86.
13. Wang EY, Mao T, Klein J, Dai Y, Huck JD, Liu F, et al. Diverse Functional Autoantibodies in Patients with COVID-19. medRxiv. 2020:2020.12.10.20247205.
14. Wang EY, Mao T, Klein J, Dai Y, Huck JD, Jaycox JR, et al. Diverse functional autoantibodies in patients with COVID-19. Nature. 2021.
15. van der Eijk AA, Vrolijk JM, Haagmans BL. Antibodies neutralizing peginterferon alfa during retreatment of hepatitis C. The New England journal of medicine. 2006;354(12):1323-4.
16. Peters AL, Van Stein D, Vlaar AP. Antibody-mediated transfusion-related acute lung injury; from discovery to prevention. Br J Haematol. 2015;170(5):597-614.
17. Middelburg RA, Van Stein D, Zupanska B, Uhrynowska M, Gajic O, Muñiz-Diaz E, et al. Female donors and transfusion-related acute lung injury: A case-referent study from the International TRALI Unisex Research Group. Transfusion. 2010;50(11):2447-54.
18. Koning R, Bastard P, Casanova JL, Brouwer MC, van de Beek D, with the Amsterdam UMCC-BI. Autoantibodies against type I interferons are associated with multi-organ failure in COVID-19 patients. Intensive Care Med. 2021:1-3.

Figures

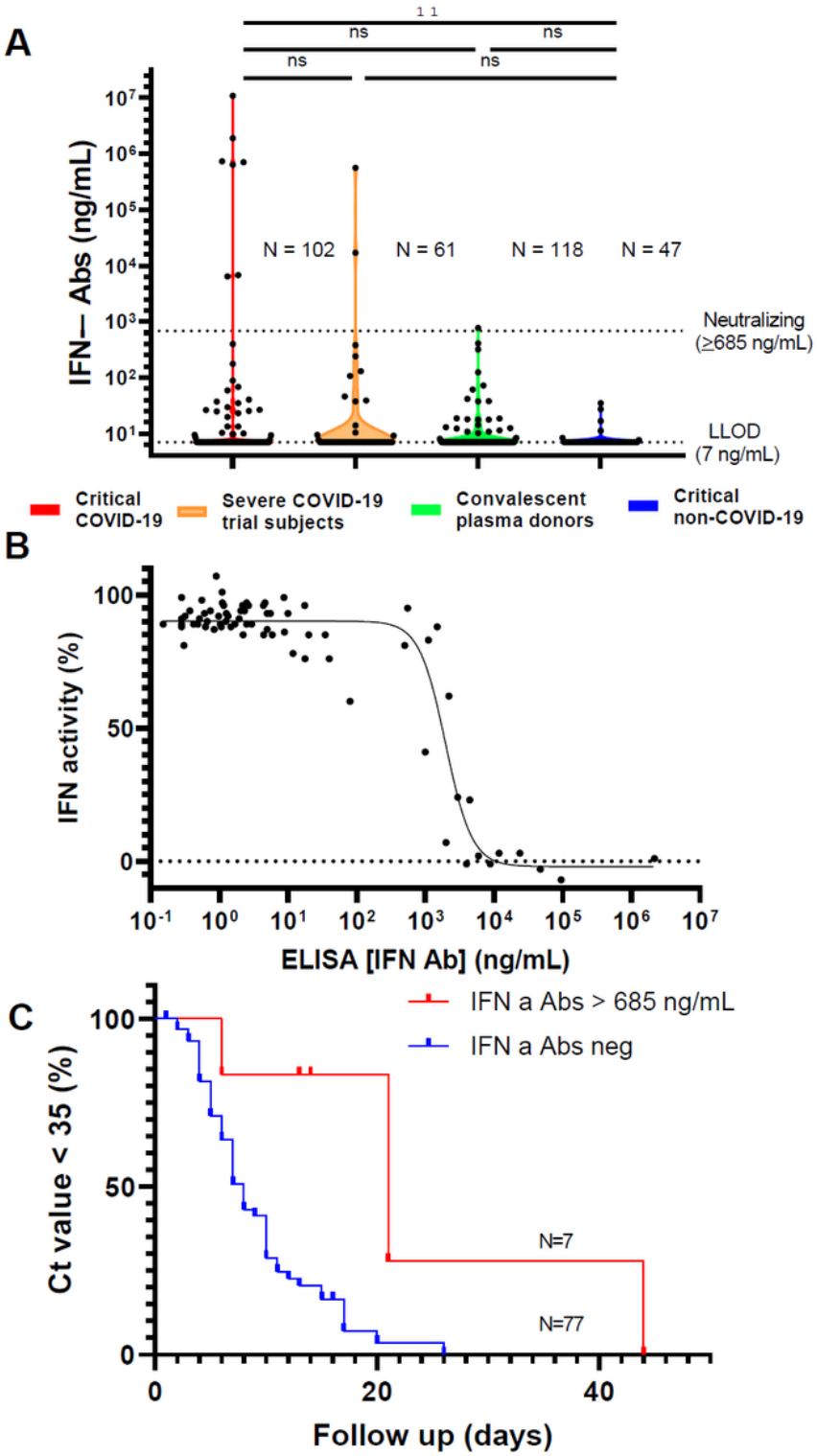

Figure 1

Quantitative levels and functional properties of IFN-α Abs in COVID-19 patients. Plot of IFN-α Abs concentrations in critically ill COVID-19 patients, convalescent plasma donors, Hospitalized patients with severe COVID-19 and ICU with respiratory illness caused by other pathogens. Dots are individual values (A). Sigmoidal IFN-α neutralization curve on a selection of individual serum samples (n = 24) covering the range of concentrations found by ELISA, in dilutions ranging from 1:5 to 1:1280 (B) Survival curve

plotting time to viral clearance in COVID-19 ICU patients with neutralizing levels if IFN-α Abs (red line, N=7) versus undetectable IFN-α Abs (blue line, N=77). (C) - ns = not significant, ** = P <0.01

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplement.pdf