Auto-antibodies against type I IFNs in patients with life-threatening COVID-19

Paul Bastard, Lindsey Rosen, Qian Zhang, Eleftherios Michailidis, Hans-Heinrich Hoffmann, Yu Zhang, Karim Dorgham, Quentin Philippot, Jérémie Rosain, Vivien Béziat, et al.

To cite this version:

HAL Id: pasteur-02958871
https://hal-pasteur.archives-ouvertes.fr/pasteur-02958871
Submitted on 6 Oct 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution| 4.0 International License
Auto-antibodies against type I IFNs in patients with life-threatening COVID-19

Paul Bastard1,2,3,5,‡, Lindsey B. Rosen4,‡, Qian Zhang6,‡, Elephantios Michailidis7,‡, Hans-Heinrich Hoffmann8,‡, Yu Zhang3,‡, Karim Dorgham9,‡, Quentin Philippot1,‡, Jérémie Rosain1,2,‡, Vivien Béziat1,2,3,‡, Jérémy Manry1,2,‡, Elana Shaw4, Liis Haljasmägi10, Pärt Peterson10, Lazaros Lorenzoz1,3, Lucy Bizien1,2, Sophie Trouillet-Assant9,‡, Kerry Dobbs4, Adriana Almeida de Jesus4, Alexandre Belot10,11,2, Anne Kallaste12, Emilie Catherinot12, Yacine Tanda Jouaoui-Lambiotte12, Jerome Le Pen12, Gaspard Kerner12, Benedetta Bigio14, Yoann Seeleuthner1,2, Rui Yang15, Alexandre Bolze16, András N. S. Spaan3,7,17, Ottavia M. Delmonte18, Michael S. Abers3, Giorgio Casari19, Vito Lampasona20, Lorenzo Piemonti20, Fabio Ciceri19, Kaya Bilguvar19, Richard P. Lifton19,20,21, Marc Vasse22, David M. Smajda14,23, Mélanie Migaud1,2, Jérôme Hadjadj24, Benjamin Terrier25, Tarragh Duffu26, Lluis Quintana-Murci27,28, Diederik van de Beek29, Lucie Roussel30,31, Donald C. Vinh30,31, Stuart G. Tangye32,33, Filomena Haerynck34, David Dalmau34, Javier Martinez-Picado35,36,37,38, Petter Brodin39,40, Michel C. Nussenzweig41,42, Stéphanie Boisson-Dupuis1,43, Carlos Rodriguez-Gallegos44,45, Guillaume Vogt45, Trine H. Mogensen46,47, Andrew J. Oler48, Jingwen Gu49, Peter D. Burbelo49, Jeffrey Cohen50, Andrea Biondi51, Laura Rachele Bettini51, Mariella D'Angelo52, Paolo Bonfanti52, Patrick Rossignol53, Julien Mayaux54, Frédéric Rieux-Laucat54, Eystein S. Husebye55,56,57, Francesca Fusco58, Matilde Valeria Ursini59, Luisa Imberti59, Alessandra Sottini60, Simone Paghera61,62, Eugenia Quiros-Roldan60,63, Camillo Rossi64, Riccardo Castagnoli62,64, Daniela Montagna65,66,67,68,69, Amelie Licari62,69, Xavier Duval63,64,65,66,67,68,69, Jade Ghosn68,69, HGID Lab5, NIAID-USUHS Immune Response to COVID Groups, COVID Clinicians, COVID-STORM Clinicians, Imagine COVID Groups, French COVID Cohort Study Groups, The Milieu Intérieur Consortium, CoV-Contact Cohort, Amsterdam UMC Covid-19 Biobank, COVID Human Genetic Effort, John S. Tsang70,71, Raphaella Goldbach-Mansky4, Kai Kissand7, Michail S. Lionakis4, Anne Puel4,5,6, Shen-Ying Zhang2,3, Steven M. Holland8,9, Guy Gorochov10,72,73, Emmanuelle Jouanguy1,2,3,7, Charles M. Rice10, Aurélie Cobat1,2,3, Luigi D. Notarangelo4, Laurent Abel1,2,3,6, Helen C. Su4,#, Jean-Laurent Casanova1,2,3,4,5,7,8,9,‡

1Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, Paris, France. 2University of Paris, Imagine Institute, Paris, France. 3St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA. 4Laboratory of Clinical Immunology and Microbiology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA. 5Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, NY, USA. 6Sorbonne University, INSERM, Centre d’Immunologie et des Maladies Infectieuses, (CIMI-Paris), Paris, France. 7Institute of Biomedical and Translational Medicine, University of Tartu, Tartu, Estonia. 8Hospices Civils de Lyon, Lyon Sud Hospital, Pierre-Bénite, France. 9International Center of Research in Infectiology, Lyon University, INSERM U1111, CNRS UMR 5308, ENS, UCBL, Lyon, France. 10International Center of Research in Infectiology, Lyon University, INSERM U1111, CNRS UMR 5308, ENS, UCBL, Lyon, France. 11National Reference Center for Rheumatic and Autoimmune and Systemic Diseases in Children (RAISE), Lyon Sud Hospital, Pierre-Bénite, France. 12Lyon Immunopathology Federation (LIFE), Hospices Civils de Lyon, Lyon, France. 13Internal Medicine Clinic, Tartu University Hospital, Tartu, Estonia. 14Pneumology Department, Foch Hospital, Suresnes, France. 15Infectious Diseases and HIV Service, Hospital Universitari Mutua Terrassa, Barcelona, Spain. 16Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, Netherlands. 17IRCCS San Raffaele Hospital and Vita-Salute San Raffaele University, Milan, Italy. 18Department of Genetics, Yale University School of Medicine, New Haven, CT, USA. 19Yale Center for Genome Analysis, Yale University School of Medicine, New Haven, CT, USA. 20Department of Infection and Immunology of Pediatric Autoimmune Diseases, INSERM U1163, University of Paris, Imagine Institute, Paris, France. 21Department of Internal Medicine, National Referral Center for Rare Systemic Autoimmune Diseases, Assistance Publique Hôpitaux de Paris-Centre (APHP-CUP), University of Paris, Paris, France. 22Lyon Genomics, The Rockefeller University, New York, NY, USA. 23Department of Microbiology and Immunology, McGill University Health Centre, Montréal, Québec, Canada. 24Laboratory of Immunoepidemiology Program, Research Institute, McGill University Health Centre, Montréal, Québec, Canada. 25Department of Biomedical Research Germans Trias i Pujol (IGTP), Badalona, Spain. 26University of Vic-Central University of Catalonia (UVic-UCC), Vic, Spain. 27Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain. 28Science for Life Laboratory, Department of Women’s and Children’s Health, Karolinska Institutet, Karolinska, Sweden. 29Department of Pediatric Rheumatology, Karolinska University Hospital, Karolinska, Sweden. 30Laboratory of Molecular Immunology, The Rockefeller University, New York, NY, USA. 31Howard Hughes Medical Institute, New York, NY, USA. 32Department of Immunology, Hospital Universitari de Gran Canaria Dr. Negrín, Canarian Health System, Las Palmas de Gran Canaria, Spain. 33University Ferdinando Pessoa Canarias, Las Palmas de Gran Canaria, Spain. 34Neglected Human Genetics Laboratory, INSERM, University of Paris, Paris, France. 35Department of Infectious Diseases, Aarhus University Hospital, Skejby, Denmark.

First release: 24 September 2020 www.sciencemag.org (Page numbers not final at time of first release)
Mycobacteriosis, staphylococcosis, and candidiasis can be driven by monogenic inborn errors of IFN-γ, IL-6, and IL-17A/F, respectively, or by their genetically driven auto-immune phenocopies, with the production of neutralizing auto-Abs against these cytokines (1–8). Type I IFNs, first described in 1957, are ubiquitously expressed cytokines that contribute to both innate immunity (via their secretion by plasmacytoid dendritic cells and other leukocytes) and cell-intrinsic immunity (in most if not all cell types) against viral infections (9–13). Their receptors are ubiquitously expressed and trigger the induction of IFN stimulated genes (ISGs) via phosphorylated STAT1-STAT2-IRF9 trimers (14). Neutralizing IgG auto-Abs against type I IFNs can occur in patients treated with IFN-α2 or IFN-β (15) and exist in almost all patients with autoimmune polyendocrinopathy syndrome type I (APS-1) (16). They are also seen in women with systemic lupus erythematosus (17).

These patients do not seem to suffer from unusually severe viral infections, although human inborn errors of type I IFNs can underlie severe viral diseases, respiratory and otherwise (18). In 1984, Ion Gresser described a patient with unexplained auto-Abs against type I IFNs suffering from severe chickenpox and shingles (19, 20). More recently, auto-Abs against type I IFNs have been found in a few patients with bi-allelic, hypomorphic RAG1 or RAG2 mutations and viral diseases, including severe chickenpox and viral pneumonias (21). Our attention was drawn to three patients with APS-1, with known pre-existing anti-type I IFN auto-Abs, and life-threatening COVID-19 pneumonia (22) (detailed case reports in Methods). While searching for inborn errors of type I IFNs (18, 23), we hypothesized that neutralizing auto-Abs against type I IFNs might also underlie life-threatening COVID-19 pneumonia.

Auto-Abs against IFN-α2 and/or IFN-ω in patients with critical COVID-19

We searched for auto-Abs against type I IFNs in 987 patients hospitalized for life-threatening COVID-19 pneumonia. We also examined 663 individuals infected with SARS-CoV-2 presenting asymptomatic or mild disease, and 1,227 healthy controls whose samples were collected before the COVID-19 pandemic. Plasma or serum samples were collected from patients with critical COVID-19 during the acute phase of disease. Multiplex particle-based flow cytometry revealed a high fluorescence intensity (FI; >1,500) for IgG auto-Abs against IFN-α2 and/or IFN-ω in 135 patients (13.7%) with life-threatening COVID-19 (Fig. 1A). We found that 49 of these 135 patients were positive for Abs against both IFN-α2 and IFN-ω, whereas 45 were positive only for Abs against IFN-α2, and 41 were positive only for Abs against IFN-ω.

We also performed ELISA and the results obtained were consistent with those obtained with Luminex technology (fig. S1A). We also found that 11 and 14 of 23 patients tested had low levels of IgM and IgA auto-Abs against IFN-ω and IFN-α2, respectively (Fig. 1B and fig. S1B). Auto-Abs against type I IFNs were detected in two unrelated patients for whom we had plasma samples obtained before SARS-CoV-2 infection, indicating that these antibodies were present before SARS-
CoV-2 infection and were not triggered by this infection. As a control, we confirmed that all 25 APS-1 patients tested had high levels of auto-Abs against IFN-α2 and IFN-ω (fig. SIC). Overall, we found that 135 of 987 patients (13.7%) with life-threatening COVID-19 pneumonia had IgG auto-Abs against at least one type I IFN.

The auto-Abs neutralize IFN-α2 and IFN-ω in vitro
We then tested whether auto-Abs against IFN-α2 and IFN-ω were neutralizing in vitro. We incubated PBMCs from healthy controls with 10 ng/mL IFN-α2 or IFN-ω in the presence of plasma from healthy individuals or from patients with auto-Abs. A complete abolition of STAT1 phosphorylation was observed in 101 patients with auto-Abs against IFN-α2 and/or IFN-ω (table S1). The antibodies detected were neutralizing against both IFN-α2 and IFN-ω in 52 of these 101 patients (51%), against IFN-α2 only in 36 patients (36%), and against IFN-ω only in 13 patients (13%), at the IFN-α2 and IFN-ω concentrations tested (Fig. 1, C and D). IgG depletion from patients with auto-Abs restored normal pSTAT1 induction after IFN-α2 and IFN-ω stimulation, whereas the purified IgG fully neutralized this induction (Fig. 1C and fig. S1D).

Furthermore, these auto-Abs neutralized high amounts of IFN-α2 (fig. S1E) and were neutralizing at high dilutions (Fig. 1E and fig. S1F). Interestingly, 15 patients with life-threatening COVID-19 and auto-Abs against IFN-α2 and/or IFN-ω also had auto-Abs against other cytokines (IFN-γ, GM-CSF, IL-6, IL-10, IL-12p70, IL-22, IL-17A, IL-17F, and/or TNF-β), only three of which (IL-12p70, IL-22, IL-6) were neutralizing (in four patients) (fig. S2, A to C). Similar proportions were observed in the other cohorts (fig. S2, D to L).

We also analyzed ISG induction after 2 hours of stimulation with IFN-α2, IFN-β or IFN-γ, in the presence of plasma from healthy individuals or from patients with auto-Abs. With plasma from 8 patients with auto-Abs against IFN-α2, the induction of ISG CXCL10 was abolished after IFN-α2 stimulation but maintained after stimulation with IFN-γ (Fig. 1F). We then found that plasma from the five patients with neutralizing auto-Abs tested neutralized the protective activity of IFN-α2 in MDBK cells infected with vesicular stomatitis virus (table S2). Overall, we found that 101 of 987 patients (10.2%), including 95 males (94%), with life-threatening COVID-19 pneumonia, had neutralizing IgG auto-Abs against at least one type I IFN. By contrast, auto-Abs were detected in only four of 1,227 healthy controls (0.33%) (Fisher exact test, p-value<10^-16) and in none of the 663 patients with asymptomatic or mild SARS-CoV-2 infection tested (Fisher exact test, p-value<10^-16).

Auto-Abs against all 13 IFN-α subtypes in patients with auto-Abs to IFN-α2
We investigated whether patients with neutralizing auto-Abs against IFN-α2 only or IFN-ω and IFN-ω also had auto-Abs against the other 15 type I IFNs. ELISA showed that all patients tested (N=22) with auto-Abs against IFN-α2 also had auto-Abs against all 13 IFN-α subtypes (IFN-α1, -α2, -α4, -α5, -α6, -α7, -α8, -α10, -α13, -α14, -α16, -α17, and -α21), whereas only two of the 22 patients tested had auto-Abs against IFN-β, one had auto-Abs against IFN-κ, and two had auto-Abs against IFN-ε (Fig. 2A). The auto-Abs against IFN-β had neutralizing activity against IFN-β (Fig. 1D). We confirmed that all the patients had auto-Abs against all 13 subtypes of IFN-α, by testing the same samples by LIPS (Fig. 2B). For IFN-β, we also screened the whole cohort in a multiplex assay. We found that 19/987 (1.9%) patients had auto-Abs against IFN-β, and that all of them were in our cohort of severe COVID-19 individuals with neutralizing auto-Abs against IFN-α and/or IFN-ω. Of these patients with auto-Abs against IFN-β, only two were neutralizing against IFN-β (Fig. 1, D and F).

Ten of the 17 genes encoding type I IFNs (IFN-α2, -α5, -α6, -α8, -α13, -α14, -α21, -β, -ω and -κ), have undergone strong negative selection, suggesting that they play an essential role in the general population, whereas the other seven IFN loci in the human genome often carry loss-of-function alleles (24). Moreover, the 13 IFN-α subtypes and IFN-ω are more closely related to each other than to the other three IFNs (IFN-β, IFN-ε and IFN-κ), which are structurally and phylogenetically more distant (Fig. 2C). Thus, all patients with neutralizing auto-Abs against IFN-α2 tested (N=22) had auto-Abs against all 13 IFN-α subtypes, and three of the 22 patients tested (14%) had auto-Abs against 14 or more type I IFNs.

The auto-Abs neutralize IFN-α2 against SARS-CoV-2 in vitro and IFN-α-1 in vivo
Plasma from eight patients with neutralizing auto-Abs against type I IFN also neutralized the ability of IFN-α2 to block the infection of Huh7.5 cells with SARS-CoV-2 (Fig. 3A). Plasma from two healthy controls or from seven SARS-CoV-2-infected patients without auto-Abs did not block the protective action of IFN-α2 (Fig. 3A and fig. S3A). These data provide compelling evidence that the patients’ blood carried sufficiently large amounts of auto-Abs to neutralize the corresponding type I IFNs and block their antiviral activity in vitro, including that against SARS-CoV-2.

We also found that all 41 patients with neutralizing auto-Abs against the 13 types of IFN-α tested had low (one patient) or undetectable (40 patients) levels of the 13 types of IFN-α in their plasma during the course of the disease (Fig. 3B) (25, 26). Type I IFNs may be degraded and/or bound to the corresponding circulating auto-Abs. The presence of circulating neutralizing auto-Abs against IFN-α is, therefore,
strongly associated with low serum IFN-α levels (Fisher exact test, \( p\)-value<10^{-4} \)). Consistently, in patients with neutralizing auto-Abs against IFN-α2, the baseline levels of type I IFN-dependent transcripts were low while they were normal for NF-κB-dependent transcripts (Fig. 3C and fig. S3B). Overall, our findings indicate that the auto-Abs against type I IFNs present in patients with life-threatening COVID-19 were neutralizing in vitro and in vivo.

**Strong excess of men in patients with auto-Abs against type I IFNs**

There was a strong excess of male patients (95 of 101, 94%) with critical COVID-19 pneumonia and neutralizing auto-Abs against type I IFNs. This proportion of males was much higher than that observed in patients with critical COVID-19 without auto-Abs (75%; Fisher exact test \( p\)-value=2.5 \times 10^{-6}), and much higher than that in male patients within the asymptomatic or pauci-symptomatic cohort (28%, Fisher exact test \( p\)-value<10^{-6}) (Table 1, Fig. 4A, and fig. S4A). Further evidence for X-linkage was provided by the observation that one of the seven women with auto-Abs and life-threatening COVID-19 had X-linked incontinentia pigmenti (IP), in which cells activate only one single X chromosome (cells having activated the X chromosome bearing the null mutation in NEMO dying in the course of development) (27). The prevalence of auto-Abs against type I IFNs in the general population was estimated at 0.33% (0.015-0.67%) in a sample of 1,227 healthy individuals, a value much lower than that in patients with life-threatening COVID-19 pneumonia, by a factor of at least 15.

The patients with auto-Abs were also slightly older than the rest of our cohort (49.5% of patients positive for auto-Abs were over 65 years of age versus 38% for the rest of the cohort, \( p=0.024 \)), suggesting that the frequency of circulating anti-type I IFNs auto-Abs increases with age (Table 1 and Fig. 4B). However, auto-Abs were found in patients aged from 25 to 87 years (fig. S4B). PCA was performed on 49: 34 European, 5 North Africans, 1 sub-Saharan African, 2 patients from the Middle East, 2 South Asians, 1 East Asian, and 1 South American (Fig. 4C). Large-scale studies will be required to determine the frequency of such auto-Abs in humans of different sexes, ages, and ancestries. Finally, the presence of auto-Abs was associated with a poor outcome, with death occurring in 37 of the 101 patients (36.6%) (table S1).

**Neutralizing auto-Abs to type I IFNs are causative of critical COVID-19**

There are multiple lines of evidence to suggest that the neutralizing auto-Abs against type I IFNs observed in these 101 patients preceded infection with SARS-CoV-2 and accounted for the severity of disease. First, the two patients for whom testing was performed before COVID-19 were found to have auto-Abs before infection. Second, three patients with APS-1 known to have neutralizing auto-Abs against type I IFN immunity before infection also had life-threatening COVID-19 (22) (supplementary methods). Third, we screened a series of 32 women with IP and found that a fourth of them had auto-Abs against type I IFNs, including one who developed critical COVID-19 (fig. S1C). Fourth, there is a marked bias in favor of men, suggesting that the production of auto-Abs against type I IFNs, whether driven by germ line or somatic genome, may be X-linked and therefore pre-existing to infection.

Moreover, IFN-α subtypes were undetectable during acute disease in the blood of patients with auto-Abs against IFN-α, suggesting a pre-existing or concomitant biological impact in vivo. It is also unlikely that patients could break self-tolerance and mount high titers of neutralizing IgG auto-Abs against type I IFN within only one, or even two weeks of infection. Finally, inborn errors of type I IFNs underlying life-threatening COVID-19 in other previously healthy adults, including autosomal recessive IFNAR1 deficiency, are reported in an accompanying paper (18). Collectively, these findings suggest that auto-Abs against type I IFNs are a cause, and not a consequence of severe SARS-CoV-2 infection, although their titers and affinity may be enhanced by the SARS-CoV-2-driven induction of type I IFNs. They also provide an explanation for the major sex bias seen in patients with life-threatening COVID-19, and perhaps also the increase in risk with age.

**Conclusion**

We report here that at least 10% of patients with life-threatening COVID-19 pneumonia have neutralizing auto-Abs against type I IFNs. With our accompanying description of patients with inborn errors of type I IFNs and life-threatening COVID-19 (18), this study highlights the crucial role of type I IFNs in protective immunity against SARS-CoV-2. These auto-Abs against type I IFNs were clinically silent until the patients were infected with SARS-CoV-2, which is a poor inducer of type I IFNs (28), suggesting that the small amounts of IFNs induced by the virus are important for protection against severe disease. The neutralizing auto-Abs against type I IFNs, like inborn errors of type I IFN production, tip the balance in favor of the virus, resulting in devastating disease, with insufficient, and even perhaps deleterious, innate and adaptive immune responses.

Our findings have direct clinical implications. First, SARS-CoV-2-infected patients can be screened to identify individuals with auto-Abs at risk of developing life-threatening pneumonia. Such patients recovering from life-threatening COVID-19 should also be excluded from donating convalescent plasma for ongoing clinical trial, or at least tested before their plasma donations are accepted (29).
Second, this unexpected finding paves the way for therapeutic intervention, including plasmapheresis, monoclonal Abs depleting plasmablasts, and the specific inhibition of type I IFN-reactive B cells (30). Finally, in this patient group, early treatment with IFN-α is unlikely to be beneficial. However, treatment with injected or nebulized IFN-β may have beneficial effects, as auto-Abs against IFN-β appear to be rare in patients with auto-Abs against type I IFNs.

**Methods**

**Subjects and samples**

We enrolled 987 patients with proven life-threatening (critical) COVID-19, 663 asymptomatic or pauci-symptomatic individuals with proven COVID-19, and 1127 healthy controls in this study. All subjects were recruited following protocols approved by local Institutional Review Boards (IRBs). All protocols followed local ethics recommendations and informed consent was obtained when required.

COVID-19 disease severity was assessed in accordance with the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia. “Life-threatening COVID-19 pneumonia” is pneumonia in patients with critical disease, whether pulmonary, with mechanical ventilation (CPAP, BIPAP, intubation, high-flow oxygen), septic shock, or damage to any other organ requiring admission in the ICU. The individuals with asymptomatic or mild SARS-CoV-2 infection we individuals infected with SARS-CoV-2 who remained asymptomatic or developed mild, self-healing, ambulatory disease with no evidence of pneumonia. The healthy controls were individuals who had not been exposed to SARS-CoV-2.

Plasma and serum samples from the patients and controls were frozen at -20°C immediately after collection. The fluid-phase luciferase immunoprecipitation systems (LIPS) assay was used to determine the levels of antibodies against the SARS-CoV-2 nucleoprotein and spike protein, as previously described (31).

**Detection of anti-cytokine autoantibodies**

**Multiplex particle-based assay**

Serum/plasma samples were screened for autoantibodies against 18 targets in a multiplex particle-based assay, in which magnetic beads with differential fluorescence were covalently coupled to recombinant human proteins. Patients with a fluorescence intensity (FI) of > 1500 for IFN-α2, IFN-β, or > 1000 IFN-ω were tested for blocking activity; as were patients positive for another cytokine.

**ELISA**

Enzyme-linked immunosorbennt assays (ELISA) was performed as previously described (5). In brief, ELISA plates were coated with rhIFN-α, or rhIFN-ω and incubated with 1:50 dilutions of plasma samples from the patients or controls. A similar protocol was used when testing for 12 subtypes of IFN-α.

**LIPS**

Levels of autoantibodies against IFN-α subtypes were measured with luciferase-based immunoprecipitation assay (LIPS), as previously described (32). IFN-α1, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α14, IFN-α16, IFN-α17 and IFN-α21 sequences were transfected in HEK293 cells and the IFN-α-luciferase fusion proteins were collected in the tissue culture supernatant. For autoantibody screening, serum samples were incubated with Protein G agarose beads and we then added 2x10⁶ luminescence units (LU) of antigen and incubated. Luminescence intensity was measured. The results are expressed in arbitrary units (AU), as a fold-difference relative to the mean of the negative control samples.

**Functional evaluation of anti-cytokine autoantibodies**

The blocking activity of anti-IFNα and anti-IFNω autoantibodies was determined by assessing STAT1 phosphorylation in healthy control cells following stimulation with the appropriate cytokines in the presence of 10% healthy control or patient serum/plasma.

We demonstrated that the IFNω blocking activity observed was due to autoantibodies and not another plasma factor, by depleting IgG from the plasma with a protein G column Without eluting the IgG, the flow-through fraction (IgG-depleted) was then collected and compared to total plasma in the phospo-STAT1 assay.

The blocking activity of anti-IFNγ, -GM-CSF, -IFNλ1, -IFNλ2, -IFNλ3, -IL-6, -IL-10, -IL-12p70, -IL-22, -IL-17A, -IL-17F, -TNFα, and -TNFβ antibodies was assessed with the assays outlined in the table in online supplementary materials, as previously reported (21).

For the neutralization of ISG induction, peripheral blood mononuclear cells (PBMCs) were left unstimulated or were stimulated for two hours with 10 ng/mL IFNα or 10 ng/mL IFNγ in a final volume of 100 µL. Quantitative real-time PCR (RT-qPCR) was performed with Applied Biosystems Taqman assays for CXCL10, and the β-glucuronidase (GUS) housekeeping gene for normalization. Results are expressed according to the ΔΔCt method, as described by the manufacturer’s kit.

**Phylogenetic reconstruction**

Protein sequences were aligned with the online version of MAFFFT v7.471 software (33), using the L-INS-i strategy (34) and the BLOSUM62 scoring matrix for amino-acid substitutions. Phylogenetic tree reconstruction was
For the NanoString assay, total RNA was extracted from whole blood samples collected in PaxGene tubes. The expression of selected genes was determined by NanoString methods and a 28-gene type I IFN score was calculated (44).

Statistical analysis

Comparison of proportions were performed using a Fisher exact test, as implemented in R (https://cran.r-project.org/).

PCA was performed with Plink v1.9 software on whole-exome and whole-genome sequencing data with the 1000 Genomes (1kG) Project phase 3 public database as a reference.

Simoa

Serum-IFNα concentrations were determined with Simoa technology, as previously described (40, 41), with reagents and procedures obtained from Quanterix Corporation.

VSV assay

The seroneutralization assay was performed as previously described (42). In brief, the incubation of IFN-α2 with Madin–Darby bovine kidney (MDBK) cells protects the cultured cells against the cytopathic effect of vesicular stomatitis virus (VSV). The titer of anti IFN alpha antibodies was defined as the last dilution causing 50% cell death.

SARS-CoV-2 experiment

SARS-CoV-2 strain USA-WAI/2020 was obtained from BEI Resources and amplified in Huh-7.5 hepatoma cells at 33°C. Viral titers were measured on Huh-7.5 cells in a standard plaque assay. Huh-7.5 cells (H. sapiens) were cultured. Plasma samples or a commercial anti-IFN-α2 antibody were serially diluted and incubated with 20 pM recombinant IFN-α2 for 1 hour at 37°C (starting concentration: plasma samples = 1/100 and anti-IFN-α2 antibody = 1/1000). The cell culture medium was then removed and replaced with the plasma/antibody-IFN-α2 mixture. The plates were incubated overnight and the plasma/antibody-IFN-α2 mixture was removed by aspiration. The cells were washed once with PBS to remove potential anti-SARS-CoV-2-neutralizing antibodies and fresh medium was then added. Cells were then infected with SARS-CoV-2 by directly adding the virus to the wells. Cells infected at a high MOI were incubated at 37°C for 24 hours, whereas cells infected at a low MOI were incubated at 33°C for 48 hours. The cells were fixed with 7% formaldehyde, stained for SARS-CoV-2 with an anti-N antibody, imaged and analyzed as previously described (43).

Nanostring

For the NanoString assay, total RNA was extracted from whole blood samples collected in PaxGene tubes. The expression of selected genes was determined by NanoString methods and a 28-gene type I IFN score was calculated (44).

REFERENCES AND NOTES


ACKNOWLEDGMENTS

We thank the patients, their families, and healthy donors for placing their trust in us. We warmly thank the “French Incontinentia pigmenti” association for their help and support. We warmly thank Y. Nemirovskaya, D. Papandrea, M. Woollert, D. Liu, C. Rivalain and C. Patissier for administrative assistance; D. Kagopianis (National Institute on Aging) for providing healthy donor samples and S. Xirasager, J. Barnett, X. Cheng, S. Weber, J. Danielson, B. Garabedian, and H. Matthews for their assistance in this study. We also thank R. Apps, B. Ryan, and Y. Belkaid of the CHI for their assistance. We thank the CRB-Institut Jérôme Lejeune, CRB-Bio.Jel. Paris, France, for their assistance. We thank M.C. García Guerrero, I. Erkizia, E. Grau, M. Massanella from irsiCaixa AIDS Research Institute, Badalona, Spain and J. Gurtart from the department of Clinical Genetics, University Hospital “ Germans Trias i Pujol”, Badalona, Spain for providing samples; as well as J. Dalmau from irsiCaixa for assistance. Funding: The Laboratory of Human Genetics of Infectious Diseases is supported by the Howard Hughes Medical Institute, the Rockefeller University, the St. Giles Foundation, the National Institutes of Health (NIH) (R01AI08364), the National Center for Advancing Translational Sciences (NCATS), NIH Clinical and Translational Science Award (CTSA) program (UL1 TR001866), a Fast Grant from Emergent Ventures, Mercatus Center at George Mason University, the Yale Center for Mendelian Genomics and the SSP Coordinating Center funded by the National Human Genome Research Institute (NHGRI) (U1HG006504 and U2HG008595), the French National Research Agency (ANR) under the “Investments for the Future” program (ANR-10-IJUH-01), the Integrative Biology of Emerging Infectious Diseases Laboratory of Excellence (ANR-10-LABX-62-IBEID), the French Foundation for Medical Research (FRM) (EQUI201903007798), the FRM and ANR GENCOVID project. ANRS-COVID, the Square Foundation, GrandR - Fonds de solidarité pour l’enfance, the SCOR Corporation Foundation for Science, Institut Institut National de la Santé à la Recherche Médicale (INSERM) and the University of Paris. Samples from San Raffaele Hospital were obtained within the Covid-BioB project and healthcare personnel of San Raffaele Hospital, San Raffaele Telethon Institute for Gene Therapy (SR-TTIG) clinical lab and clinical research Unit; funded by the Program Project COVID-19 CSR-UNISRI and Fondazione Telethon. The French COVID Cohort study group was sponsored by Inserm and supported by the REACTing consortium and by a grant from the French Ministry of Health (PHRC 20-0244). The Cov-Contact Cohort was supported by the REACTing consortium, the French Ministry of Health, and the European Commission (RECOVER WP 6). The “Milieu Intérieur” cohort was supported by was supported by the French Government’s Investissement d’Avenir Program, Laboratoire d’Excellence “Milieu Intérieur” Grant (ANR-10-LABX-69-01) (PI: L. Quintana-Murci & D Duffy). The Simoa experiment was supported by the PHRC-20-0375 COVID-19 grant “DIGITAL COVID” (PI: G Gorochov). SGT is supported by a Leadership 3 Investigator Grant awarded by the National Health and Medical Research Council of Australia, and a COVID19 Rapid Response Grant awarded by UNSW Sydney. CRG and colleagues were supported by Instituto de Salud Carlos III (COV20_0133 and COV20_0134, Spanish Ministry of Science and Innovation -RTC-2017-6471-1: AEI/FEDER, UE), and Cabildo Insular de Tenerife (CIGIEU000219140 and “Apuestas científicas del ITER para colaborar en la lucha contra la COVID-19”). SA and AB were supported by ANR-20-COVI-0064 (PI: A Belot). This work is supported by the French Ministry of Health “Programme Hospitalier de Recherche Clinique Interregional 2013” by the Contrat de Plan Etat-Lorraine and FEDER Lorraine, and a public grant overseen by the French National Research Agency (ANR) as part of the second “Investissements d’Avenir” program FIGHT-HF (reference: ANR-15-RHU-0004) and by the French PIA project “Lorraine Université d’Excellence”, reference ANR-15-IDEX-04-LU46 and biobanking is performed by the Biological Resource Center Lorrain BB-0033-00035. This study was supported by the Fonds IMMUNOV, for Innovation in Immunopathology and by a grant from the Agence Nationale de la Recherche (ANR-flash Covid19 “AIROCovid” to FRL), and by the FAST Foundation (French Friends of Sheba Tel Hashomer Hospital). Work in the Laboratory of Virology and Infectious Disease was supported by NIH grants P01AI138398-S1, 2101111825, and 2101019707-1051, a George Mason University Fast Grant, and the G. Harold and Leila Y. Mathers Charitable Foundation. The Amsterdam UMC Covid-19 Biobank was supported by grants of the Amsterdam Corona Research Fund, Dr. C. J. Vaillant Fund, and Networks Organization for Health Research and Development (ZonMw; NWO-Vici Grant [grant number 918.19.627 to DvDb]). This work was also supported by the Division of Intramural Research of the National Institute of Dental Craniofacial Research and National Institute of Allergy and Infectious Diseases, National Institutes of Health, and by Regione Lombardia, Italy (project “Risposta immune in pazienti con COVID-19 e comorbilità”). The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense. JH holds an Institut Imagine MD-PhD fellowship from the Fondation Bettencourt Schueller. JR is supported by the Inserm PhD program ("poste d'accueil Inserm"). PB was supported by the French Foundation for Medical Research (FRM, EA2107638020) and the MD-PhD program of the Imagine Institute (with the support of the Fondation Bettencourt-Schueller). We thank the Association “Turner et vous” for their help and support. Sample processing at irsiCaixa was possible thanks to the crowdfunding initiative YoMeCorono, DCV is supported by the Fonds de la recherche en santé du québec clinician-scientist scholar program. K. Kisand was supported by the Estonian Research Council grant PUT1367. We thank the GEN-COVID Multicenter Study (https://sites.google.com/ibm.unisit.js/gen-covid). We thank the NIAID Office of Cyber Infrastructure and Computational Biology, Bioinformatics and Computational Biosciences Branch (Contract HHSN362201300006W/HHSN27200002 to MSC, Inc) and Operations Engineering Branch for developing the HGRepo system to enable streamlined access to the data and the NCI Advanced Biomedical Computational Science (ABCS) for data transformation support. Author contributions: PB, LBR, QZ, EM, HHY, YZ, KD, QP, JR, VB, JM, ES, LH, PP, LL, LB, SA, KD, AADJ, AB, LP, DD, ESH, JST, RGM, KK, AP, SHZ, SMH, GG, EJ, CMR, LDN, HCS, JLC provided supervised provision of experiments, generated and analyzed data, and contributed to the manuscript by providing figures and tables. JLP, KB, BS, RY, AB, KB, RL, MM, AC, LA, performed computational analysis of data. PB, AK, EC, YTL, ANS, OMD, MSA, AA, GC, VL, FC, MV, DMS, JH, BT, DD, LQ, DVB, LR, DCV, SGT, FH, DD, TM, PB, JMP, MCN, SBD, CRG, VG, AJ, JG, PDB, JG, RB, LMB, MDA, PB, PR, FRL, FF, MVU, LI, AS, SP, EQR, CR, PC, DA, AL, GLM, XD, JG, MSL, GG, evaluated and recruited patients to COVID and/or control cohorts of patients. PB, QZ, AC, EJ, LA and JLC wrote the manuscript. JLC, supervised the project. All authors edited the manuscript. Competing interests: Helen Su is adjunct faculty at the University of Pennsylvania. Jean-Laurent Casanova is
listed as an inventor on patent application US 63/055,155 filed by The Rockefeller University that encompasses aspects of this publication. Richard Lifton is a non-executive director of Roche and its subsidiary Genentech. The authors declare no other competing interests.

**Data and materials availability:**
All data are available in the manuscript or in the supplementary materials.

Plasma, cells, and genomic DNA are available from Dr. Jean-Laurent Casanova/Dr. Donald Vinh under a material agreement with The Rockefeller University and Apath, LLC. Materials and reagents used are almost exclusively commercially available and non-provisional. Requests for materials derivated from human samples may be made available, subject to any underlying restrictions on such samples. Jean-Laurent Casanova can make material transfer agreements available through The Rockefeller University. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/. This license does not apply to figures/photos/artwork or other content included in the article that is credited to a third party; obtain authorization from the rights holder before using such material.

**HGHD Lab**
Andrés Augusto Arias13, Bertrand Boisson2, Soraya Boucherit2, Jacinta Bustamante1, Marwa Chibihi1, Jie Chen1, Maya Chrabieh2, Tatiana Kochetkov1, Tom Le Voyer1, Dana Li1, Yelena Nemirovskaya1, Masato Ogishi1, Dominick Papandrea1, Cécile Patissier1, Franck Rapaport1, Manon Roynard2, Natasha Vladikine2, Mark Woollett1, Peng Zhang1

1St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, Laboratory of Human Genetics of Infectious Diseases, Neckar Branch, INSERM U1163, Neckar Hospital for Sick Children. School of Microbiology and Group of Primary Immunodeficiencies, University of Antioquia UdeA, Medellín, Colombia.

**NIAID-USUHS Immune Response to COVID Group**
Anuj Kashyap1, Li Ding1, Marita Bosticardo1, Qinlu Wang2, Sebastian Ochoa1, Hui Liu1, Li Ding1, Anuj Kashyap1, Li Ding1, Marita Bosticardo1, Qinlu Wang2, Sebastian Ochoa1, Hui Liu1, Sevket Arslan16, Sophie Assant17, Anna-Sophie Rebillat68, Ismail Reisli169, Pilar Ricart170, Jean-Christophe Richon171, Denis Morand172, Catherine Soler173, Pere Soler-Palacín174, Yuri Stepanovskiy192, Anna-Maria Escribà-Sánchez200, Josep Trenado Alvarez199, Sophie Trouillet-Assant200, Jean-Christophe Richard1, Nadia Rivet1, Jacques G. Rivière2, Gemma Rocamora Blanch1, Carlos Rodriguez2, Carlos Rodríguez-Gallego217, Agustí Rodríguez-Palomero214, Carolina Soler-Campos197, Ayana Rothenburger196, Flore Rozenberg195, María Yolanda Razo del Prado190, Joan Sabater Riera190, Oliver Sanchez217, Silvia Sánchez-Ramón219, Agatha Schletter220, Matthieu Schmidt221, Cyril E. Schweitzer222, Francesco Scollanari23, Anna Sediva199, Luis M. Seijo200, Damien Sené201, Sevap Sengul202, Mikko Sepänikkö203, Alex Serra Ilovich204, Mohammad Shahrooee205, David Smadja206, Ali Sobhi207, Xavier Soleran Moreiro208, José Solér Vilaplana208, Catherine Solers209, Pere Soler-Palacín208, Yuli Stopanovskyy210, Anna-Louise Stoclin211, Fabio Taccone212, Yacine Tanjiaoui-Lambiotte214, Jean-Luc Taupin195, Simon J. Tavernier196, Benjamin Terrier197, Caroline Thumerelle105, Gabriele Tomasoni198, Julie Toubiana199, Josep Trenado Alvarez199, Sophie Trouillet-Assant199, Jesús Troya200, Alessandra Tucci201, Matilde Valeria Ursini84, Yurdagul Uzunhan203, Pierre Vabres204, Juan Valencia-Ramos205, Ana Maria Van Den Rym197, Isabello Vanderwee206, Hulya Vatansev197, Valentina Vélez-Santamaría207, Sébastien Vila208, Cédric Vila209, Marie E. Vilaire210, Audrey Vincent211, Guilhena Violeit212, Fanny Vuotto213, Agler Yusonkaya214, Barnaby E. Young215, Fathi Yuce216, Faez Zannad201, Mayana Zatz217, Alexandre Belo218

1University Hospital and Research Institute “Germans Trias i Pujol”. Badalona, Spain.
2Navarra Health Service Hospital, Pamplona, Spain. Spain. Division of Pediatric Infectious Diseases, NeceRmB Ekaravan University, Meram Medical Faculty, Konya, Turkey. Turkey. Department of Infectious Diseases, Lógham Hákam Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. 3Hospital Nacional Egdaro Rebagliati Martins, Lima, Peru. 4Parc Sanitari Sant Joan de Déu, Sant Boi de Llobregat Spain. 5Virology Research Center, National institutes of Tuberculosis and Lung diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. 6Division of Pediatric Infectious Diseases, Faculty of Medicine, Selcuk University, Konya, Turkey. Intensive care unit, Hôpital Européen, Marseille, France. 7Immunology Department, University Hospital 12 de Octubre, Research Institute iams12, Complutense University, Madrid, Spain. 8Hospital Sant Joan de Déu, Barcelona, Spain. 9Department of Biological Immunology, Neckler Hospital for Sick Children, APHP and INEM, Paris, France. 10Internal
SUPPLEMENTARY MATERIALS

science.sciencemag.org/cgi/content/full/science.abd4585/DC1

Supplementary Materials and Methods

Tables S1 to S3

Figs. S1 to S4

Tables S1 to S3

Data S1

22 June 2020; accepted 16 September 2020

Published online 24 September 2020

10.1126/science.abd4585

First release: 24 September 2020 www.sciencemag.org (Page numbers not final at time of first release)
Fig. 1. Neutralizing auto-Abs against IFN-α2 and/or IFN-ω in patients with life-threatening COVID-19. (A) Multiplex particle-based assay for auto-Abs against IFN-α2 and IFN-ω in patients with life-threatening COVID-19 (N=782), or asymptomatic or mild SARS-CoV-2 infection (N=443), and in healthy controls not infected with SARS-CoV2 (N=1160). (B) Anti-IFN-ω Ig isotypes in 23 patients with life-threatening COVID-19 and auto-Abs to type 1 IFNs. (C) Representative FACS plots depicting IFN-α2- or IFN-ω-induced pSTAT1 in healthy control cells (gated on CD14+ monocytes) in the presence of 10% healthy control or anti-IFN-α2/ω-auto-Abs-containing patient plasma (top panel) or an IgG-depleted plasma fraction (bottom panel). (D) Plot of anti-IFN-α2 auto-Ab levels against their neutralization capacity. The stimulation index (stimulated/unstimulated conditions) for the plasma from each was normalized against that of healthy control plasma from the same experiment. Spearman’s rank correlation coefficient = −0.6805, p-value <0.0001. (E) IC50 curves representing IFN-α2- and IFN-ω-induced pSTAT1 levels in healthy donor cells in the presence of serial dilutions of patient plasma. The stimulation index (stimulated/unstimulated conditions) for patient plasma was normalized against that of 10% healthy control plasma. IFN-α2: IC50 = 0.016%, R2 = 0.983; IFN-ω: IC50 = 0.0353%, R2 = 0.926. (F) Neutralizing effect on CXLC10 induction after stimulation with IFN-α2, IFN-β or IFN-γ, of plasma from healthy controls (N=4), patients with life-threatening COVID-19 and auto-Abs against IFN-α2 (N=8) and APS-1 patients (N=2).
Fig. 2. Auto-Abs against the different type I IFN subtypes. (A) Enzyme-linked immunosorbent assay (ELISA) for auto-Abs against the 13 different IFN-α subtypes, IFN-ω, -β, -κ, and -ε in patients with life-threatening COVID-19 and auto-Abs against IFN-α2 (N=22), APS-1 patients (N=2) and healthy controls (N=2). (B) Luciferase-based immunoprecipitation assay (LIPS) for the 12 different IFN-α subtypes tested in patients with auto-Abs against IFN-α2 (N=22), and healthy controls (N=2). (C) Neighbor-joining phylogenetic tree of the 17 human type I IFN proteins. Horizontal branches are drawn to scale (bottom left, number of substitutions per site). Thinner, intermediate and thicker internal branches have bootstrap support <50%, ≥50 and >80%, respectively. The bootstrap value for the branch separating IFN-ω from all IFN-α is 100%.
Fig. 3. Enhanced SARS-CoV-2 replication, despite the presence of IFN-α2, in the presence of plasma from patients with auto-Abs against IFN-α2 and low in vivo levels of IFN-α. (A) SARS-CoV-2 replication, measured 24h (left panel) and 48h (right panel) after infection, in Huh7.5 cells treated with IFN-α2 in the presence of plasma from patients with life-threatening COVID-19 and neutralizing auto-Abs against IFN-α2 (N=8); a commercial anti-IFN-α2 antibody; or control plasma (N=2). (B) IFN-α levels in the plasma or serum of patients with neutralizing Auto-Abs (N=41), healthy controls (N=5), COVID-19 patients without auto-Abs (N=21) and patients with life-threatening COVID-19 and loss-of-function (LOF) variants (N=10) as assessed by Simoa ELISA. (C) z-scores for type I IFN gene responses in whole blood of COVID-19 patients with (N=8) or without neutralizing Auto-Abs (N=51), or healthy uninfected controls (N=22). The median ± interquartile range is shown. Z-scores were significantly lower for patients with neutralizing auto-Abs compared with patients without auto-Abs (Mann-Whitney test, p=0.01).
Fig. 4. Demographic and ethnic information about the patients and controls. (A) Gender distribution in patients with life-threatening COVID-19 and auto-Ab to type I IFNs, patients with life-threatening COVID-19 and without auto-Ab to type I IFNs and individuals with asymptomatic or mild SARS-CoV-2. (B) Age distribution in patients with life-threatening COVID-19 and auto-Ab to type I IFNs, patients with life-threatening COVID-19 and without auto-Ab to type I IFNs and individuals with asymptomatic or mild SARS-CoV-2. (C) Principal component analysis (PCA) on 49 patients with life-threatening COVID-19 and auto-Ab against type I IFNs.
Table 1. Sex and age distribution of patients with critical COVID-19 with and without autoAbs. Age and sex of the patients and controls, and information about auto-Abs against IFN-α2 and IFN-ω by age and sex. OR: odds ratio.

<table>
<thead>
<tr>
<th>Life-threatening COVID-19</th>
<th>N total</th>
<th>N auto-Abs positive (%)</th>
<th>OR [95% CI]</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>226</td>
<td>6 (2.7%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>761</td>
<td>95 (12.5%)</td>
<td>5.22 [2.27-14.80]</td>
<td>2.5 $10^{-6}$</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>602</td>
<td>51 (8.5%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>≥65 years</td>
<td>385</td>
<td>50 (13.0%)</td>
<td>1.61 [1.04 - 2.49]</td>
<td>0.024</td>
</tr>
</tbody>
</table>

*p-value were derived from Fisher's exact test, as implemented in R ([https://cran.r-project.org/](https://cran.r-project.org/)).
Auto-antibodies against type I IFNs in patients with life-threatening COVID-19

Helen C. Su and Jean-Laurent Casanova


published online September 24, 2020