Preexisting autoantibodies to type I IFNs underlie critical COVID-19 pneumonia in patients with APS-1

Pre-existing autoantibodies to type I IFNs underlie critical COVID-19 pneumonia in patients with autoimmune polyendocrine syndrome type 1

Paul Bastard[1,2,3,@], Elizaveta Orlova[4], Leila Sozaeva[4], Romain Lévy[1,5], Alyssa James[6], Monica M. Schmitt[6], Sebastian Ochoa[6], Maria Kareva[4], Yulia Rodina[7], Adrian Gervais[1,2], Tom Le Voyer[1,2], Jérémie Rosain[1,2], Quentin Philippot[1,2], Anna-Lena Neehus[1,2], Elana Shaw[6], Mélanie Migaud[1], Lucy Bizien[1], Olov Ekwall[8,9], Stefan Berg[8], Guglielmo Beccuti[10], Lucia Ghizzoni[10], Gérard Thiriez[11], Arthur Pavot[12], Cécile Goujard[13], Marie-Louise Frémond[5,14], Edwin Carter[15], Anya Rothenbuhler[16], Agnès Linglard[16], Brigite Mignot[17], Aurélie Comte[17], Nathalie Cheik[18], Olivier Hermine[19], Lars Breivik[20], Eystein S. Husebye[20,21,22], Sébastien Humbert[23], Pierre Rohrlich[24], Alain Coaquette[25], Fanny Vuoto[26], Karine Faure[26], Nizar Mahlaoui[27], Primož Kotnik[28], Tadej Battelino[28], Katarina Trebušak Podkrajšek[29], Kai Kisand[30], Elise M. N. Ferré[6], Thomas DiMaggio[6], Lindsey B. Rosen[6], Peter D. Burbelo[31], Martin McIntyre[32], Nelli Y. Kann[7], Anna Shcherbina[7], Maria Pavlova[33], Anna Kolodkina[4], Steven M. Holland[6], Yanick Crow[14,15], Luigi D. Notarangelo[6], Helen C. Su[6], Laurent Abel[1,2,3], Mark S. Anderson[34], Emmanuelle Jouanguy[1,2,3], Bénédicte Neven[2,5], Anne Puel[1,2,3], Jean-Laurent Casanova[1,2,3,35,@] and Michail S. Lionakis[6,@]

1. Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, Paris, France
2. University of Paris, Imagine Institute, Paris, France
3. St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA
4. Endocrinology Research Centre, Moscow, Russia
5. Pediatric Hematology and Immunology Unit, Necker Hospital for Sick Children, AP-HP, Paris, France
6. Laboratory 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
7. Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia
8. Department of Pediatrics, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Sweden
9. Department of Rheumatology and Inflammation Research, Institute of Medicine, The Sahlgrenska Academy, University of Gothenburg, Sweden
10. Department of Medical Sciences, University of Turin, Turin, Italy
11. Intensive Care Unit, Besançon Hospital, Besançon, France
12. Intensive Care Unit, Kremlin-Bicêtre Hospital, Kremlin-Bicêtre, France
13. Internal medicine department, Bicêtre Hospital, Assistance Publique Hôpitaux de Paris, Paris Saclay University, Inserm U1018, Le Kremlin-Bicêtre, France
14. Laboratory of Neurogenetics and Neuroinflammation, Université de Paris, Imagine Institute, Paris, France
15. Centre for Genomic and Experimental Medicine, Medical Research Council Institute of Genetics and Molecular Medicine, Edinburgh, UK
16. Pediatric endocrinology department, Bicêtre Hospital, Assistance Publique Hôpitaux de Paris, Paris Saclay University, Le Kremlin-Bicêtre, France
17. Pediatric Medicine Unit, University Hospital of Besançon, Besançon, France
18. Pediatric Hematology Unit, University Hospital of Besançon, Besançon, France

19. Hematology department, University of Paris, Necker Hospital for Sick Children, AP-HP, Paris, France
20. Department of Clinical Science and K.G. Jebsen Center for Autoimmune Disorders, University of Bergen, Bergen, Norway.
21. Department of Medicine, Haukeland University Hospital, Bergen, Norway.
22. Department of Medicine (Solna), Karolinska Institutet, Stockholm, Sweden.
23. Internal Medicine Unit, Besançon Hospital, Besançon, France
24. Pediatric Hematology and Oncology unit, CHU de Nice, Nice, France
25. Laboratory of Virology, Besancon Hospital, Besançon, France
26. Infectious Disease Unit, Lille Hospital, Lille, France
27. Centre de Référence Déficits Immunitaires Héréditaires, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France.
28. Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
29. University Medical Centre Ljubljana, University Children's Hospital, Ljubljana, Slovenia
30. Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
31. National Institute of Dental and Craniofacial Research (NIDCR), Bethesda, MD, USA
32. Royal Alexandra Hospital, Paisley, Scotland, United Kingdom
33. Department of Endocrinology №1, Sechenov University, Moscow, Russia
34. Diabetes Center, University of California, San Francisco, California, USA
35. Howard Hughes Medical Institute, New York, NY, USA

@ corresponding authors
lionakism@mail.nih.gov; casanova@rockefeller.edu; paul.bastard@institutimagine.org

Abstract

Patients with biallelic loss-of-function variants of *AIRE* suffer from autoimmune polyendocrine syndrome type-1 (APS-1) and produce a broad range of autoantibodies (auto-Abs), including circulating auto-Abs neutralizing most type-I interferons (IFNs). These auto-Abs were recently reported to account for at least 10% of cases of life-threatening COVID-19 pneumonia in the general population. We report 22 APS-1 patients from 21 kindreds in seven countries, aged between 8 and 48 years and infected with SARS-CoV-2 since February 2020. The 21 patients tested had auto-Abs neutralizing IFN-α subtypes and/or IFN-ω, one had anti-IFN-β, another anti-IFN-ε, but none had anti-IFN-κ. Strikingly, nineteen patients (86%) were hospitalized for COVID-19 pneumonia, including fifteen (68%) admitted to an intensive care unit, eleven (50%) who required mechanical ventilation, and four (19%) who died. Ambulatory disease in three patients (14%) was possibly accounted for by prior or early specific interventions. Pre-existing auto-Abs neutralizing type-I IFNs in APS-1 patients confer a very high risk of life-threatening COVID-19 pneumonia at any age.

Short summary (40 words)

Patients with autoimmune polyendocrine syndrome type-1 (APS-1) have circulating auto-Abs neutralizing most type-I interferons. These auto-Abs can underlie life-threatening COVID-19 pneumonia in the general population. We report 22 APS-1 patients infected with SARS-CoV-2 including fifteen (68%) who developed life-threatening disease.

Running title (50 characters max)

Autoimmune polyendocrine syndrome type-1 and COVID-19

Abbreviation list

AIRE: Auto-immune regulator
APS-1: Autoimmune polyendocrine syndrome type-1,
APECED: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy,
IFNs: Interferons,
MMR: Measles-mumps-rubella,
CMC: Chronic mucocutaneous candidiasis
YFV: Yellow fever virus,
monoclonal antibody (mAb)
intravenous immunoglobulin (IVIg)
JAK: Janus Kinase
ICU: Intensive Care Unit
ALC: Absolute lymphocyte count,
AST: Aspartate transaminase
ECMO: Extracorporeal membrane oxygenation,
p02: Partial pressure of oxygen,
CT-scan: Computed tomography scan,
mmHg: Millimetre of mercury,
CRP: C-reactive protein,
LDH: Lactate deshydrogenase,
ISG: Interferon stimulated gene

Introduction

Autoimmune polyendocrine syndrome type 1 (APS-1), also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a monogenic inborn error of immunity typically caused by biallelic deleterious variants of the autoimmune regulator gene (*AIRE*)[1-5]. Heterozygous variants can also underlie autosomal dominant forms[6]. Patients with APS-1 have defective central T-cell tolerance, leading to the thymic escape of auto-reactive T cells and the development, from early childhood, of a broad range of autoantibodies (auto-Abs) against various autoantigens, including endocrine and other tissue antigens and cytokines[7-12]. Among these anti-cytokine auto-Abs, neutralizing auto-Abs against IL-17A and IL-17F phenocopy inborn errors of IL-17A/F and underlie chronic mucocutaneous candidiasis (CMC)[13-15]. High mucosal concentrations of IFN-γ were also proposed to contribute to CMC[16]. Virtually all patients with APS-1 produce auto-Abs against type I IFNs, generally against the 13 individual IFN-α subtypes and IFN-ω[11,12,17-19]. These auto-Abs were long thought to be clinically silent. This was surprising, as these auto-Abs are neutralizing and type I IFNs are potent antiviral molecules, acting through both innate immunity (via their secretion by plasmacytoid dendritic cells and other leukocytes) and cell-intrinsic immunity (in most cell types)[20-26]. Moreover, the essential role of type I IFNs in fending off viruses in humans was confirmed by the description of patients with autosomal recessive, complete IFNAR1 or IFNAR2 deficiency and adverse reactions to measles-mumps-rubella (MMR) vaccine or yellow fever virus (YFV-17D) live-attenuated viral vaccine[27-29], herpes simplex encephalitis[30], or critical COVID-19 pneumonia[30-32]. Nevertheless, the viral phenotype of these patients is not as broad as initially predicted, as neatly illustrated by two IFNAR1-deficient adults (26 and 38 years old) who had never been hospitalized for severe viral disease until they were admitted for critical COVID-19 pneumonia[31].

In this context, we and others recently reported that three unrelated patients with APS-1 had life-threatening COVID-19 pneumonia [33,34]. These cases suggested that the auto-Abs neutralizing type I IFN were pathogenic and contributed to the discovery that they can also underlie life-threatening COVID-19 pneumonia in previously healthy individuals without APS-1, accounting for at least 10% of the cases in an international cohort of patients[33]. These auto-Abs were more frequent in men (95%) than women (5%), and in elderly patients with critical COVID-19, half of the patients with auto-Abs being over the age of 65 years[33]. These auto-Abs typically neutralized the 13 individual IFN-α, or IFN-ω, or both, but only rarely IFN-β, -κ, and -ε. These findings were replicated in other cohorts[35-39]. Subjects with inborn errors of type I IFN immunity or neutralizing auto-Abs against type I IFN are, thus, at high risk of critical COVID-19 pneumonia, with impaired control of viral replication in the first few days of SARS-CoV-2 infection resulting in a secondary phase of pulmonary and systemic hyperinflammation[32]. Subjects with such auto-Abs are also at high risk of YFV-17D disease, with these antibodies accounting for three of the eight cases studied[40]. Interestingly, the three patients with YFV-17D disease had auto-Abs that neutralized both the 13 IFN-α and IFN-ω, and two also had auto-Abs against IFN-β, these proportions being higher than those reported for COVID-19 patients. It is unknown whether patients with APS-1 have ever been vaccinated with YFV-17D. Following on from the brief description of three APS-1 patients with critical COVID-19 pneumonia, we report here the immunological and clinical features of 22 patients with APS-1 during the course of SARS-CoV-2 infection.

Results

Baseline characteristics of the patients

The 22 patients studied were aged 8 to 48 years at the time of infection with SARS-CoV-2 (median: 24.5 years). Nine were male and 13 were female. Eight were children under the age of 16 years (Table 1). All had undergone vaccination according to the schedules in

force in their country of origin, including vaccination with the live attenuated MMR, with no overt adverse events, between the ages of one to two years. None of the patients had a previous history of severe viral infection, and only one had a history of pneumonia. The 22 patients came from 21 unrelated families (two patients were siblings). The patients originated from and lived in England (*n*=1), France (*n*=5), Italy (*n*=1), Russia (*n*=11), Scotland (*n*=1), Sweden (*n*=1), and the United States of America (*n*=2). Twenty-one of the 22 patients had a typical clinical diagnosis of APS-1 (i.e., any two of the classic triad of manifestations: hypoparathyroidism, adrenal insufficiency, and CMC), with confirmation of the presence of homozygous or compound heterozygous loss-of function variants of *AIRE* in the 17 tested patients. One 16-year-old patient with biallelic loss-of-function *AIRE* mutations (p.R257X) presented only CMC. Twenty-one of the 22 patients had a history of severe tissue auto-immunity, including hypoparathyroidism (*n*=20), adrenal insufficiency (*n*=20), hypogonadism (*n*=9), enteropathy (*n*=9), pernicious anemia (*n*=6), alopecia (*n*=6), autoimmune hepatitis (*n*=3), and vitiligo (*n*=3), and all but four had a history of CMC. One patient was on immunosuppressive treatment with the B cell-depleting monoclonal antibody (mAb) rituximab and monthly intravenous immunoglobulin (IVIg) substitution, another was on treatment with the JAK inhibitor ruxolitinib, a third patients was receiving treatment with the calcineurin inhibitor tacrolimus, and another two patients were on monthly IVIg treatment. Other treatments included endocrine replacement therapy (hydrocortisone and/or fludrocortisone, *n*=20; levothyroxine, *n*=5) and antifungal prophylaxis (fluconazole, *n*=8).

Clinical, radiographic, and laboratory characteristics of COVID-19 infection in 22 patients with APS-1, in chronological order

Patient 1 is a 32-year-old Italian woman with a history of the classic triad manifestations, enteropathy and pernicious anemia[34]. She was diagnosed with bilateral COVID-19 pneumonia in February 2020 and hospitalized for 37 days in Northern Italy. She developed hypoxemia requiring ICU admission and mechanical ventilation for six days. She developed secondary pneumococcal pneumonia and sepsis-induced ventricular dysfunction. She was treated with corticosteroids and broad-spectrum antibiotics. Two months after discharge from hospital, her pulmonary function was persistently impaired (i.e., diffusing capacity for carbon monoxide, 55%).

Patient 2 is a 35-year-old Scottish woman with a history of hypoparathyroidism, adrenal insufficiency and hypogonadism, but not CMC[33]. She was diagnosed with bilateral COVID-19 pneumonia in March 2020 and hospitalized for 12 days. She developed hypoxemia and was intubated and mechanically ventilated in the ICU for five days. She developed lymphopenia (ALC, 600/mm^3) and a mild increase in transaminase levels (AST, 89 U/L). She was treated with corticosteroids; she recovered and was discharged home.

Patient 3 is a 48-year-old American woman of Danish ancestry with a history of the classic triad manifestations, hypogonadism, hypothyroidism, and Sjögren's syndrome[33]. She was diagnosed with bilateral COVID-19 pneumonia and hospitalized in March 2020 for 17 days. She developed hypoxemia and was intubated in the ICU for 11 days. She developed lymphopenia (ALC, 650/mm^3), an increase in transaminase levels (AST, 1668 U/L), hyperferritinemia (14,679 μg/dL), and high D-dimer levels. She was treated with corticosteroids and broad-spectrum antibiotics; she recovered and was discharged home.

Patient 4 is a 21-year-old French man with a history of the classic triad manifestations, type 1 diabetes, asplenia, and myocarditis[14]. He was on monthly IVIg substitution at the time of infection. He developed a high fever and mild respiratory symptoms in May 2020. He was hospitalized, with radiographic evidence of mild pneumonia not requiring oxygen therapy.

Patient 5 is a 34-year-old Russian man with a history of hypoparathyroidism, adrenal insufficiency, and enteropathy, but no CMC. He was hospitalized for 10 days in January 2021

with bilateral COVID-19 pneumonia. He developed hypoxemia requiring oxygen supplementation, lymphopenia (ALC, 380/mm^3) and an increase in D-dimer levels. He received corticosteroids, tocilizumab, and broad-spectrum antibiotics, and made a full recovery.

Patient 6 is a 13-year-old Russian girl with a history of the classic triad manifestations, autoimmune hepatitis, and enteropathy. She was diagnosed with COVID-19 infection while asymptomatic during a SARS-CoV-2 PCR test performed for screening purposes before a routine clinic visit in July 2020. Interestingly, she had been on rituximab since October 2017, and was also receiving IVIg substitution (0.5 g/kg monthly).

Patient 7 was a 28-year-old Russian man with a history of the classic triad manifestations, enteropathy, and alopecia, who was hospitalized in the ICU for bilateral COVID-19 pneumonia in October 2020. He suffered severe hypoxemia requiring mechanical ventilation for four weeks, complicated by secondary bacterial sepsis, acute renal failure requiring hemodialysis, and two episodes of pneumothorax. He developed lymphopenia (ALC, 100/mm^3), high transaminase levels (ALT, 225 U/L), and high D-dimer levels. He was treated with broad-spectrum antibiotics, with the initiation of corticosteroids and tofacitinib three to eight days after the onset of hypoxemia. He died after 47 days in the hospital.

Patient 8 was a 32-year-old Russian woman with a history of the classic triad manifestations, autoimmune hepatitis, alopecia and a previous episode of pneumonia, which is seen in the setting of autoimmune pneumonitis in APS-1 patients[41]. She was hospitalized in the ICU of a hospital for COVID-19 for 15 days in October 2020. She developed severe hypoxemia requiring mechanical ventilation, and died of respiratory failure. She was treated with corticosteroids from day 13 of hospitalization, two days before her death.

Patient 9 is a 14-year-old Russian adolescent with a history of CMC, adrenal insufficiency, alopecia and type 1 diabetes. He was hospitalized for bilateral COVID-19 pneumonia for 15 days in October 2020. He developed hypoxemia requiring oxygen supplementation by a nasal cannula for six days. He recovered and was discharged home.

Patient 10 is an eight-year-old Russian girl with a history of the classic triad manifestations, enteropathy, autoimmune hepatitis and autoimmune encephalitis. She was receiving IVIg substitution (0.5 g/kg monthly) at the time of infection. She was diagnosed with asymptomatic COVID-19 following screening by serological SARS-CoV-2 IgG testing after her mother was diagnosed with mild COVID-19 infection.

Patient 11 is a 28-year-old Russian woman with a history of CMC, hypoparathyroidism, and enteropathy. She was hospitalized in October 2020 for 12 days for bilateral COVID-19 pneumonia not requiring oxygen supplementation. She developed lymphopenia (ALC, 190/mm^3). She recovered and was discharged home.

Patient 12 is a 16-year-old Russian man with a history of CMC and enamel hypoplasia. He was hospitalized for 26 days in October 2020 for bilateral COVID-19 pneumonia. He developed hypoxemia requiring ICU admission and mechanical ventilation. He developed lymphopenia (ALC, 600/mm^3) and his D-dimer levels increased. He was treated with corticosteroids, tocilizumab, and broad-spectrum antibiotics. He recovered and was discharged home.

Patient 13 was a 20-year-old Russian woman with a history of the classic triad manifestations and hypothyroidism. She was hospitalized for 14 days for COVID-19 pneumonia. She was already hypoxemic at admission and her hospital course was further complicated by worsening hypoxemia, requiring ICU admission and mechanical ventilation on day 9 of hospitalization. She was then treated with corticosteroids and tocilizumab, but died from respiratory failure five days after intubation.

Patient 14 is a 31-year-old French woman with a history of the classic triad manifestations, hypogonadism, and pernicious anemia. She was hospitalized for more than 60

days for COVID-19 pneumonia in November 2020. She developed hypoxemia requiring ICU admission, mechanical ventilation, and extracorporeal membrane oxygenation (ECMO). She suffered from multiple secondary bacterial infections, including pneumonia, bacteremia, and sepsis, and ventricular tachycardia. She developed mild increases in transaminase (AST, 77 U/L) and D-dimer levels. She was treated with corticosteroids. She survived but required tracheostomy and intensive respiratory rehabilitation due to persistent respiratory insufficiency.

Patient 15 is a 45-year-old American man of Danish ancestry, brother of Patient 3, with a history of the classic triad manifestations, enteropathy, alopecia, pernicious anemia, hypothyroidism, and end-stage renal disease on hemodialysis. He was hospitalized in November 2020 as a prophylactic measure, to facilitate close monitoring after his diagnosis with COVID-19 at an external facility. He was febrile upon admission, with mild respiratory symptoms, no hypoxemia, and bilateral pneumonia on imaging. He was treated with remdesivir and corticosteroids, while ruxolitinib was continued to prevent progression to hypoxemia and rebound inflammation. His hospital course was complicated by pulmonary embolism (Fig. S1B), which was treated with anticoagulation. He recovered without needing oxygen supplementation or ICU admission and was discharged home after an 18-day stay in hospital.

Patient 16 was a 38-year-old French woman with a history of the classic triad manifestations, hypogonadism, pernicious anemia, myocarditis, and cutaneous lupus. She was hospitalized for 13 days in November 2020. She developed hypoxemia requiring ICU admission and mechanical ventilation. She developed multiple bacterial superinfections and pneumothorax. She was treated with corticosteroids after intubation. She developed lymphopenia (ALC, 290/mm^3), and her transaminase (AST, 76 U/L) and D-dimer levels increased slightly. She died of respiratory failure after 12 days of intubation.

Patient 17 is an eight-year-old Swedish girl with a history of hypoparathyroidism, adrenal insufficiency, and vitiligo, but not CMC. She was hospitalized for bilateral COVID-19 pneumonia at the end of November 2020. She developed hypoxemia requiring ICU admission and mechanical ventilation for four days. She was treated with corticosteroids, plasmapheresis, which successfully decreased type I IFN auto-Ab titers (Fig. 1C), and IVIg substitution. She recovered and was discharged home after a 20-day stay in hospital.

Patient 18 is an 11-year-old French boy with the classic triad manifestations and hypothyroidism. He was hospitalized for 56 days in December 2020 for bilateral COVID-19 pneumonia. His course was complicated by hypoxemia requiring ICU admission and mechanical ventilation. He developed lymphopenia (ALC, 300/mm^3) and increases in D-dimer and transaminase (AST, 48 U/L) levels. He was receiving tacrolimus before COVID-19. He was treated with corticosteroids, IFN-β (45 μg, AVONEX, 3 injections), convalescent plasma, and plasmapheresis, which decreased type I IFN auto-Ab titers (Fig. 1D).

Patient 19 is an 18-year-old British man with a history of the classic triad manifestations, hypogonadism, type 1 diabetes, and alopecia. He was diagnosed with COVID-19 infection at the end of December 2020 after the diagnosis of his parents. He developed a high fever and mild cough and was instructed to initiate stress-dose corticosteroid treatment and to continue until the symptoms had completely resolved, to prevent secondary hyperinflammation. He remained at home without the need for hospitalization and recovered after seven days.

Patient 20 is a 15-year-old French girl with a history of the hypoparathyroidism, adrenal insufficiency, and hypogonadism, hypogonadism and retinitis. She had weekly methotrexate treatment for her retinitis. She was diagnosed with mild COVID-19 pneumonia in early January 2021. She had radiological evidence of bilateral COVID-19 pneumonia (Fig. S1C). After multidisciplinary discussion, she was hospitalized for treatment with three

injections of IFN-β (45 μg, AVONEX) and convalescent plasma therapy to prevent progression to hypoxemic COVID-19 pneumonia. She developed high fever for 72h and recovered without requiring oxygen supplementation and was discharged home.

Patient 21 is a 10-year-old Russian boy with a history of the classic triad manifestations, enteropathy, and retinitis. He was hospitalized for 24 days in January 2021 for bilateral COVID-19 pneumonia. He developed hypoxemia requiring oxygen supplementation by nasal cannula. He developed lymphopenia (ALC, 840/mm^3) and his D-dimer levels increased. He was treated with corticosteroids, tocilizumab, prophylactic anticoagulation, and broad-spectrum antibiotics. He recovered and was discharged home.

Patient 22 is a 30-year-old Russian woman with a history of hypoparathyroidism, adrenal insufficiency, and hypogonadism. She was hospitalized for six days in January 2021 for COVID-19 pneumonia. She developed hypoxemia requiring oxygen supplementation by a nasal cannula. She presented a mild increase in transaminase levels (ALT, 128 U/L). She received corticosteroids, tofacitinib, faripiravir, and prophylactic anticoagulation. She recovered and was discharged home.

Auto-Abs against type I IFNs in the patients

All the patients tested (*n*=21, P6 not tested) had high titers of neutralizing auto-Abs against IFN-α2 and/or IFN-ω, and one (P3) also had auto-Abs against IFN-β (Table 1). All patients but 2 had been tested for the auto-Abs before COVID-19 pandemic. We also tested for the presence of auto-Abs against the 17 individual type I IFNs for all patients for whom serum or plasma samples were available. Eight patients were tested for the presence of auto-Abs against all 13 individual IFN-α and IFN-ω, and they all tested positive (Figure 1A). Only one patient had auto-Abs against IFN-β and one other had auto-Abs against IFN-ε while none of the patients tested had auto-Abs against IFN-κ. We then confirmed that these auto-Abs had neutralizing activity (Fig. 1B), against IFN-α2 and IFN-ω in all patients, and against IFN-β in the only patient positive for auto-Abs against this cytokine. We could not test the neutralizing activity of the auto-Abs to IFN-ε. The serum and plasma samples from patients without detectable auto-Abs against IFN-β did not neutralize the activity of this cytokine. Pre- and post-COVID serum samples were available for 4 patients, and we found no significant differences in titer or neutralization capacity of anti-IFN auto-Abs before and after SARS-CoV-2 infection. We also tested for lung-targeted auto-Abs against the lung antigens KCNRG and BPIFB1 in 8 patients (5 severe and 3 mild/moderate)[41]. All examined patients were negative for KCNRG auto-Abs but two patients, one with severe (P17) and another with mild COVID-19 (P19), tested positive for BPIFB1 auto-Abs (Fig. S2).

Life-threatening COVID-19 pneumonia in 15 APS-1 patients

All 15 patients with hypoxemic COVID-19 pneumonia had positive SARS-CoV-2 PCR results. They had a median age of 30 years (range: 8-48 years). Six were male and nine were female (Tables 1 and 2). Five were children under the age of 16 years. The patients were admitted to hospital between 2 and 10 days after the onset of clinical manifestations (median: 5 days) and were hospitalized for a median of 16 days (range: 6-50 days). We applied the NIH ordinal scale (range: 1-8)[42] to assess the severity of COVID-19 in these patients. They were found to have a median ordinal scale score of 7 (range: 5-8). The degree of hypoxemia was variable, with a median nadir pO_2 of 82 mmHg (range: 60-93 mmHg). Eleven patients required intubation and mechanical ventilation for a median of six days (range: 1-27 days), and one patient required ECMO for 42 days. All patients had a chest CT-scan or X ray showing extensive bilateral ground-glass opacities due to severe COVID-19 pneumonia (Fig. S1A). Four patients suffered from bacterial superinfections, including ventilator-associated pneumonia, bacteremia, and sepsis. Two patients developed pneumothorax requiring chest

tube placement, twice in one patient, and ventricular tachycardia and sepsis-induced cardiomyopathy occurred in one patient each. One patient was discharged with a tracheostomy. All patients had high CRP levels, eight had lymphopenia, seven had high D-dimer levels, six had high transaminase levels, and four had high ferritin and LDH levels.

Managements of the 15 patients with life threatening COVID-19

Thirteen patients received high-dose corticosteroids (>0.5 mg/kg prednisone equivalent/day) in the form of dexamethasone, betamethasone, hydrocortisone, methylprednisolone, or prednisone (Table 2); all 10 patients given corticosteroids within 24 hours of the onset of hypoxemia survived, whereas all four patients receiving corticosteroids later in the course of their hypoxemic disease died (P=0.002; chi-squared test with Yates' correction). Six patients received broad-spectrum antibacterial antibiotics and three patients received antiviral treatment with faripiravir, ribavirin, or a combination of lopinavir/ritonavir with ribavirin. Four patients received anti-IL-6 receptor therapy (tocilizumab) and two patients received the JAK-inhibitor tofacitinib. One patient (P20) received convalescent plasma (twice, 24 hours apart) and intramuscular recombinant IFN-β (Avonex, 45 μg every 48 hours, three injections). Plasmapheresis was performed in two patients (daily, five times for P17 and six times for P18), resulting in a decrease in type I IFN auto-Ab titers in both (Fig. 1C, D). One patient (P18) also received 3 injections of intramuscular IFN-β as well as convalescent plasma, after the first three plasmapheresis sessions. We monitored the blood interferon stimulated gene (ISG) response in this patient using Nanostring. Interestingly, we found a clear increase of ISGs after the initiation of plasmapheresis and IFN-β treatment (Fig. 1D and S3). Four patients (18%) died from sepsis and/or respiratory failure. All the patients who died were adults (aged 20, 28, 32 and 38 years old). The 11 survivors, aged 8 to 48 years, have been discharged from hospital, including one patient suffering from chronic respiratory failure and still dependent on oxygen therapy at most recent follow-up.

Mild non-hypoxemic COVID-19 infection in seven APS-1 patients and the efficacy of early treatment in three of these patients

Seven of 22 patients (32%) had SARS-CoV-2 infection without developing hypoxemia (Tables 1 and 2). The median age of these patients was 18 years (range: 8-45 years). Three were male and four were female. Three were children under the age of 15 years. Interestingly, two of these patients were receiving monthly IVIg therapy at the time of infection; one remained asymptomatic and was treated as an outpatient whereas the other was hospitalized with a high fever and bilateral pneumonia but did not develop hypoxemia. Another patient with asymptomatic infection was receiving IVIg and had also received rituximab eight months before the diagnosis of COVID-19. Moreover, an American man on ruxolitinib treatment was admitted for prophylactic monitoring when he developed a high fever and pneumonia. Treatment with corticosteroids and a 10-day course of remdesivir were initiated in this patient, with the aim of preventing progression to hypoxemic COVID-19. In addition, a British patient harboring BPIFB1 auto-Abs recovered at home following the early initiation and prolonged administration of stress-dose corticosteroid therapy after the development of a high fever with symptoms of pneumonia. Finally, a French patient whose family was made aware of the risk of severe COVID-19 in APS-1 was hospitalized prophylactically two days after symptom onset, while presenting mild radiographic lesions on a chest CT scan (Fig. S1C). She was treated with subcutaneous recombinant IFN-β (Avonex, 45 μg dose every 48 hours, 3 doses) and convalescent plasma therapy for two consecutive days, with the goal of preventing progression to hypoxemic COVID-19. She recovered fully without the need for oxygen supplementation and was discharged home without sequelae.

Pre-existing auto-Abs to type I IFNs underlie life-threatening COVID-19 in APS-1 patients

We describe 22 patients with APS-1 from 21 kindreds from seven countries who were infected with SARS-CoV-2 between February 2020 and January 2021. Nineteen patients (86%) were hospitalized; 15 (68%) developed life-threatening bilateral COVID-19 pneumonia with hypoxemia requiring admission to an ICU, 11 of whom required mechanical ventilation, including five who developed life-threatening secondary complications such as sepsis, pneumothorax, arrhythmias and/or pulmonary embolism, and four of whom died (18%). As we do not know how many SARS-CoV-2-infected APS-1 patients there are worldwide and our series probably reflects an ascertainment bias, we cannot rigorously estimate the proportion of life-threatening cases. However, our findings strongly suggest that APS-1 patients are at very high risk of critical COVID-19 pneumonia. Our previous report of auto-Abs against type I IFNs in at least 10% of patients with critical COVID-19 pneumonia, and in none of the subjects with asymptomatic or benign SARS-CoV-2 infection tested[33] further suggests that APS-1 patients are at high risk of developing critical disease because of their neutralizing auto-Abs against type I IFNs. This very poor outcome seems to be independent of age, sex, European ancestry, and the nature of any other autoimmune manifestations. Importantly, our findings confirm that auto-Abs neutralizing type I IFNs present before SARS-CoV-2 infection, as opposed to other auto-Abs potentially triggered by this infection, confer a very high risk of critical COVID-19[31,33,36-39]. We also found similar levels of auto-Abs prior to and after COVID-19 in the patients tested, further suggesting that the infection does not significantly trigger their production.

Vaccination or early treatment to avoid life-threatening COVID-19 pneumonia

Patients with APS-1 should be prioritized for vaccination against COVID-19. In the meantime, all necessary measures should be taken to avoid infection. Our report of seven patients with SARS-CoV-2 infection following a mild or moderate, non-hypoxemic course is of interest in this respect. Three of these seven patients were on monthly IVIg treatment, which may have decreased the pathogenicity of the auto-Abs against type I IFNs or acted through other mechanisms. Consistently, one of these patients was also receiving rituximab at the time of COVID-19 diagnosis, which may have altered the nature or decreased the titer of auto-Abs against type I IFNs. In addition, three patients whose medical teams had been informed by us of the risk of critical COVID-19 were treated early in the course of infection, one with an early and prolonged course of stress-dose corticosteroids, another by prophylactic admission with the administration of corticosteroids and remdesivir, and the third by early administration of subcutaneous IFN-β. We thus recommend that infected patients should be hospitalized promptly. In patients diagnosed early, ideally before the development of pneumonia, several treatments may be considered. First, cocktails of mAb against the SARS-CoV-2 spike protein may be given to accelerate the decline in viral load[43,44]; these antibodies should be preferred over convalescent plasma, the composition of which is unknown, and which may also contain auto-Abs against type I IFNs or other detrimental components and have not shown efficacy in severe COVID-19 pneumonia[45]. Intramuscular or nebulized IFN-β, or subcutaneous pegylated-IFN-β, may also be considered in patients without auto-Abs against IFN-β[46], as successfully reported for intramuscular IFN-α2 in patients with inborn errors of type I IFN[47] and for IFN-β in a patient with incontinentia pigmenti and auto-Abs against type I IFNs[48]. Obviously, the administration of IFN-α2 is not indicated in APS-1 patients. In patients treated with IFN-β, a monitoring of anti-IFN-β auto-Abs will be important. In the small minority of APS-1 patients carrying auto-Abs against IFN-β, alternative options could be considered.

Rescue treatment in patients with APS-1 and life-threatening COVID-19

When patients present with hypoxemia in the later phase of COVID-19, the administration of mAbs against the SARS-CoV-2 spike protein and of IFN-β should be avoided, given the potential risk of worsening the hyperinflammation and hypoxemia[49,50]. In hypoxemic patients, the early initiation of high-dose corticosteroid treatment is crucial, to prevent a worsening of lung injury and death, as suggested by the observation that patients receiving high-dose corticosteroids at or within 24 hours of the onset of hypoxemia recovered, whereas the later initiation of corticosteroids was associated with death [51]. Indeed, two symptomatic patients without hypoxemic disease who received corticosteroids did not progress to severe disease, further suggesting that early corticosteroid treatment might prevent or attenuate the secondary hyperinflammatory phase of disease[32]. The prompt initiation of corticosteroid treatment is of particular importance in APS-1 patients with pre-existing autoimmune pneumonitis, a frequently overlooked manifestation of APS-1 that affects up to ~40% of patients[41], as the inflammation-prone lung tissue in these patients may confer a predisposition to a worsening of lung injury. Two of the eight patients tested here had auto-Abs against the lung auto-Ab BPIFB1. Such patients are often misdiagnosed as having a prior history of reactive airway disease or recurrent pneumonia[41]. Finally, both in the early phase of disease and after the development of COVID-19 pneumonia, plasmapheresis should be considered, as it has been safely performed in two APS-1 patients (this report) and four patients without APS-1[38]. This procedure can lower the titers of circulating auto-Abs against type I IFNs without lowering the titers of anti-viral Abs[38], and it may be more beneficial when performed early in the course of hospitalization.

No previous viral disease before severe COVID-19

None of the 22 APS-1 patients had previously suffered from severe viral infections, consistent with the history of most patients with APS-1[10]. By inference from our recent observation that auto-Abs against type I IFNs can underlie life-threatening disease due to the YFV-17D live attenuated virus vaccine[40], APS-1 patients should not be vaccinated against YFV. None of the 22 patients described here reported having been inoculated with the YFV-17D vaccine. It is striking that these and other APS-1 patients have not been reported to suffer from other severe viral infections, including MMR disease and herpes simplex virus encephalitis, which have been reported in patients with IFNAR1 or IFNAR2 deficiency[27-30]. This may reflect the residual activity of some of the 17 type I IFNs, including IFN-β in particular, or that at the age of vaccination or HSV-1 infection, the auto-Abs were not yet present, or not as potent, or did not target all the type I IFNs neutralized in older APS-1 patients. There is, nevertheless, one case report of an APS-1 patient suffering from recurrent cutaneous HSV-1 infection[52]. The paucity of viral infections in patients with inherited IFNAR1 or IFNAR2 deficiency is, itself, intriguing[27,28,30,32,53]. Careful retrospective and prospective studies of viral infections and viral diseases in APS-1 patients are therefore warranted. More generally, a careful study of viral infections and viral diseases in patients with inherited IFNAR1 or IFNAR2 deficiency, and in patients with auto-Abs against type I IFNs, regardless of their etiology, is also warranted.

Acknowledgments

We thank the patients and their families for placing their trust in us. We warmly thank the members of both branches of the Laboratory of Human Genetics of Infectious Diseases. We warmly thank Y. Nemirovskaya, M. Woollet, D. Liu, S. Boucherit, C. Rivalain, M. Chrabieh and L. Lorenzo for administrative 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) (R01AI088364), 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 GSP Coordinating Center funded by the National Human Genome Research Institute (NHGRI) (UM1HG006504 and U24HG008956), the Yale High Performance Computing Center (S10OD018521), the Fisher Center for Alzheimer's Research Foundation, the Meyer Foundation, the French National Research Agency (ANR) under the "Investments for the Future" program (ANR-10-IAHU-01), the Integrative Biology of Emerging Infectious Diseases Laboratory of Excellence (ANR-10-LABX-62-IBEID), the ANR project AABIFNCOV (ANR-20-CO11-0001), the French Foundation for Medical Research (FRM) (EQU201903007798), the FRM and ANR GENCOVID project, ANRS-COV05, the Square Foundation, *Grandir - Fonds de solidarité pour l'enfance*, the Fondation du Souffle, the SCOR Corporate Foundation for Science, Institut National de la Santé et de la Recherche Médicale (INSERM) and the University of Paris. This work was further supported in part by the Intramural Research Program of the NIAID and NIDCR, NIH. The work was also supported in part by the Russian Science Foundation (project number 17-75-30035). PB was supported by the French Foundation for Medical Research (FRM, EA20170638020). PB and TLV were supported by the MD-PhD program of the Imagine Institute (with the support of the Fondation Bettencourt-Schueller). ALN was supported by the Foundation Bettencourt-Schueller and the International PhD program of the Imagine Institute. The content is the manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the any of the funding sources.

Author contributions:

PB, EO, LS, RL, AJ, MMS, SO, MK, YR, AG, TLV, JR, QP, ALN, ES, MM, LB, OE, SB, GB, LG, GT, AP, CG, MLF, EC, AR, AL, BM, AC, NC, OH, LB, ESH, SH, PR, AC, FV, KF, NM, PK, TB, KTP, KK, EMNF, TD, LBR, PDB, MM, NYK, AS, MP, SMH, YC, LDN, HCS, LA, MSA, EJ, BN, AP, collected the clinical data, recruited and/or treated the patients. MSL and JLC supervised the project. PB, JLC and MSL wrote the manuscript. All the authors edited the manuscript.

Conflict of interest statement: The authors have no competing financial interests to declare.

Methods

Patients and study approval

Written informed consent was obtained from patients or their parents in the country in which they were followed, in accordance with local regulations. The study was approved by the institutional review boards of The Rockefeller University and *Institut National de la Santé et de la Recherche Médicale* (INSERM), the NIAID/NIH, the Endocrinology Research Center of Russia, and the University of Gothenburg, Sweden. Experiments were conducted in the United States of America and France, in accordance with local regulations and with the approval of the institutional review boards of The Rockefeller University, NIAID/NIH and INSERM. Anonymized samples were studied at the NIAID under non-human subject research conditions; no additional IRB consent was required at the NIH. APS-1 patients gave consent under IRB-approved protocols 11-I-0187 (clinicaltrials.gov NCT01386437) at the NIAID/NIH, study no 779-11. The Swedish patient was enrolled in study no 779-11,

approved by the Central Ethical Review Board at the University of Gothenburg. The study has been approved by the local ethics committee at Endocrinology Research Center of Russia (Protocol №11 from 23.10.2013) and all patients or their parents or guardians singed the informed consent.

Detection of anti-cytokine auto-Abs using a cell-based assay

All Russian patients were tested for neutralizing auto-Abs against IFN-α2 and/or IFN-ω using a cell-based assay as previously described[12,54].

Detection of anti-cytokine auto-Abs in a multiplex particle-based assay

Serum/plasma samples were screened for auto-Abs against IFN-α2 and IFN-ω targets in a multiplex particle-based assay, in which magnetic beads with differential fluorescence were covalently coupled to recombinant human proteins (2.5 μg/reaction). Beads were combined and incubated with 1:100 diluted serum/plasma samples for 30 minutes. Each sample was tested once. The beads were then washed and incubated with PE-labeled goat anti-human IgG antibody (1 μg/mL) for 30 minutes. They were washed again and used in a multiplex assay run on a BioPlex X200 instrument. Patients with a fluorescence intensity (FI) > 1500 for IFN-α2 or IFN-β, or > 1000 for IFN-ω were tested for blocking activity.

Enzyme-linked immunosorbent assays (ELISA) for anti-cytokine auto-Abs

ELISA was performed as previously described[33]. In brief, 96-well ELISA plates (MaxiSorp; Thermo Fisher Scientific) were coated by incubation overnight at 4°C with 2 μg/mL rhIFN-α, and rhIFN-ω (R&D Systems). Plates were then washed (PBS/0.005% Tween), blocked by incubation with 5% nonfat milk powder in the same buffer, washed, and incubated with 1:50 dilutions of plasma from the patients or controls for 2 h at room temperature (or with specific mAbs as positive controls). Each sample was tested once. Plates were thoroughly washed. Horseradish peroxidase (HRP)–conjugated Fc-specific IgG fractions from polyclonal goat antiserum against human IgG or IgA (Nordic Immunological Laboratories) were added to a final concentration of 2 μg/mL. Plates were incubated for 1 h at room temperature and washed. Substrate was added and the optical density (OD) was measured. A similar protocol was used to test for antibodies against 12 subtypes of IFN-α, except that the plates were coated with cytokines from PBL Assay Science (catalog #11002-1).

Functional evaluation of anti-cytokine auto-Abs

The blocking activity of auto-Abs against IFN-α2 and IFN-ω was assessed by evaluating STAT1 phosphorylation in healthy control cells following stimulation with the appropriate cytokines in the presence of 10% serum/plasma from a healthy control or a patient. Surface-stained healthy control PBMCs (350,000/reaction) were cultured in serum-free RPMI medium supplemented with 10% healthy control or patient serum/plasma and were either left unstimulated or were stimulated with IFN-α2 and IFN-ω (10 ng/mL) for 15 minutes at 37°C. Each sample was tested once. Cells were fixed, permeabilized, and stained for intranuclear phospho-STAT1 (Y701). Cells were acquired on a BD LSRFortessa cytometer with gating on $CD14^+$ monocytes and analyzed with FlowJo software.

Luciferase immunoprecipitation systems (LIPS) assay for lung-targeted auto-Abs

We used the LIPS immunoassay to detect auto-Ab immunoreactivity against the lung targeting the potassium regulator KCNRG and bactericidal/permeability-increasing fold-containing B1 (BPIFB1) in APS-1 patient sera. Seropositivity was defined as a value greater than the mean for healthy donors plus three standard deviations, as previously described[41].

IFN score[55]

Total RNA was extracted from whole blood with a PAXgene (PreAnalytix) RNA isolation kit. RNA concentration was assessed with a spectrophotometer (FLUOstar Omega, Labtech). Analysis of 24 genes and 3 housekeeping genes was conducted using the NanoString customer designed CodeSets according to the manufacturer's recommendations (NanoString Technologies, Seattle, WA). Agilent Tapestation was used to assess the quality of the RNA. 100ng of total RNA was loaded for each sample. Data were processed with nSolver software (NanoString Technologies Seattle, WA). The data was normalized relative to the internal positive and negative calibrators, the 3 reference probes and the control samples. The median of the 24 probes for each of 27 healthy control samples was calculated. The mean NanoString score of the 27 healthy controls +2SD of the mean was calculated. Scores above this value (>2.724) were designated as positive. The list of probes used in NanoString ISG analysis is supplied in Table S1.

Online supplemental material

Table S1 provides additional data on the probes used in the NanoString ISG analysis. **Figure S1** provides radiological images of COVID-19 in the patients. **Figure S2** shows the auto-Ab result for lung-targeted auto-Abs (KCNRG and BPIFB1). **Figure S3** shows the ISGs used in the Nanostring, at the different time-points as well as the neutrophil score.

Table legends

Table 1: Baseline demographic, genetic and clinical characteristics of the 22 APS-1 patients with SARS-CoV-2 infection included in this study

AIRE, autoimmune regulator; APS-1 autoimmune polyglandular syndrome type 1; AI, adrenal insufficiency; HP, hypoparathyroidism, CMC, chronic mucocutaneous candidiasis; HT, hypothyroidism; PA, pernicious anemia; DM, diabetes mellitus; PTH, parathyroid hormone; HRT, hormone replacement therapy; NT, not tested; F, female; M, male; IFN, interferon.

Table 2: Clinical features of 22 APS-1 patients with SARS-CoV-2 infection

EF, ejection fraction; DLCO, diffusing capacity for carbon monoxide; LOP/RIT, lopinavir/ritonavir; HCQ, hydroxychloroquine; GGO, ground-glass opacities; CRP, C-reactive protein; AST, aspartate aminotransferase; ALC, absolute lymphocyte count; ECMO, extracorporeal membrane oxygenation; GI, gastrointestinal; N/A, not available. *Hypoxemia defined as SpO2 <94 mmHg.

Figure legends

Figure 1: APS-1 patients have neutralizing auto-Abs against type I IFNs, the titers of which can be decreased by plasmapheresis. (A) Titers of auto-Ab titers against the 17 type I IFNs in APS-1 patients infected with SARS-CoV-2 (n=8). **(B)** Neutralization of IFN-α2 by various dilutions of auto-Ab-containing serum from APS-1 patients with COVID-19 (*n*=5). **(C)** Plasmapheresis (PE) decreased the titers of type I IFN auto-Abs in one APS-1 patient (P17) with COVID-19 pneumonia. The titers of auto-Abs against IFN-α2 are shown for one of the APS-1 patients treated by plasmapheresis. **(D)** Plasmapheresis (PE) decreased the titers of type I IFN auto-Abs in another APS-1 patient (P18) with COVID-19 pneumonia, treated with plasmapheresis, convalescent plasma and IFN-β (as shown with arrows). The titers of auto-Abs against IFN-α2 are shown for the APS-1 patients treated by plasmapheresis

in the upper panel. In the lower panel, ISG scores -evaluated by Nanostring- show an increase after the initiation of treatments. ISG score cut-off for positivity is 2,758.

Figure S1: Imaging of COVID-19 pneumonia in APS-1 patients. (A) Course of COVID-19 pneumonia in an APS-1 patient. Bilateral (left>right) ground glass opacities are seen on initial chest CT six days after symptom onset (left upper panel). Persistence of bilateral ground glass opacities with a worsening of radiographic signs in the left lung base on day 10 after symptom onset (right upper panel). Improvement of ground glass opacities on days 16 (left lower panel) and 37 (right lower panel) after symptom onset. **(B)** Coronal chest CT angiogram demonstrating non-occlusive segmental pulmonary embolus to the distal pulmonary arterial branches of the right lower lobe. **(C)** Chest CT scan of an APS-1 patient showing bilateral alveolo-interstitial lesions of COVID-19 pneumonia.

Figure S2: Analysis of lung-targeting auto-Abs against KCNRG and BPIFB1 in APS-1 patients with COVID-19. Auto-Ab titers to KCNRG **(A)** and BPIFB1 **(B)** in APS-1 patients with COVID-19 (n=8). Positive and negative control sample results are also shown.

Figure S3: ISG score and neutrophil score at different time points in an APS-1 patient, with severe COVID-19, and treated with plasmapheresis, convalescent plasma and IFN-β. (A) 24 ISGs are shown at each time point, and **(B)** 6 neutrophil signature genes are shown. ISG score are higher during treatment, while the neutrophile score diminishes.

References

1. Nagamine K, Peterson P, Scott HS, et al. Positional cloning of the APECED gene. Nat Genet 1997;17:393-8.
2. Finnish-German AC. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 1997;17:399-403.
3. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 1990;322:1829-36.
4. Ferre EM, Rose SR, Rosenzweig SD, et al. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. JCI Insight 2016;1.
5. Husebye ES, Anderson MS, Kampe O. Autoimmune Polyendocrine Syndromes. N Engl J Med 2018;378:2543-4.
6. Oftedal BE, Hellesen A, Erichsen MM, et al. Dominant Mutations in the Autoimmune Regulator AIRE Are Associated with Common Organ-Specific Autoimmune Diseases. Immunity 2015;42:1185-96.
7. Chan AY, Anderson MS. Central tolerance to self revealed by the autoimmune regulator. Ann N Y Acad Sci 2015;1356:80-9.
8. Proekt I, Miller CN, Lionakis MS, Anderson MS. Insights into immune tolerance from AIRE deficiency. Curr Opin Immunol 2017;49:71-8.
9. Guo CJ, Leung PSC, Zhang W, Ma X, Gershwin ME. The immunobiology and clinical features of type 1 autoimmune polyglandular syndrome (APS-1). Autoimmun Rev 2018;17:78-85.
10. Constantine GM, Lionakis MS. Lessons from primary immunodeficiencies: Autoimmune regulator and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Immunol Rev 2019;287:103-20.

11. Bruserud O, Oftedal BE, Landegren N, et al. A Longitudinal Follow-up of Autoimmune Polyendocrine Syndrome Type 1. J Clin Endocrinol Metab 2016;101:2975-83.
12. Orlova EM, Sozaeva LS, Kareva MA, et al. Expanding the Phenotypic and Genotypic Landscape of Autoimmune Polyendocrine Syndrome Type 1. J Clin Endocrinol Metab 2017;102:3546-56.
13. Kisand K, Boe Wolff AS, Podkrajsek KT, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 2010;207:299-308.
14. Puel A, Doffinger R, Natividad A, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 2010;207:291-7.
15. Puel A, Cypowyj S, Bustamante J, et al. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 2011;332:65-8.
16. Break TJ, Oikonomou V, Dutzan N, et al. Aberrant type 1 immunity drives susceptibility to mucosal fungal infections. Science 2021;371.
17. Meager A, Visvalingam K, Peterson P, et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 2006;3:e289.
18. Levin M. Anti-interferon auto-antibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 2006;3:e292.
19. Meyer S, Woodward M, Hertel C, et al. AIRE-Deficient Patients Harbor Unique High-Affinity Disease-Ameliorating Autoantibodies. Cell 2016;166:582-95.
20. Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 1957;147:258-67.
21. Isaacs A, Lindenmann J, Valentine RC. Virus interference. II. Some properties of interferon. Proc R Soc Lond B Biol Sci 1957;147:268-73.
22. Gresser I. Wherefore interferon? J Leukoc Biol 1997;61:567-74.
23. Hoffmann HH, Schneider WM, Rice CM. Interferons and viruses: an evolutionary arms race of molecular interactions. Trends Immunol 2015;36:124-38.
24. Lazear HM, Schoggins JW, Diamond MS. Shared and Distinct Functions of Type I and Type III Interferons. Immunity 2019;50:907-23.
25. Jing H, Su HC. New immunodeficiency syndromes that help us understand the IFN-mediated antiviral immune response. Curr Opin Pediatr 2019;31:815-20.
26. Duncan CJA, Randall RE, Hambleton S. Genetic Lesions of Type I Interferon Signalling in Human Antiviral Immunity. Trends Genet 2021;37:46-58.
27. Duncan CJ, Mohamad SM, Young DF, et al. Human IFNAR2 deficiency: Lessons for antiviral immunity. Sci Transl Med 2015;7:307ra154.
28. Hernandez N, Bucciol G, Moens L, et al. Inherited IFNAR1 deficiency in otherwise healthy patients with adverse reaction to measles and yellow fever live vaccines. J Exp Med 2019;216:2057-70.
29. Gothe F, Hatton CF, Truong L, et al. A novel case of homozygous IFNAR1 deficiency with haemophagocytic lymphohistiocytosis. Clin Infect Dis 2020.
30. Bastard P, Manry J, Chen J, et al. Herpes simplex encephalitis in a patient with a distinctive form of inherited IFNAR1 deficiency. J Clin Invest 2020;131(1).
31. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science 2020;370.
32. Zhang Q, Bastard P, Bolze A, et al. Life-Threatening COVID-19: Defective Interferons Unleash Excessive Inflammation. Med (N Y) 2020;1:14-20.
33. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020;370.

34. Beccuti G, Ghizzoni L, Cambria V, et al. A COVID-19 pneumonia case report of autoimmune polyendocrine syndrome type 1 in Lombardy, Italy: letter to the editor. J Endocrinol Invest 2020;43:1175-7.
35. Wang EY, Mao T, Klein J, et al. Diverse Functional Autoantibodies in Patients with COVID-19. medRxiv 2020.
36. Wijst MGPvd, Vazquez SE, Hartoularos GC, et al. Longitudinal single-cell epitope and RNA-sequencing reveals the immunological impact of type 1 interferon autoantibodies in critical COVID-19. Submitted
37. Jesús Troya García, Bastard P, Planas-Serra L, et al. Neutralizing autoantibodies to type I IFNs in >10% of patients with severe COVID-19 pneumonia hospitalized in Madrid, Spain. Submitted.
38. de Prost N, Bastard P, Arrestier R, et al. Plasma Exchange to Rescue Patients with Autoantibodies Against Type I Interferons and Life-Threatening COVID-19 Pneumonia. J Clin Immunol 2021.
39. Rutger Koning Paul Bastard SdB, Amsterdam UMC Covid-19 Biobank, Jean-Laurent Casanova, Alexander P.J. Vlaar, Matthijs C. Brouwer, Diederik van de Beek. Autoantibodies against type I interferons are associated with multi-organ failure in COVID-19 patients. Intensive Care Medicine 2021;In Press.
40. Bastard P, Michailidis E, Hoffmann HH, et al. Auto-antibodies to type I IFNs can underlie adverse reactions to yellow fever live attenuated vaccine. J Exp Med 2021;218.
41. Ferre EMN, Break TJ, Burbelo PD, et al. Lymphocyte-driven regional immunopathology in pneumonitis caused by impaired central immune tolerance. Sci Transl Med 2019;11.
42. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med 2020;383:1813-26.
43. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. N Engl J Med 2021;384:229-37.
44. Weinreich DM, Sivapalasingam S, Norton T, et al. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. N Engl J Med 2021;384:238-51.
45. Simonovich VA, Burgos Pratx LD, Scibona P, et al. A Randomized Trial of Convalescent Plasma in Covid-19 Severe Pneumonia. N Engl J Med 2021;384:619-29.
46. Monk PD, Marsden RJ, Tear VJ, et al. Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Respir Med 2021;9:196-206.
47. Levy R, Bastard P, Lanternier F, Lecuit M, Zhang SY, Casanova JL. IFN-alpha2a Therapy in Two Patients with Inborn Errors of TLR3 and IRF3 Infected with SARS-CoV-2. J Clin Immunol 2021:26–7.
48. Bastard P, Levy R, Henriquez S, Bodemer C, Szwebel TA, Casanova JL. Interferon-beta Therapy in a Patient with Incontinentia Pigmenti and Autoantibodies against Type I IFNs Infected with SARS-CoV-2. J Clin Immunol 2021.
49. Consortium WHOST, Pan H, Peto R, et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med 2020.
50. Hung IF, Lung KC, Tso EY, et al. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet 2020;395:1695-704.
51. Group RC, Horby P, Lim WS, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. N Engl J Med 2020.
52. Nagafuchi S, Umene K, Yamanaka F, et al. Recurrent herpes simplex virus infection in a patient with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy

associated with L29P and IVS9-1G>C compound heterozygous autoimmune regulator gene mutations. J Intern Med 2007;261:605-10.
53. Meyts I, Casanova JL. Viral infections in humans and mice with genetic deficiencies of the type I IFN response pathway. Eur J Immunol 2021.
54. Breivik L, Oftedal BE, Boe Wolff AS, Bratland E, Orlova EM, Husebye ES. A novel cell-based assay for measuring neutralizing autoantibodies against type I interferons in patients with autoimmune polyendocrine syndrome type 1. Clin Immunol 2014;153:220-7.
55. Rice GI, Forte GM, Szynkiewicz M, et al. Assessment of interferon-related biomarkers in Aicardi-Goutieres syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. Lancet Neurol 2013;12:1159-69.

Table 1: Baseline demographic, genetic and clinical characteristics of the 22 APS-1 patients with SARS-CoV-2 infection included in the study

Patient #	Ancestry/residence	Age	Sex	*AIRE* variants	IFN-α, IFN-β, IFN-ω auto-Ab positivity	Prior non-infectious clinical manifestations	Prior infections	Treatments at the time of SARS-CoV-2 infection diagnosis
1	European/Italy	32	F	R203X/R203X	IFN-α and IFN-ω positive	AI, HP, ectodermal dystrophy, hypogonadism PA, enteropathy	CMC	Hydrocortisone, fludrocortisone, calcium, iron, magnesium, vitamin B12, folic acid, cholecalciferol, PTH, HRT, mesalasine, pancreatic enzyme replacement therapy, rifaximin
2	European/Scotland	35	F	L323SfsX51/ L323SfsX51	IFN-α and IFN-ω positive	AI, HP, hypogonadism, gastroparesis	None	Hydrocortisone, fludrocortisone, estrogen, PTH
3	European/USA	48	F	L323SfsX51/ S64TfsX71	IFN-α, IFN-β, and IFN-ω positive	AI, HP, HT, hypogonadism, Sjögren's syndrome	CMC	Hydrocortisone, fludrocortisone, calcitriol, levothyroxine, HRT
4	European/France	21	M	R257X/P539L	IFN-α and IFN-ω positive	AI, HP, enteropathy, DM, HT, asplenia, exocrine pancreatic insufficiency, myocarditis	CMC	Hydrocortisone, fludrocortisone, levothyroxine, insulin, fluconazole, trimethoprim/sulfamethoxazole, pancreatic enzymes**,** monthly IVIg

5	European/Russia	34	M	NT	IFN-ω positive	AI, HP, enteropathy, nail dystrophy	None	Hydrocortisone, fludrocortisone, calcitriol
6	European/Russia	13	F	R257X/ R257X	NT	AI, HP, autoimmune hepatitis, enteropathy, pancreatitis, nephritis	CMC	Hydrocortisone, fludrocortisone, calcium, amlodipine, enalapril, rituximab (treatment initiation in October 2017 with re-dosing every 6 months; last dose 8 months prior to SARS-CoV-2 infection diagnosis), monthly IVig
7	European/Russia	28	M	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, enteropathy, alopecia, ptosis, enamel dysplasia	CMC	Hydrocortisone, fludrocortisone, calcitriol, fluconazole
8	European/Russia	32	F	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, hypogonadism, enteropathy, autoimmune hepatitis, alopecia, vitiligo, asplenia, Sjögren's syndrome, PA, deep vein thrombosis, ptosis, enamel dysplasia, cataract	CMC, pneumonia	Hydrocortisone, fludrocortisone, calcium carbonate, alfacalcidol, fluconazole, rivaroxaban

9	European/Russia	14	M	R257X/R257X	IFN-α and IFN-ω positive	AI, DM, alopecia, enamel dysplasia, asthma	CMC	Hydrocortisone, fludrocortisone, fluconazole
10	European/Russia	8	F	R257X/E298X	IFN-α and IFN-ω positive	AI, HP, enteropathy, alopecia, PA, autoimmune hepatitis, autoimmune encephalitis	CMC	Hydrocortisone, fludrocortisone, calcitriol, fluconazole, monthly IVIg
11	European/Russia	28	F	R257X/R257X	IFN-α and IFN-ω positive	HP	CMC	Alfacalcidol, fluconazole
12	European/Russia	16	M	R257X/R257X	IFN-α and IFN-ω positive	enamel hypoplasia	CMC	Fluconazole
13	European/Russia	20	F	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, hypogonadism, HT	CMC	Hydrocortisone, fludrocortisone, alfacalcidol, levothyroxine, HRT
14	European/France	31	F	NT	IFN-α and IFN-ω positive	AI, HP, hypogonadism, PA, hypopituitarism, achalasia	CMC	Hydrocortisone, levothyroxine
15	European/USA	45	M	S64TfsX71/ L323SfsX51	IFN-α and IFN-ω positive	AI, HP, HT, end-stage renal disease, alopecia, PA, vitiligo, enteropathy	CMC	Hydrocortisone, fludrocortisone, calcium, ruxolitinib
16	European/France	38	F	NT	IFN-α	AI, HP,	CMC,	Hydrocortisone, fludrocortisone,

					and IFN-ω positive	myocarditis, PA, hypogonadism, cutaneous lupus	urinary tract infections	PTH, iron, magnesium, 1-0-HRT, perindopril, vitamin C, posaconazole
17	European/Sweden	8	F	P538L/P538L	IFN-α and IFN-ω positive	AI, HP, urticarial eruption, vitiligo, lupus-like systemic inflammation	None	Hydrocortisone, fludrocortisone, alfacalcidol calcium, magnesium
18	European/France	11	M	NT	IFN-α and IFN-ω positive	AI, HP, HT	CMC	Hydrocortisone, fludrocortisone, calcium, levothyroxine, tacrolimus
19	European/England	18	M	c.242T>C/C.1265delC	IFN-α and IFN-ω positive	AI, HP, urticarial eruption, DM, alopecia, hypogonadism	CMC	Hydrocortisone, fludrocortisone, calcium
20	European/France	15	F	NT	IFN-α and IFN-ω positive	AI, HP, hypogonadism, retinitis	CMC	Hydrocortisone, fludrocortisone, calcium, weekly methotrexate
21	European/Russia	10	M	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, urticarial eruption, enteropathy, retinitis	CMC	Hydrocortisone, fludrocortisone, calcium, calcitriol, fluconazole
22	European/Russia	30	F	R257X/ L323SfsX51	IFN-ω positive	AI, HP, hypogonadism	None	Hydrocortisone, fludrocortisone, calcitriol

Table 2. Clinical features of 22 APS-1 patients with SARS-CoV-2 infection

Patient #	Days from symptom onset to hospital admission	COVID-19 severity (NIH ordinal scale score)	COVID-19 complications (other than hypoxemia-related)	Duration of hospital stay (days)	Hypoxemia support* (nadir SpO_2)	Intubation (duration in days)	Laboratory abnormalities	Radiographic abnormalities	Treatments	Outcomes
1	4	Critical (7)	Hypotension requiring dobutamine/ norepinephrine infusion; pneumococcal pneumonia; sepsis-induced ventricular dysfunction (EF, 30%); *Clostridium difficile* infection	37	Mechanical ventilation (N/A)	6	N/A	Bilateral, multiple GGO	High-dose hydrocortisone, LOP/RIT, ribavirin, HCQ, piperacillin/ tazobactam	Survival; low DLCO (55%) two months after discharge
2	8	Critical (7)	None	12	Mechanical ventilation (N/A)	5	↑CRP, ↓ALC, ↑AST	Bilateral, multiple GGO	High-dose hydrocortisone	Survival
3	7	Critical (7)	None	17	Mechanical ventilation (80%)	11	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑ferritin, ↑LDH, ↑D-dimer	Bilateral, multiple GGO	High-dose methyl-prednisolone, azithromycin, ceftriaxone, HCQ	Survival

4	4	Mild (4)	None	3	No	No	N/A	Bilateral GGO	None	Survival
5	10	Moderate-severe (5)	Antibiotic-associated diarrhea	10	No (93%)	No	↑CRP, ↓ALC, ↑LDH, ↑D-dimer	Bilateral, multiple GGO	High-dose prednisone, tocilizumab, vancomycin, ertapenem, levofloxacin, HCQ	Survival
6	Not hospitalized	Mild (1)	None	0	No	No	Not tested	Not performed	None	Survival
7	3	Critical (8)	Bacterial sepsis (*Acinetobacter Klebsiella*), pneumothorax (twice), acute renal failure (requiring hemodialysis)	47	Mechanical ventilation (60%)	28	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑creatinine, ↑D-dimer, ↑IL-6	Bilateral, multiple GGO	High-dose prednisone, tofacitinib, cefepime, sulbactam, polymixin B, linezolid, caspofungin	Death
8	4	Critical (8)	None	15	Mechanical ventilation (82%)	1	N/A	Bilateral, multiple GGO	High-dose dexamethasone	Death

9	7	Moderate-severe (5)	None	15	Nasal cannula (86%)	No	↑CRP, ↑LDH	Bilateral, multiple GGO	No	Survival
10	Not hospitalized	Mild (1)	None	0	No	No	Not tested	Not performed	No	Survival
11	5	Mild (4)	None	12	No	No	↓ALC	Bilateral GGO	No	Survival
12	5	Critical (7)	None	26	Mechanical ventilation (82%)	N/A	↑CRP, ↓ALC, ↑LDH, ↑D-dimer	Bilateral, multiple GGO	High-dose dexamethasone, tocilizumab, ribavirin, azithromycin, cefepime, vancomycin, voriconazole	Survival
13	3	Critical (8)	None	14	Mechanical ventilation (N/A)	5	N/A	N/A	High-dose dexamethasone, tocilizumab	Death
14	9	Critical (7)	Bacterial pneumonia, bacteremia, and sepsis	>60 days	Mechanical ventilation and ECMO (N/A)	42	↑CRP, ↑ferritin, ↓ALT, ↑AST,	Bilateral, multiple GGO	High-dose dexamethasone	Survival, tracheostomy

			(*Klebsiella*, *Serratia*, *Enterobacter*, *E. coli*), ventricular arrhythmia				↑D-dimer			
15	4	Moderate (4)	Pulmonary embolism	18	No	No	↑CRP, ↓ALC, ↑D-dimer	Bilateral GGO	High-dose hydrocortisone, remdesivir, azithromycin, ceftriaxone, apixaban	Survival
16	7	Critical (8)	Bacterial pneumonia (*Enterobacter*) pneumothorax	13	Mechanical ventilation (60%)	12	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑D-dimer	Bilateral, multiple GGO	High-dose dexamethasone	Death
17	2	Critical (7)	Transient diabetes insipidus	20	Mechanical ventilation (80%)	4	↑CRP, ↑AST, ↑IL-6	Bilateral, multiple GGO	High-dose betamethasone, plasmapheresis	Survival
18	2	Critical (7)	Hemoptysis	56	Mechanical ventilation (87%)	25	↑CRP, ↑ferritin, ↓ALC, ↑AST, ↑D-dimer	Bilateral, multiple GGO	High-dose dexamethasone, IFN-β, convalescent plasma, plasmapheresis	Survival
19	Not hospitalized	Mild (2)	None	0	No	No	Not tested	Not performed	Prolonged course of stress-dose steroids	Survival
20	5	Mild (4)	None	7	No	No	N/A	Bilateral GGO	IFN-β, convalescent plasma	Survival
21	5	Moderate-severe (5)	GI bleeding	21 days	Nasal cannula (87%)	No	↑CRP, ↓ALC, ↑LDH, ↑D-dimer,	Bilateral, multiple GGO	High-dose dexamethasone, tocilizumab, meropenem, fluconazole, IVIg	Survival
22	8	Moderate-severe (5)	None	6	Nasal cannula (89%)	No	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑ferritin	Bilateral, multiple GGO	High-dose dexamethasone, tofacitinib,	Survival

favipiravir, amoxicillin-clavulanic acid, IVIg

Table S1. Probes used for NanoString ISG analysis

Probes of interest (n = 24)	*IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1, CMPK2, DDX60, EPSTI1, FBXO39, HERC5, HES4, IFI44, IFI6, IFIH1, IRF7, LAMP3, LY6E, MX1, NRIR, OAS1, OASL, OTOF, SPATS2L*
Reference probes (n = 3)	*NRDC, OTUD5, TUBB*

+ Neutrophilic score

CEACAM6, CRISP3, DEFA4, LCN2, LTF, MMP8

Figure 1

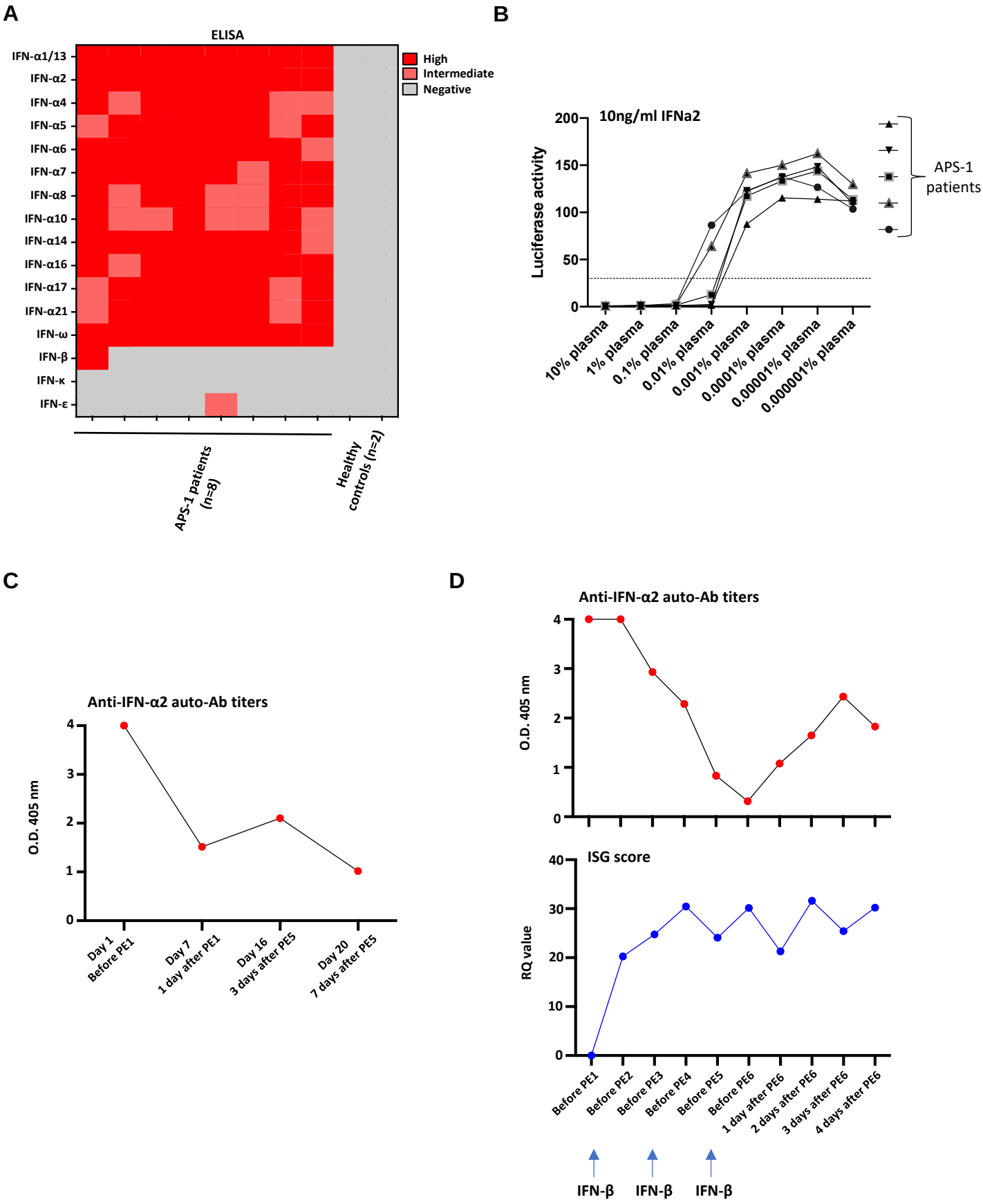
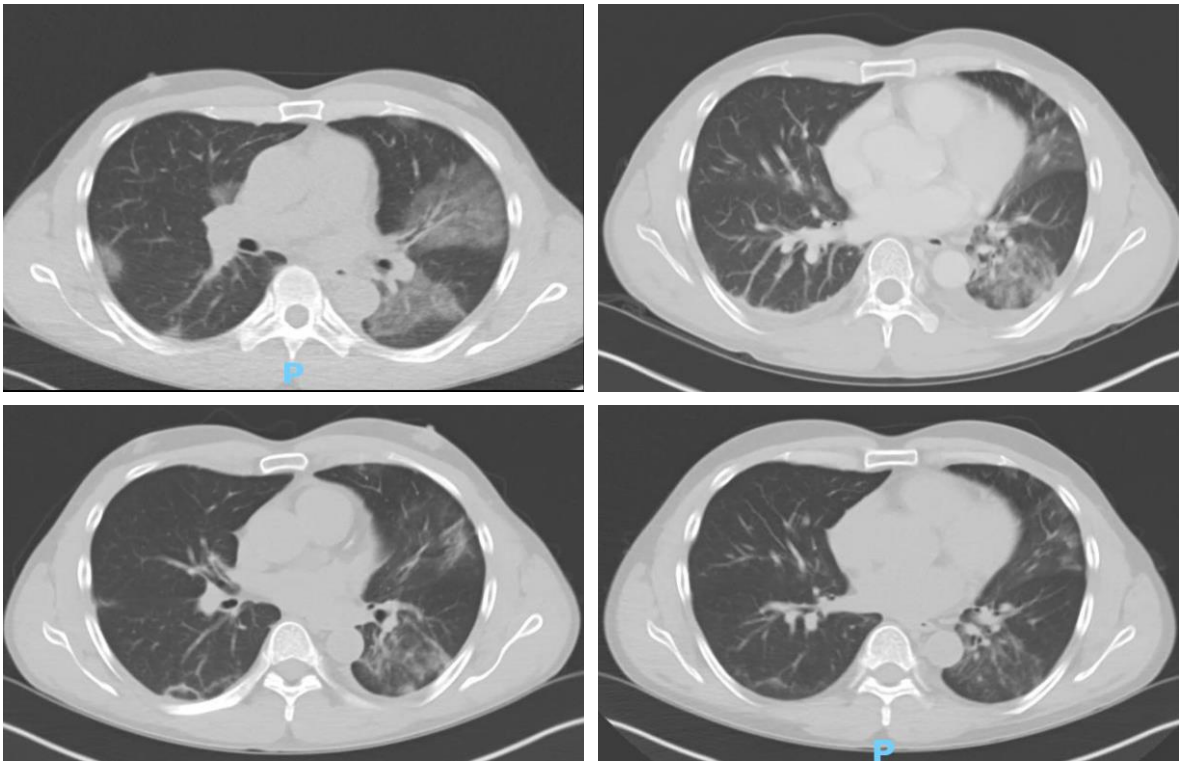
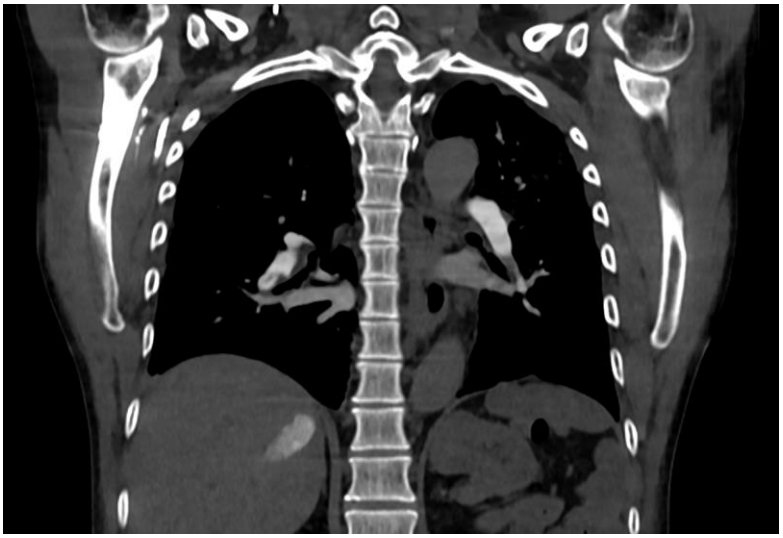

A
ELISA
IFN-α1/13
IFN-α2
IFN-α4
IFN-α5
IFN-α6
IFN-α7
IFN-α8
IFN-α10
IFN-α14
IFN-α16
IFN-α17
IFN-α21
IFN-ω
IFN-β
IFN-κ
IFN-ε
High
Intermediate
Negative
APS-1 patients (n=8)
Healthy controls (n=2)
B
10ng/ml IFNa2
Luciferase activity
200
150
100
50
0
10% plasma
1% plasma
0.1% plasma
0.01% plasma
0.001% plasma
0.0001% plasma
0.00001% plasma
0.000001% plasma
APS-1 patients
C
Anti-IFN-α2 auto-Ab titers
O.D. 405 nm
4
3
2
1
0
Day 1 Before PE1
Day 7 1 day after PE1
Day 16 3 days after PE5
Day 20 7 days after PE5
D
Anti-IFN-α2 auto-Ab titers
O.D. 405 nm
4
3
2
1
0
ISG score
RQ value
40
30
20
10
0
Before PE1
Before PE2
Before PE3
Before PE4
Before PE5
Before PE6
1 day after PE6
2 days after PE6
3 days after PE6
4 days after PE6
IFN-β
IFN-β
IFN-β

Figure S1

A



B



C

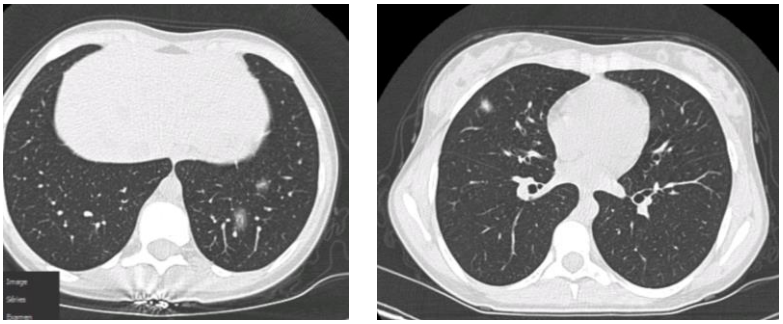


Figure S2

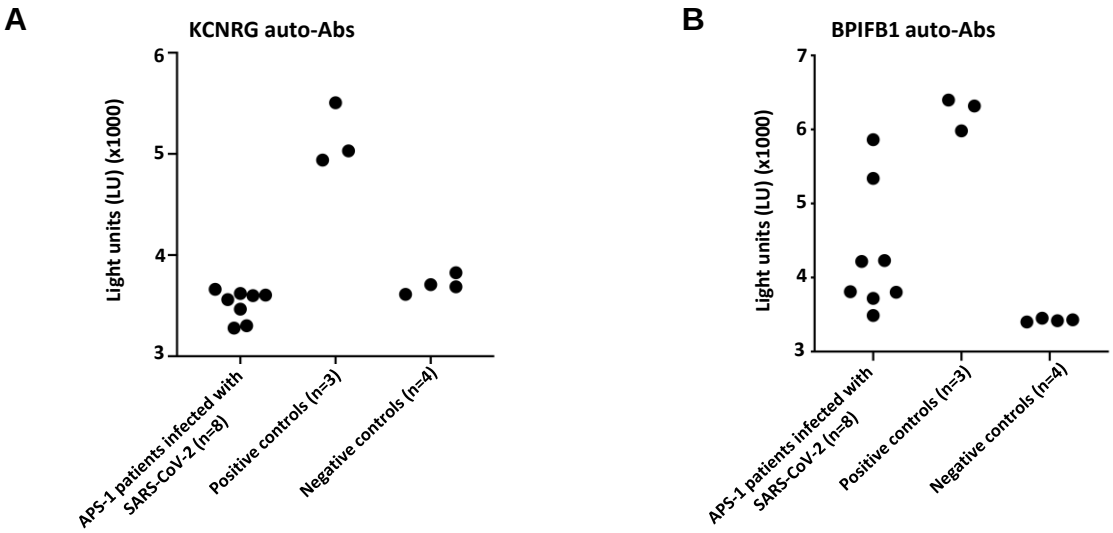
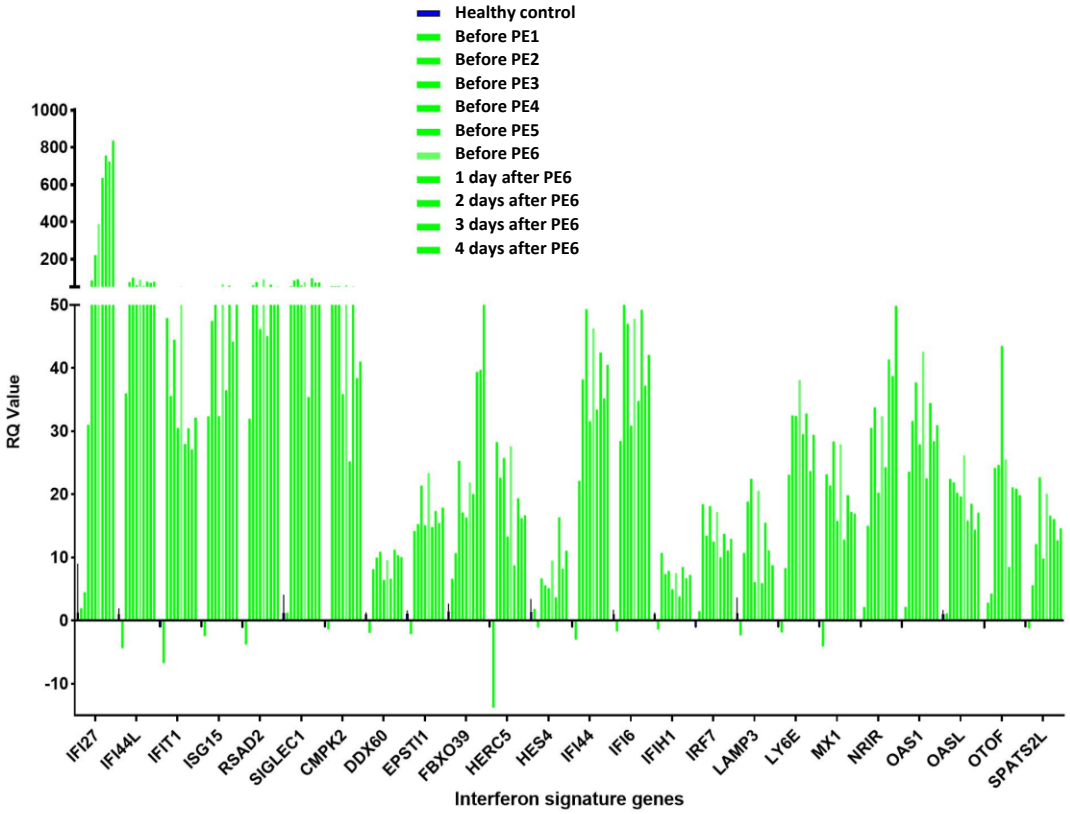
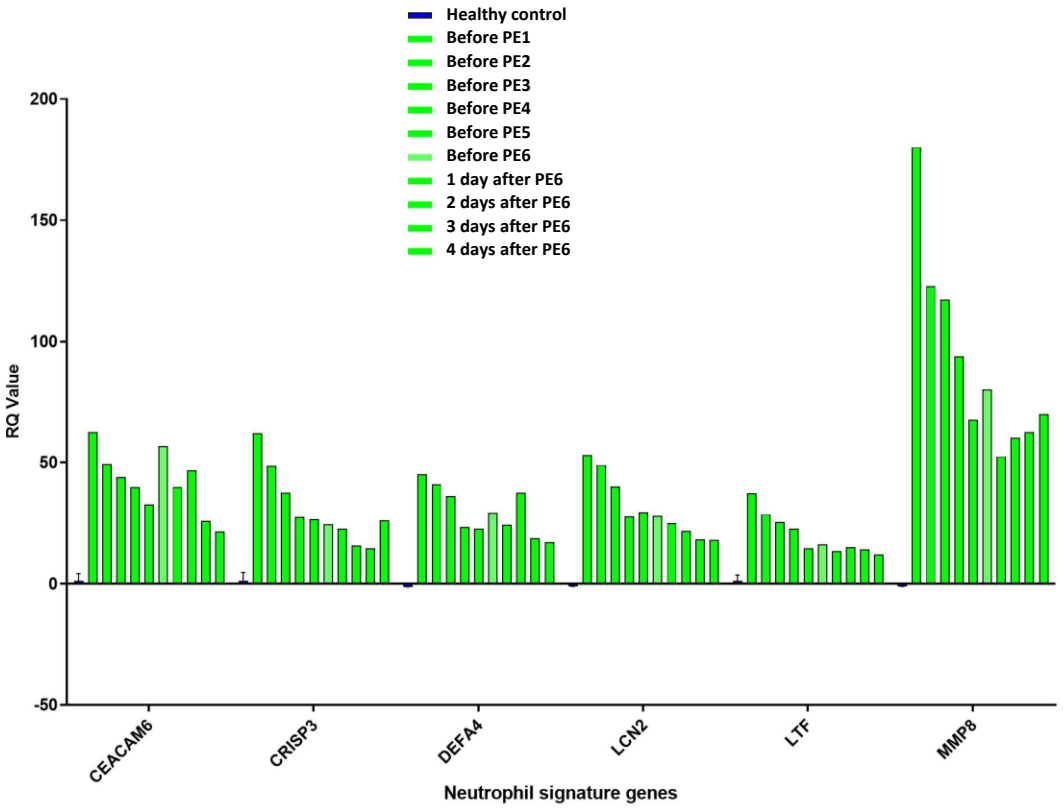

A
KCNRG auto-Abs
Light units (LU) (x1000)
6
5
4
3
APS-1 patients infected with SARS-CoV-2 (n=8)
Positive controls (n=3)
Negative controls (n=4)
B
BPIFB1 auto-Abs
Light units (LU) (x1000)
7
6
5
4
3
APS-1 patients infected with SARS-CoV-2 (n=8)
Positive controls (n=3)
Negative controls (n=4)

Figure S3

A



B



ISG score cut-off 2.758; Neutrophil score cut-off 6.088

Pre-existing autoantibodies to type I IFNs underlie critical COVID-19 pneumonia in patients with autoimmune polyendocrine syndrome type 1

Paul Bastard[1,2,3,@], Elizaveta Orlova[4], Leila Sozaeva[4], Romain Lévy[1,5], Alyssa James[6], Monica M. Schmitt[6], Sebastian Ochoa[6], Maria Kareva[4], Yulia Rodina[7], Adrian Gervais[1,2], Tom Le Voyer[1,2], Jérémie Rosain[1,2], Quentin Philippot[1,2], Anna-Lena Neehus[1,2], Elana Shaw[6], Mélanie Migaud[1], Lucy Bizien[1], Olov Ekwall[8,9], Stefan Berg[8], Guglielmo Beccuti[10], Lucia Ghizzoni[10], Gérard Thiriez[11], Arthur Pavot[12], Cécile Goujard[13], Marie-Louise Frémond[5,14], Edwin Carter[15], Anya Rothenbuhler[16], Agnès Linglard[16], Brigite Mignot[17], Aurélie Comte[17], Nathalie Cheik[18], Olivier Hermine[19], Lars Breivik[20], Eystein S. Husebye[20,21,22], Sébastien Humbert[23], Pierre Rohrlich[24], Alain Coaquette[25], Fanny Vuoto[26], Karine Faure[26], Nizar Mahlaoui[27], Primož Kotnik[28], Tadej Battelino[28], Katarina Trebušak Podkrajšek[29], Kai Kisand[30], Elise M. N. Ferré[6], Thomas DiMaggio[6], Lindsey B. Rosen[6], Peter D. Burbelo[31], Martin McIntyre[32], Nelli Y. Kann[7], Anna Shcherbina[7], Maria Pavlova[33], Anna Kolodkina[4], Steven M. Holland[6], Yanick Crow[14,15], Luigi D. Notarangelo[6], Helen C. Su[6], Laurent Abel[1,2,3], Mark S. Anderson[34], Emmanuelle Jouanguy[1,2,3], Bénédicte Neven[2,5], Anne Puel[1,2,3], Jean-Laurent Casanova[1,2,3,35,@] and Michail S. Lionakis[6,@]

1. Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, Paris, France
2. University of Paris, Imagine Institute, Paris, France
3. St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA
4. Endocrinology Research Centre, Moscow, Russia
5. Pediatric Hematology and Immunology Unit, Necker Hospital for Sick Children, AP-HP, Paris, France
6. Laboratory 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
7. Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia
8. Department of Pediatrics, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Sweden
9. Department of Rheumatology and Inflammation Research, Institute of Medicine, The Sahlgrenska Academy, University of Gothenburg, Sweden
10. Department of Medical Sciences, University of Turin, Turin, Italy
11. Intensive Care Unit, Besançon Hospital, Besançon, France
12. Intensive Care Unit, Kremlin-Bicêtre Hospital, Kremlin-Bicêtre, France
13. Internal medicine department, Bicêtre Hospital, Assistance Publique Hôpitaux de Paris, Paris Saclay University, Inserm U1018, Le Kremlin-Bicêtre, France
14. Laboratory of Neurogenetics and Neuroinflammation, Université de Paris, Imagine Institute, Paris, France
15. Centre for Genomic and Experimental Medicine, Medical Research Council Institute of Genetics and Molecular Medicine, Edinburgh, UK
16. Pediatric endocrinology department, Bicêtre Hospital, Assistance Publique Hôpitaux de Paris, Paris Saclay University, Le Kremlin-Bicêtre, France
17. Pediatric Medicine Unit, University Hospital of Besançon, Besançon, France
18. Pediatric Hematology Unit, University Hospital of Besançon, Besançon, France

19. Hematology department, University of Paris, Necker Hospital for Sick Children, AP-HP, Paris, France
20. Department of Clinical Science and K.G. Jebsen Center for Autoimmune Disorders, University of Bergen, Bergen, Norway.
21. Department of Medicine, Haukeland University Hospital, Bergen, Norway.
22. Department of Medicine (Solna), Karolinska Institutet, Stockholm, Sweden.
23. Internal Medicine Unit, Besançon Hospital, Besançon, France
24. Pediatric Hematology and Oncology unit, CHU de Nice, Nice, France
25. Laboratory of Virology, Besancon Hospital, Besançon, France
26. Infectious Disease Unit, Lille Hospital, Lille, France
27. Centre de Référence Déficits Immunitaires Héréditaires, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France.
28. Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
29. University Medical Centre Ljubljana, University Children's Hospital, Ljubljana, Slovenia
30. Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
31. National Institute of Dental and Craniofacial Research (NIDCR), Bethesda, MD, USA
32. Royal Alexandra Hospital, Paisley, Scotland, United Kingdom
33. Department of Endocrinology №1, Sechenov University, Moscow, Russia
34. Diabetes Center, University of California, San Francisco, California, USA
35. Howard Hughes Medical Institute, New York, NY, USA

@ corresponding authors
lionakism@mail.nih.gov; casanova@rockefeller.edu; paul.bastard@institutimagine.org

Abstract

Patients with biallelic loss-of-function variants of *AIRE* suffer from autoimmune polyendocrine syndrome type-1 (APS-1) and produce a broad range of autoantibodies (auto-Abs), including circulating auto-Abs neutralizing most type-I interferons (IFNs). These auto-Abs were recently reported to account for at least 10% of cases of life-threatening COVID-19 pneumonia in the general population. We report 22 APS-1 patients from 21 kindreds in seven countries, aged between 8 and 48 years and infected with SARS-CoV-2 since February 2020. The 21 patients tested had auto-Abs neutralizing IFN-α subtypes and/or IFN-ω, one had anti-IFN-β, another anti-IFN-ε, but none had anti-IFN-κ. Strikingly, nineteen patients (86%) were hospitalized for COVID-19 pneumonia, including fifteen (68%) admitted to an intensive care unit, eleven (50%) who required mechanical ventilation, and four (19%) who died. Ambulatory disease in three patients (14%) was possibly accounted for by prior or early specific interventions. Pre-existing auto-Abs neutralizing type-I IFNs in APS-1 patients confer a very high risk of life-threatening COVID-19 pneumonia at any age.

Short summary (40 words)

Patients with autoimmune polyendocrine syndrome type-1 (APS-1) have circulating auto-Abs neutralizing most type-I interferons. These auto-Abs can underlie life-threatening COVID-19 pneumonia in the general population. We report 22 APS-1 patients infected with SARS-CoV-2 including fifteen (68%) who developed life-threatening disease.

Running title (50 characters max)

Autoimmune polyendocrine syndrome type-1 and COVID-19

Abbreviation list

AIRE: Auto-immune regulator
APS-1: Autoimmune polyendocrine syndrome type-1,
APECED: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy,
IFNs: Interferons,
MMR: Measles-mumps-rubella,
CMC: Chronic mucocutaneous candidiasis
YFV: Yellow fever virus,
monoclonal antibody (mAb)
intravenous immunoglobulin (IVIg)
JAK: Janus Kinase
ICU: Intensive Care Unit
ALC: Absolute lymphocyte count,
AST: Aspartate transaminase
ECMO: Extracorporeal membrane oxygenation,
p02: Partial pressure of oxygen,
CT-scan: Computed tomography scan,
mmHg: Millimetre of mercury,
CRP: C-reactive protein,
LDH: Lactate deshydrogenase,
ISG: Interferon stimulated gene

Introduction

Autoimmune polyendocrine syndrome type 1 (APS-1), also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a monogenic inborn error of immunity typically caused by biallelic deleterious variants of the autoimmune regulator gene (*AIRE*)[1-5]. Heterozygous variants can also underlie autosomal dominant forms[6]. Patients with APS-1 have defective central T-cell tolerance, leading to the thymic escape of auto-reactive T cells and the development, from early childhood, of a broad range of autoantibodies (auto-Abs) against various autoantigens, including endocrine and other tissue antigens and cytokines[7-12]. Among these anti-cytokine auto-Abs, neutralizing auto-Abs against IL-17A and IL-17F phenocopy inborn errors of IL-17A/F and underlie chronic mucocutaneous candidiasis (CMC)[13-15]. High mucosal concentrations of IFN-γ were also proposed to contribute to CMC[16]. Virtually all patients with APS-1 produce auto-Abs against type I IFNs, generally against the 13 individual IFN-α subtypes and IFN-ω[11,12,17-19]. These auto-Abs were long thought to be clinically silent. This was surprising, as these auto-Abs are neutralizing and type I IFNs are potent antiviral molecules, acting through both innate immunity (via their secretion by plasmacytoid dendritic cells and other leukocytes) and cell-intrinsic immunity (in most cell types)[20-26]. Moreover, the essential role of type I IFNs in fending off viruses in humans was confirmed by the description of patients with autosomal recessive, complete IFNAR1 or IFNAR2 deficiency and adverse reactions to measles-mumps-rubella (MMR) vaccine or yellow fever virus (YFV-17D) live-attenuated viral vaccine[27-29], herpes simplex encephalitis[30], or critical COVID-19 pneumonia[30-32]. Nevertheless, the viral phenotype of these patients is not as broad as initially predicted, as neatly illustrated by two IFNAR1-deficient adults (26 and 38 years old) who had never been hospitalized for severe viral disease until they were admitted for critical COVID-19 pneumonia[31].

In this context, we and others recently reported that three unrelated patients with APS-1 had life-threatening COVID-19 pneumonia [33,34]. These cases suggested that the auto-Abs neutralizing type I IFN were pathogenic and contributed to the discovery that they can also underlie life-threatening COVID-19 pneumonia in previously healthy individuals without APS-1, accounting for at least 10% of the cases in an international cohort of patients[33]. These auto-Abs were more frequent in men (95%) than women (5%), and in elderly patients with critical COVID-19, half of the patients with auto-Abs being over the age of 65 years[33]. These auto-Abs typically neutralized the 13 individual IFN-α, or IFN-ω, or both, but only rarely IFN-β, -κ, and -ε. These findings were replicated in other cohorts[35-39]. Subjects with inborn errors of type I IFN immunity or neutralizing auto-Abs against type I IFN are, thus, at high risk of critical COVID-19 pneumonia, with impaired control of viral replication in the first few days of SARS-CoV-2 infection resulting in a secondary phase of pulmonary and systemic hyperinflammation[32]. Subjects with such auto-Abs are also at high risk of YFV-17D disease, with these antibodies accounting for three of the eight cases studied[40]. Interestingly, the three patients with YFV-17D disease had auto-Abs that neutralized both the 13 IFN-α and IFN-ω, and two also had auto-Abs against IFN-β, these proportions being higher than those reported for COVID-19 patients. It is unknown whether patients with APS-1 have ever been vaccinated with YFV-17D. Following on from the brief description of three APS-1 patients with critical COVID-19 pneumonia, we report here the immunological and clinical features of 22 patients with APS-1 during the course of SARS-CoV-2 infection.

Results

Baseline characteristics of the patients

The 22 patients studied were aged 8 to 48 years at the time of infection with SARS-CoV-2 (median: 24.5 years). Nine were male and 13 were female. Eight were children under the age of 16 years (Table 1). All had undergone vaccination according to the schedules in

force in their country of origin, including vaccination with the live attenuated MMR, with no overt adverse events, between the ages of one to two years. None of the patients had a previous history of severe viral infection, and only one had a history of pneumonia. The 22 patients came from 21 unrelated families (two patients were siblings). The patients originated from and lived in England (*n*=1), France (*n*=5), Italy (*n*=1), Russia (*n*=11), Scotland (*n*=1), Sweden (*n*=1), and the United States of America (*n*=2). Twenty-one of the 22 patients had a typical clinical diagnosis of APS-1 (i.e., any two of the classic triad of manifestations: hypoparathyroidism, adrenal insufficiency, and CMC), with confirmation of the presence of homozygous or compound heterozygous loss-of function variants of *AIRE* in the 17 tested patients. One 16-year-old patient with biallelic loss-of-function *AIRE* mutations (p.R257X) presented only CMC. Twenty-one of the 22 patients had a history of severe tissue auto-immunity, including hypoparathyroidism (*n*=20), adrenal insufficiency (*n*=20), hypogonadism (*n*=9), enteropathy (*n*=9), pernicious anemia (*n*=6), alopecia (*n*=6), autoimmune hepatitis (*n*=3), and vitiligo (*n*=3), and all but four had a history of CMC. One patient was on immunosuppressive treatment with the B cell-depleting monoclonal antibody (mAb) rituximab and monthly intravenous immunoglobulin (IVIg) substitution, another was on treatment with the JAK inhibitor ruxolitinib, a third patients was receiving treatment with the calcineurin inhibitor tacrolimus, and another two patients were on monthly IVIg treatment. Other treatments included endocrine replacement therapy (hydrocortisone and/or fludrocortisone, *n*=20; levothyroxine, *n*=5) and antifungal prophylaxis (fluconazole, *n*=8).

Clinical, radiographic, and laboratory characteristics of COVID-19 infection in 22 patients with APS-1, in chronological order

Patient 1 is a 32-year-old Italian woman with a history of the classic triad manifestations, enteropathy and pernicious anemia[34]. She was diagnosed with bilateral COVID-19 pneumonia in February 2020 and hospitalized for 37 days in Northern Italy. She developed hypoxemia requiring ICU admission and mechanical ventilation for six days. She developed secondary pneumococcal pneumonia and sepsis-induced ventricular dysfunction. She was treated with corticosteroids and broad-spectrum antibiotics. Two months after discharge from hospital, her pulmonary function was persistently impaired (i.e., diffusing capacity for carbon monoxide, 55%).

Patient 2 is a 35-year-old Scottish woman with a history of hypoparathyroidism, adrenal insufficiency and hypogonadism, but not CMC[33]. She was diagnosed with bilateral COVID-19 pneumonia in March 2020 and hospitalized for 12 days. She developed hypoxemia and was intubated and mechanically ventilated in the ICU for five days. She developed lymphopenia (ALC, 600/mm^3) and a mild increase in transaminase levels (AST, 89 U/L). She was treated with corticosteroids; she recovered and was discharged home.

Patient 3 is a 48-year-old American woman of Danish ancestry with a history of the classic triad manifestations, hypogonadism, hypothyroidism, and Sjögren's syndrome[33]. She was diagnosed with bilateral COVID-19 pneumonia and hospitalized in March 2020 for 17 days. She developed hypoxemia and was intubated in the ICU for 11 days. She developed lymphopenia (ALC, 650/mm^3), an increase in transaminase levels (AST, 1668 U/L), hyperferritinemia (14,679 µg/dL), and high D-dimer levels. She was treated with corticosteroids and broad-spectrum antibiotics; she recovered and was discharged home.

Patient 4 is a 21-year-old French man with a history of the classic triad manifestations, type 1 diabetes, asplenia, and myocarditis[14]. He was on monthly IVIg substitution at the time of infection. He developed a high fever and mild respiratory symptoms in May 2020. He was hospitalized, with radiographic evidence of mild pneumonia not requiring oxygen therapy.

Patient 5 is a 34-year-old Russian man with a history of hypoparathyroidism, adrenal insufficiency, and enteropathy, but no CMC. He was hospitalized for 10 days in January 2021

with bilateral COVID-19 pneumonia. He developed hypoxemia requiring oxygen supplementation, lymphopenia (ALC, 380/mm^3) and an increase in D-dimer levels. He received corticosteroids, tocilizumab, and broad-spectrum antibiotics, and made a full recovery.

Patient 6 is a 13-year-old Russian girl with a history of the classic triad manifestations, autoimmune hepatitis, and enteropathy. She was diagnosed with COVID-19 infection while asymptomatic during a SARS-CoV-2 PCR test performed for screening purposes before a routine clinic visit in July 2020. Interestingly, she had been on rituximab since October 2017, and was also receiving IVIg substitution (0.5 g/kg monthly).

Patient 7 was a 28-year-old Russian man with a history of the classic triad manifestations, enteropathy, and alopecia, who was hospitalized in the ICU for bilateral COVID-19 pneumonia in October 2020. He suffered severe hypoxemia requiring mechanical ventilation for four weeks, complicated by secondary bacterial sepsis, acute renal failure requiring hemodialysis, and two episodes of pneumothorax. He developed lymphopenia (ALC, 100/mm^3), high transaminase levels (ALT, 225 U/L), and high D-dimer levels. He was treated with broad-spectrum antibiotics, with the initiation of corticosteroids and tofacitinib three to eight days after the onset of hypoxemia. He died after 47 days in the hospital.

Patient 8 was a 32-year-old Russian woman with a history of the classic triad manifestations, autoimmune hepatitis, alopecia and a previous episode of pneumonia, which is seen in the setting of autoimmune pneumonitis in APS-1 patients[41]. She was hospitalized in the ICU of a hospital for COVID-19 for 15 days in October 2020. She developed severe hypoxemia requiring mechanical ventilation, and died of respiratory failure. She was treated with corticosteroids from day 13 of hospitalization, two days before her death.

Patient 9 is a 14-year-old Russian adolescent with a history of CMC, adrenal insufficiency, alopecia and type 1 diabetes. He was hospitalized for bilateral COVID-19 pneumonia for 15 days in October 2020. He developed hypoxemia requiring oxygen supplementation by a nasal cannula for six days. He recovered and was discharged home.

Patient 10 is an eight-year-old Russian girl with a history of the classic triad manifestations, enteropathy, autoimmune hepatitis and autoimmune encephalitis. She was receiving IVIg substitution (0.5 g/kg monthly) at the time of infection. She was diagnosed with asymptomatic COVID-19 following screening by serological SARS-CoV-2 IgG testing after her mother was diagnosed with mild COVID-19 infection.

Patient 11 is a 28-year-old Russian woman with a history of CMC, hypoparathyroidism, and enteropathy. She was hospitalized in October 2020 for 12 days for bilateral COVID-19 pneumonia not requiring oxygen supplementation. She developed lymphopenia (ALC, 190/mm^3). She recovered and was discharged home.

Patient 12 is a 16-year-old Russian man with a history of CMC and enamel hypoplasia. He was hospitalized for 26 days in October 2020 for bilateral COVID-19 pneumonia. He developed hypoxemia requiring ICU admission and mechanical ventilation. He developed lymphopenia (ALC, 600/mm^3) and his D-dimer levels increased. He was treated with corticosteroids, tocilizumab, and broad-spectrum antibiotics. He recovered and was discharged home.

Patient 13 was a 20-year-old Russian woman with a history of the classic triad manifestations and hypothyroidism. She was hospitalized for 14 days for COVID-19 pneumonia. She was already hypoxemic at admission and her hospital course was further complicated by worsening hypoxemia, requiring ICU admission and mechanical ventilation on day 9 of hospitalization. She was then treated with corticosteroids and tocilizumab, but died from respiratory failure five days after intubation.

Patient 14 is a 31-year-old French woman with a history of the classic triad manifestations, hypogonadism, and pernicious anemia. She was hospitalized for more than 60

days for COVID-19 pneumonia in November 2020. She developed hypoxemia requiring ICU admission, mechanical ventilation, and extracorporeal membrane oxygenation (ECMO). She suffered from multiple secondary bacterial infections, including pneumonia, bacteremia, and sepsis, and ventricular tachycardia. She developed mild increases in transaminase (AST, 77 U/L) and D-dimer levels. She was treated with corticosteroids. She survived but required tracheostomy and intensive respiratory rehabilitation due to persistent respiratory insufficiency.

Patient 15 is a 45-year-old American man of Danish ancestry, brother of Patient 3, with a history of the classic triad manifestations, enteropathy, alopecia, pernicious anemia, hypothyroidism, and end-stage renal disease on hemodialysis. He was hospitalized in November 2020 as a prophylactic measure, to facilitate close monitoring after his diagnosis with COVID-19 at an external facility. He was febrile upon admission, with mild respiratory symptoms, no hypoxemia, and bilateral pneumonia on imaging. He was treated with remdesivir and corticosteroids, while ruxolitinib was continued to prevent progression to hypoxemia and rebound inflammation. His hospital course was complicated by pulmonary embolism (Fig. S1B), which was treated with anticoagulation. He recovered without needing oxygen supplementation or ICU admission and was discharged home after an 18-day stay in hospital.

Patient 16 was a 38-year-old French woman with a history of the classic triad manifestations, hypogonadism, pernicious anemia, myocarditis, and cutaneous lupus. She was hospitalized for 13 days in November 2020. She developed hypoxemia requiring ICU admission and mechanical ventilation. She developed multiple bacterial superinfections and pneumothorax. She was treated with corticosteroids after intubation. She developed lymphopenia (ALC, 290/mm^3), and her transaminase (AST, 76 U/L) and D-dimer levels increased slightly. She died of respiratory failure after 12 days of intubation.

Patient 17 is an eight-year-old Swedish girl with a history of hypoparathyroidism, adrenal insufficiency, and vitiligo, but not CMC. She was hospitalized for bilateral COVID-19 pneumonia at the end of November 2020. She developed hypoxemia requiring ICU admission and mechanical ventilation for four days. She was treated with corticosteroids, plasmapheresis, which successfully decreased type I IFN auto-Ab titers (Fig. 1C), and IVIg substitution. She recovered and was discharged home after a 20-day stay in hospital.

Patient 18 is an 11-year-old French boy with the classic triad manifestations and hypothyroidism. He was hospitalized for 56 days in December 2020 for bilateral COVID-19 pneumonia. His course was complicated by hypoxemia requiring ICU admission and mechanical ventilation. He developed lymphopenia (ALC, 300/mm^3) and increases in D-dimer and transaminase (AST, 48 U/L) levels. He was receiving tacrolimus before COVID-19. He was treated with corticosteroids, IFN-β (45 μg, AVONEX, 3 injections), convalescent plasma, and plasmapheresis, which decreased type I IFN auto-Ab titers (Fig. 1D).

Patient 19 is an 18-year-old British man with a history of the classic triad manifestations, hypogonadism, type 1 diabetes, and alopecia. He was diagnosed with COVID-19 infection at the end of December 2020 after the diagnosis of his parents. He developed a high fever and mild cough and was instructed to initiate stress-dose corticosteroid treatment and to continue until the symptoms had completely resolved, to prevent secondary hyperinflammation. He remained at home without the need for hospitalization and recovered after seven days.

Patient 20 is a 15-year-old French girl with a history of the hypoparathyroidism, adrenal insufficiency, and hypogonadism, hypogonadism and retinitis. She had weekly methotrexate treatment for her retinitis. She was diagnosed with mild COVID-19 pneumonia in early January 2021. She had radiological evidence of bilateral COVID-19 pneumonia (Fig. S1C). After multidisciplinary discussion, she was hospitalized for treatment with three

injections of IFN-β (45 μg, AVONEX) and convalescent plasma therapy to prevent progression to hypoxemic COVID-19 pneumonia. She developed high fever for 72h and recovered without requiring oxygen supplementation and was discharged home.

Patient 21 is a 10-year-old Russian boy with a history of the classic triad manifestations, enteropathy, and retinitis. He was hospitalized for 24 days in January 2021 for bilateral COVID-19 pneumonia. He developed hypoxemia requiring oxygen supplementation by nasal cannula. He developed lymphopenia (ALC, 840/mm^3) and his D-dimer levels increased. He was treated with corticosteroids, tocilizumab, prophylactic anticoagulation, and broad-spectrum antibiotics. He recovered and was discharged home.

Patient 22 is a 30-year-old Russian woman with a history of hypoparathyroidism, adrenal insufficiency, and hypogonadism. She was hospitalized for six days in January 2021 for COVID-19 pneumonia. She developed hypoxemia requiring oxygen supplementation by a nasal cannula. She presented a mild increase in transaminase levels (ALT, 128 U/L). She received corticosteroids, tofacitinib, faripiravir, and prophylactic anticoagulation. She recovered and was discharged home.

Auto-Abs against type I IFNs in the patients

All the patients tested (*n*=21, P6 not tested) had high titers of neutralizing auto-Abs against IFN-α2 and/or IFN-ω, and one (P3) also had auto-Abs against IFN-β (Table 1). All patients but 2 had been tested for the auto-Abs before COVID-19 pandemic. We also tested for the presence of auto-Abs against the 17 individual type I IFNs for all patients for whom serum or plasma samples were available. Eight patients were tested for the presence of auto-Abs against all 13 individual IFN-α and IFN-ω, and they all tested positive (Figure 1A). Only one patient had auto-Abs against IFN-β and one other had auto-Abs against IFN-ε while none of the patients tested had auto-Abs against IFN-κ. We then confirmed that these auto-Abs had neutralizing activity (Fig. 1B), against IFN-α2 and IFN-ω in all patients, and against IFN-β in the only patient positive for auto-Abs against this cytokine. We could not test the neutralizing activity of the auto-Abs to IFN-ε. The serum and plasma samples from patients without detectable auto-Abs against IFN-β did not neutralize the activity of this cytokine. Pre- and post-COVID serum samples were available for 4 patients, and we found no significant differences in titer or neutralization capacity of anti-IFN auto-Abs before and after SARS-CoV-2 infection. We also tested for lung-targeted auto-Abs against the lung antigens KCNRG and BPIFB1 in 8 patients (5 severe and 3 mild/moderate)[41]. All examined patients were negative for KCNRG auto-Abs but two patients, one with severe (P17) and another with mild COVID-19 (P19), tested positive for BPIFB1 auto-Abs (Fig. S2).

Life-threatening COVID-19 pneumonia in 15 APS-1 patients

All 15 patients with hypoxemic COVID-19 pneumonia had positive SARS-CoV-2 PCR results. They had a median age of 30 years (range: 8-48 years). Six were male and nine were female (Tables 1 and 2). Five were children under the age of 16 years. The patients were admitted to hospital between 2 and 10 days after the onset of clinical manifestations (median: 5 days) and were hospitalized for a median of 16 days (range: 6-50 days). We applied the NIH ordinal scale (range: 1-8)[42] to assess the severity of COVID-19 in these patients. They were found to have a median ordinal scale score of 7 (range: 5-8). The degree of hypoxemia was variable, with a median nadir pO_2 of 82 mmHg (range: 60-93 mmHg). Eleven patients required intubation and mechanical ventilation for a median of six days (range: 1-27 days), and one patient required ECMO for 42 days. All patients had a chest CT-scan or X ray showing extensive bilateral ground-glass opacities due to severe COVID-19 pneumonia (Fig. S1A). Four patients suffered from bacterial superinfections, including ventilator-associated pneumonia, bacteremia, and sepsis. Two patients developed pneumothorax requiring chest

tube placement, twice in one patient, and ventricular tachycardia and sepsis-induced cardiomyopathy occurred in one patient each. One patient was discharged with a tracheostomy. All patients had high CRP levels, eight had lymphopenia, seven had high D-dimer levels, six had high transaminase levels, and four had high ferritin and LDH levels.

Managements of the 15 patients with life threatening COVID-19

Thirteen patients received high-dose corticosteroids (>0.5 mg/kg prednisone equivalent/day) in the form of dexamethasone, betamethasone, hydrocortisone, methylprednisolone, or prednisone (Table 2); all 10 patients given corticosteroids within 24 hours of the onset of hypoxemia survived, whereas all four patients receiving corticosteroids later in the course of their hypoxemic disease died (P=0.002; chi-squared test with Yates' correction). Six patients received broad-spectrum antibacterial antibiotics and three patients received antiviral treatment with faripiravir, ribavirin, or a combination of lopinavir/ritonavir with ribavirin. Four patients received anti-IL-6 receptor therapy (tocilizumab) and two patients received the JAK-inhibitor tofacitinib. One patient (P20) received convalescent plasma (twice, 24 hours apart) and intramuscular recombinant IFN-β (Avonex, 45 μg every 48 hours, three injections). Plasmapheresis was performed in two patients (daily, five times for P17 and six times for P18), resulting in a decrease in type I IFN auto-Ab titers in both (Fig. 1C, D). One patient (P18) also received 3 injections of intramuscular IFN-β as well as convalescent plasma, after the first three plasmapheresis sessions. We monitored the blood interferon stimulated gene (ISG) response in this patient using Nanostring. Interestingly, we found a clear increase of ISGs after the initiation of plasmapheresis and IFN-β treatment (Fig. 1D and S3). Four patients (18%) died from sepsis and/or respiratory failure. All the patients who died were adults (aged 20, 28, 32 and 38 years old). The 11 survivors, aged 8 to 48 years, have been discharged from hospital, including one patient suffering from chronic respiratory failure and still dependent on oxygen therapy at most recent follow-up.

Mild non-hypoxemic COVID-19 infection in seven APS-1 patients and the efficacy of early treatment in three of these patients

Seven of 22 patients (32%) had SARS-CoV-2 infection without developing hypoxemia (Tables 1 and 2). The median age of these patients was 18 years (range: 8-45 years). Three were male and four were female. Three were children under the age of 15 years. Interestingly, two of these patients were receiving monthly IVIg therapy at the time of infection; one remained asymptomatic and was treated as an outpatient whereas the other was hospitalized with a high fever and bilateral pneumonia but did not develop hypoxemia. Another patient with asymptomatic infection was receiving IVIg and had also received rituximab eight months before the diagnosis of COVID-19. Moreover, an American man on ruxolitinib treatment was admitted for prophylactic monitoring when he developed a high fever and pneumonia. Treatment with corticosteroids and a 10-day course of remdesivir were initiated in this patient, with the aim of preventing progression to hypoxemic COVID-19. In addition, a British patient harboring BPIFB1 auto-Abs recovered at home following the early initiation and prolonged administration of stress-dose corticosteroid therapy after the development of a high fever with symptoms of pneumonia. Finally, a French patient whose family was made aware of the risk of severe COVID-19 in APS-1 was hospitalized prophylactically two days after symptom onset, while presenting mild radiographic lesions on a chest CT scan (Fig. S1C). She was treated with subcutaneous recombinant IFN-β (Avonex, 45 μg dose every 48 hours, 3 doses) and convalescent plasma therapy for two consecutive days, with the goal of preventing progression to hypoxemic COVID-19. She recovered fully without the need for oxygen supplementation and was discharged home without sequelae.

Pre-existing auto-Abs to type I IFNs underlie life-threatening COVID-19 in APS-1 patients

We describe 22 patients with APS-1 from 21 kindreds from seven countries who were infected with SARS-CoV-2 between February 2020 and January 2021. Nineteen patients (86%) were hospitalized; 15 (68%) developed life-threatening bilateral COVID-19 pneumonia with hypoxemia requiring admission to an ICU, 11 of whom required mechanical ventilation, including five who developed life-threatening secondary complications such as sepsis, pneumothorax, arrhythmias and/or pulmonary embolism, and four of whom died (18%). As we do not know how many SARS-CoV-2-infected APS-1 patients there are worldwide and our series probably reflects an ascertainment bias, we cannot rigorously estimate the proportion of life-threatening cases. However, our findings strongly suggest that APS-1 patients are at very high risk of critical COVID-19 pneumonia. Our previous report of auto-Abs against type I IFNs in at least 10% of patients with critical COVID-19 pneumonia, and in none of the subjects with asymptomatic or benign SARS-CoV-2 infection tested[33] further suggests that APS-1 patients are at high risk of developing critical disease because of their neutralizing auto-Abs against type I IFNs. This very poor outcome seems to be independent of age, sex, European ancestry, and the nature of any other autoimmune manifestations. Importantly, our findings confirm that auto-Abs neutralizing type I IFNs present before SARS-CoV-2 infection, as opposed to other auto-Abs potentially triggered by this infection, confer a very high risk of critical COVID-19[31,33,36-39]. We also found similar levels of auto-Abs prior to and after COVID-19 in the patients tested, further suggesting that the infection does not significantly trigger their production.

Vaccination or early treatment to avoid life-threatening COVID-19 pneumonia

Patients with APS-1 should be prioritized for vaccination against COVID-19. In the meantime, all necessary measures should be taken to avoid infection. Our report of seven patients with SARS-CoV-2 infection following a mild or moderate, non-hypoxemic course is of interest in this respect. Three of these seven patients were on monthly IVIg treatment, which may have decreased the pathogenicity of the auto-Abs against type I IFNs or acted through other mechanisms. Consistently, one of these patients was also receiving rituximab at the time of COVID-19 diagnosis, which may have altered the nature or decreased the titer of auto-Abs against type I IFNs. In addition, three patients whose medical teams had been informed by us of the risk of critical COVID-19 were treated early in the course of infection, one with an early and prolonged course of stress-dose corticosteroids, another by prophylactic admission with the administration of corticosteroids and remdesivir, and the third by early administration of subcutaneous IFN-β. We thus recommend that infected patients should be hospitalized promptly. In patients diagnosed early, ideally before the development of pneumonia, several treatments may be considered. First, cocktails of mAb against the SARS-CoV-2 spike protein may be given to accelerate the decline in viral load[43,44]; these antibodies should be preferred over convalescent plasma, the composition of which is unknown, and which may also contain auto-Abs against type I IFNs or other detrimental components and have not shown efficacy in severe COVID-19 pneumonia[45]. Intramuscular or nebulized IFN-β, or subcutaneous pegylated-IFN-β, may also be considered in patients without auto-Abs against IFN-β[46], as successfully reported for intramuscular IFN-α2 in patients with inborn errors of type I IFN[47] and for IFN-β in a patient with incontinentia pigmenti and auto-Abs against type I IFNs[48]. Obviously, the administration of IFN-α2 is not indicated in APS-1 patients. In patients treated with IFN-β, a monitoring of anti-IFN-β auto-Abs will be important. In the small minority of APS-1 patients carrying auto-Abs against IFN-β, alternative options could be considered.

Rescue treatment in patients with APS-1 and life-threatening COVID-19

When patients present with hypoxemia in the later phase of COVID-19, the administration of mAbs against the SARS-CoV-2 spike protein and of IFN-β should be avoided, given the potential risk of worsening the hyperinflammation and hypoxemia[49,50]. In hypoxemic patients, the early initiation of high-dose corticosteroid treatment is crucial, to prevent a worsening of lung injury and death, as suggested by the observation that patients receiving high-dose corticosteroids at or within 24 hours of the onset of hypoxemia recovered, whereas the later initiation of corticosteroids was associated with death [51]. Indeed, two symptomatic patients without hypoxemic disease who received corticosteroids did not progress to severe disease, further suggesting that early corticosteroid treatment might prevent or attenuate the secondary hyperinflammatory phase of disease[32]. The prompt initiation of corticosteroid treatment is of particular importance in APS-1 patients with pre-existing autoimmune pneumonitis, a frequently overlooked manifestation of APS-1 that affects up to ~40% of patients[41], as the inflammation-prone lung tissue in these patients may confer a predisposition to a worsening of lung injury. Two of the eight patients tested here had auto-Abs against the lung auto-Ab BPIFB1. Such patients are often misdiagnosed as having a prior history of reactive airway disease or recurrent pneumonia[41]. Finally, both in the early phase of disease and after the development of COVID-19 pneumonia, plasmapheresis should be considered, as it has been safely performed in two APS-1 patients (this report) and four patients without APS-1[38]. This procedure can lower the titers of circulating auto-Abs against type I IFNs without lowering the titers of anti-viral Abs[38], and it may be more beneficial when performed early in the course of hospitalization.

No previous viral disease before severe COVID-19

None of the 22 APS-1 patients had previously suffered from severe viral infections, consistent with the history of most patients with APS-1[10]. By inference from our recent observation that auto-Abs against type I IFNs can underlie life-threatening disease due to the YFV-17D live attenuated virus vaccine[40], APS-1 patients should not be vaccinated against YFV. None of the 22 patients described here reported having been inoculated with the YFV-17D vaccine. It is striking that these and other APS-1 patients have not been reported to suffer from other severe viral infections, including MMR disease and herpes simplex virus encephalitis, which have been reported in patients with IFNAR1 or IFNAR2 deficiency[27-30]. This may reflect the residual activity of some of the 17 type I IFNs, including IFN-β in particular, or that at the age of vaccination or HSV-1 infection, the auto-Abs were not yet present, or not as potent, or did not target all the type I IFNs neutralized in older APS-1 patients. There is, nevertheless, one case report of an APS-1 patient suffering from recurrent cutaneous HSV-1 infection[52]. The paucity of viral infections in patients with inherited IFNAR1 or IFNAR2 deficiency is, itself, intriguing[27,28,30,32,53]. Careful retrospective and prospective studies of viral infections and viral diseases in APS-1 patients are therefore warranted. More generally, a careful study of viral infections and viral diseases in patients with inherited IFNAR1 or IFNAR2 deficiency, and in patients with auto-Abs against type I IFNs, regardless of their etiology, is also warranted.

Acknowledgments

We thank the patients and their families for placing their trust in us. We warmly thank the members of both branches of the Laboratory of Human Genetics of Infectious Diseases. We warmly thank Y. Nemirovskaya, M. Woollet, D. Liu, S. Boucherit, C. Rivalain, M. Chrabieh and L. Lorenzo for administrative 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) (R01AI088364), 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 GSP Coordinating Center funded by the National Human Genome Research Institute (NHGRI) (UM1HG006504 and U24HG008956), the Yale High Performance Computing Center (S10OD018521), the Fisher Center for Alzheimer's Research Foundation, the Meyer Foundation, the French National Research Agency (ANR) under the "Investments for the Future" program (ANR-10-IAHU-01), the Integrative Biology of Emerging Infectious Diseases Laboratory of Excellence (ANR-10-LABX-62-IBEID), the ANR project AABIFNCOV (ANR-20-CO11-0001), the French Foundation for Medical Research (FRM) (EQU201903007798), the FRM and ANR GENCOVID project, ANRS-COV05, the Square Foundation, *Grandir - Fonds de solidarité pour l'enfance*, the Fondation du Souffle, the SCOR Corporate Foundation for Science, Institut National de la Santé et de la Recherche Médicale (INSERM) and the University of Paris. This work was further supported in part by the Intramural Research Program of the NIAID and NIDCR, NIH. The work was also supported in part by the Russian Science Foundation (project number 17-75-30035). PB was supported by the French Foundation for Medical Research (FRM, EA20170638020). PB and TLV were supported by the MD-PhD program of the Imagine Institute (with the support of the Fondation Bettencourt-Schueller). ALN was supported by the Foundation Bettencourt-Schueller and the International PhD program of the Imagine Institute. The content is the manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the any of the funding sources.

Author contributions:

PB, EO, LS, RL, AJ, MMS, SO, MK, YR, AG, TLV, JR, QP, ALN, ES, MM, LB, OE, SB, GB, LG, GT, AP, CG, MLF, EC, AR, AL, BM, AC, NC, OH, LB, ESH, SH, PR, AC, FV, KF, NM, PK, TB, KTP, KK, EMNF, TD, LBR, PDB, MM, NYK, AS, MP, SMH, YC, LDN, HCS, LA, MSA, EJ, BN, AP, collected the clinical data, recruited and/or treated the patients. MSL and JLC supervised the project. PB, JLC and MSL wrote the manuscript. All the authors edited the manuscript.

Conflict of interest statement: The authors have no competing financial interests to declare.

Methods

Patients and study approval

Written informed consent was obtained from patients or their parents in the country in which they were followed, in accordance with local regulations. The study was approved by the institutional review boards of The Rockefeller University and *Institut National de la Santé et de la Recherche Médicale* (INSERM), the NIAID/NIH, the Endocrinology Research Center of Russia, and the University of Gothenburg, Sweden. Experiments were conducted in the United States of America and France, in accordance with local regulations and with the approval of the institutional review boards of The Rockefeller University, NIAID/NIH and INSERM. Anonymized samples were studied at the NIAID under non-human subject research conditions; no additional IRB consent was required at the NIH. APS-1 patients gave consent under IRB-approved protocols 11-I-0187 (clinicaltrials.gov NCT01386437) at the NIAID/NIH, study no 779-11. The Swedish patient was enrolled in study no 779-11,

approved by the Central Ethical Review Board at the University of Gothenburg. The study has been approved by the local ethics committee at Endocrinology Research Center of Russia (Protocol №11 from 23.10.2013) and all patients or their parents or guardians singed the informed consent.

Detection of anti-cytokine auto-Abs using a cell-based assay

All Russian patients were tested for neutralizing auto-Abs against IFN-α2 and/or IFN-ω using a cell-based assay as previously described[12,54].

Detection of anti-cytokine auto-Abs in a multiplex particle-based assay

Serum/plasma samples were screened for auto-Abs against IFN-α2 and IFN-ω targets in a multiplex particle-based assay, in which magnetic beads with differential fluorescence were covalently coupled to recombinant human proteins (2.5 μg/reaction). Beads were combined and incubated with 1:100 diluted serum/plasma samples for 30 minutes. Each sample was tested once. The beads were then washed and incubated with PE-labeled goat anti-human IgG antibody (1 μg/mL) for 30 minutes. They were washed again and used in a multiplex assay run on a BioPlex X200 instrument. Patients with a fluorescence intensity (FI) > 1500 for IFN-α2 or IFN-β, or > 1000 for IFN-ω were tested for blocking activity.

Enzyme-linked immunosorbent assays (ELISA) for anti-cytokine auto-Abs

ELISA was performed as previously described[33]. In brief, 96-well ELISA plates (MaxiSorp; Thermo Fisher Scientific) were coated by incubation overnight at 4°C with 2 μg/mL rhIFN-α, and rhIFN-ω (R&D Systems). Plates were then washed (PBS/0.005% Tween), blocked by incubation with 5% nonfat milk powder in the same buffer, washed, and incubated with 1:50 dilutions of plasma from the patients or controls for 2 h at room temperature (or with specific mAbs as positive controls). Each sample was tested once. Plates were thoroughly washed. Horseradish peroxidase (HRP)–conjugated Fc-specific IgG fractions from polyclonal goat antiserum against human IgG or IgA (Nordic Immunological Laboratories) were added to a final concentration of 2 μg/mL. Plates were incubated for 1 h at room temperature and washed. Substrate was added and the optical density (OD) was measured. A similar protocol was used to test for antibodies against 12 subtypes of IFN-α, except that the plates were coated with cytokines from PBL Assay Science (catalog #11002-1).

Functional evaluation of anti-cytokine auto-Abs

The blocking activity of auto-Abs against IFN-α2 and IFN-ω was assessed by evaluating STAT1 phosphorylation in healthy control cells following stimulation with the appropriate cytokines in the presence of 10% serum/plasma from a healthy control or a patient. Surface-stained healthy control PBMCs (350,000/reaction) were cultured in serum-free RPMI medium supplemented with 10% healthy control or patient serum/plasma and were either left unstimulated or were stimulated with IFN-α2 and IFN-ω (10 ng/mL) for 15 minutes at 37°C. Each sample was tested once. Cells were fixed, permeabilized, and stained for intranuclear phospho-STAT1 (Y701). Cells were acquired on a BD LSRFortessa cytometer with gating on $CD14^+$ monocytes and analyzed with FlowJo software.

Luciferase immunoprecipitation systems (LIPS) assay for lung-targeted auto-Abs

We used the LIPS immunoassay to detect auto-Ab immunoreactivity against the lung targeting the potassium regulator KCNRG and bactericidal/permeability-increasing fold-containing B1 (BPIFB1) in APS-1 patient sera. Seropositivity was defined as a value greater than the mean for healthy donors plus three standard deviations, as previously described[41].

IFN score[55]

Total RNA was extracted from whole blood with a PAXgene (PreAnalytix) RNA isolation kit. RNA concentration was assessed with a spectrophotometer (FLUOstar Omega, Labtech). Analysis of 24 genes and 3 housekeeping genes was conducted using the NanoString customer designed CodeSets according to the manufacturer's recommendations (NanoString Technologies, Seattle, WA). Agilent Tapestation was used to assess the quality of the RNA. 100ng of total RNA was loaded for each sample. Data were processed with nSolver software (NanoString Technologies Seattle, WA). The data was normalized relative to the internal positive and negative calibrators, the 3 reference probes and the control samples. The median of the 24 probes for each of 27 healthy control samples was calculated. The mean NanoString score of the 27 healthy controls +2SD of the mean was calculated. Scores above this value (>2.724) were designated as positive. The list of probes used in NanoString ISG analysis is supplied in Table S1.

Online supplemental material

Table S1 provides additional data on the probes used in the NanoString ISG analysis. **Figure S1** provides radiological images of COVID-19 in the patients. **Figure S2** shows the auto-Ab result for lung-targeted auto-Abs (KCNRG and BPIFB1). **Figure S3** shows the ISGs used in the Nanostring, at the different time-points as well as the neutrophil score.

Table legends

Table 1: Baseline demographic, genetic and clinical characteristics of the 22 APS-1 patients with SARS-CoV-2 infection included in this study

AIRE, autoimmune regulator; APS-1 autoimmune polyglandular syndrome type 1; AI, adrenal insufficiency; HP, hypoparathyroidism, CMC, chronic mucocutaneous candidiasis; HT, hypothyroidism; PA, pernicious anemia; DM, diabetes mellitus; PTH, parathyroid hormone; HRT, hormone replacement therapy; NT, not tested; F, female; M, male; IFN, interferon.

Table 2: Clinical features of 22 APS-1 patients with SARS-CoV-2 infection

EF, ejection fraction; DLCO, diffusing capacity for carbon monoxide; LOP/RIT, lopinavir/ritonavir; HCQ, hydroxychloroquine; GGO, ground-glass opacities; CRP, C-reactive protein; AST, aspartate aminotransferase; ALC, absolute lymphocyte count; ECMO, extracorporeal membrane oxygenation; GI, gastrointestinal; N/A, not available. *Hypoxemia defined as SpO2 <94 mmHg.

Figure legends

Figure 1: APS-1 patients have neutralizing auto-Abs against type I IFNs, the titers of which can be decreased by plasmapheresis. (A) Titers of auto-Ab titers against the 17 type I IFNs in APS-1 patients infected with SARS-CoV-2 (n=8). **(B)** Neutralization of IFN-α2 by various dilutions of auto-Ab-containing serum from APS-1 patients with COVID-19 (*n*=5). **(C)** Plasmapheresis (PE) decreased the titers of type I IFN auto-Abs in one APS-1 patient (P17) with COVID-19 pneumonia. The titers of auto-Abs against IFN-α2 are shown for one of the APS-1 patients treated by plasmapheresis. **(D)** Plasmapheresis (PE) decreased the titers of type I IFN auto-Abs in another APS-1 patient (P18) with COVID-19 pneumonia, treated with plasmapheresis, convalescent plasma and IFN-β (as shown with arrows). The titers of auto-Abs against IFN-α2 are shown for the APS-1 patients treated by plasmapheresis

in the upper panel. In the lower panel, ISG scores -evaluated by Nanostring- show an increase after the initiation of treatments. ISG score cut-off for positivity is 2,758.

Figure S1: Imaging of COVID-19 pneumonia in APS-1 patients. (A) Course of COVID-19 pneumonia in an APS-1 patient. Bilateral (left>right) ground glass opacities are seen on initial chest CT six days after symptom onset (left upper panel). Persistence of bilateral ground glass opacities with a worsening of radiographic signs in the left lung base on day 10 after symptom onset (right upper panel). Improvement of ground glass opacities on days 16 (left lower panel) and 37 (right lower panel) after symptom onset. **(B)** Coronal chest CT angiogram demonstrating non-occlusive segmental pulmonary embolus to the distal pulmonary arterial branches of the right lower lobe. **(C)** Chest CT scan of an APS-1 patient showing bilateral alveolo-interstitial lesions of COVID-19 pneumonia.

Figure S2: Analysis of lung-targeting auto-Abs against KCNRG and BPIFB1 in APS-1 patients with COVID-19. Auto-Ab titers to KCNRG **(A)** and BPIFB1 **(B)** in APS-1 patients with COVID-19 (n=8). Positive and negative control sample results are also shown.

Figure S3: ISG score and neutrophil score at different time points in an APS-1 patient, with severe COVID-19, and treated with plasmapheresis, convalescent plasma and IFN-β. (A) 24 ISGs are shown at each time point, and **(B)** 6 neutrophil signature genes are shown. ISG score are higher during treatment, while the neutrophile score diminishes.

References

1. Nagamine K, Peterson P, Scott HS, et al. Positional cloning of the APECED gene. Nat Genet 1997;17:393-8.
2. Finnish-German AC. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 1997;17:399-403.
3. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 1990;322:1829-36.
4. Ferre EM, Rose SR, Rosenzweig SD, et al. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. JCI Insight 2016;1.
5. Husebye ES, Anderson MS, Kampe O. Autoimmune Polyendocrine Syndromes. N Engl J Med 2018;378:2543-4.
6. Oftedal BE, Hellesen A, Erichsen MM, et al. Dominant Mutations in the Autoimmune Regulator AIRE Are Associated with Common Organ-Specific Autoimmune Diseases. Immunity 2015;42:1185-96.
7. Chan AY, Anderson MS. Central tolerance to self revealed by the autoimmune regulator. Ann N Y Acad Sci 2015;1356:80-9.
8. Proekt I, Miller CN, Lionakis MS, Anderson MS. Insights into immune tolerance from AIRE deficiency. Curr Opin Immunol 2017;49:71-8.
9. Guo CJ, Leung PSC, Zhang W, Ma X, Gershwin ME. The immunobiology and clinical features of type 1 autoimmune polyglandular syndrome (APS-1). Autoimmun Rev 2018;17:78-85.
10. Constantine GM, Lionakis MS. Lessons from primary immunodeficiencies: Autoimmune regulator and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Immunol Rev 2019;287:103-20.

11. Bruserud O, Oftedal BE, Landegren N, et al. A Longitudinal Follow-up of Autoimmune Polyendocrine Syndrome Type 1. J Clin Endocrinol Metab 2016;101:2975-83.
12. Orlova EM, Sozaeva LS, Kareva MA, et al. Expanding the Phenotypic and Genotypic Landscape of Autoimmune Polyendocrine Syndrome Type 1. J Clin Endocrinol Metab 2017;102:3546-56.
13. Kisand K, Boe Wolff AS, Podkrajsek KT, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 2010;207:299-308.
14. Puel A, Doffinger R, Natividad A, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 2010;207:291-7.
15. Puel A, Cypowyj S, Bustamante J, et al. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 2011;332:65-8.
16. Break TJ, Oikonomou V, Dutzan N, et al. Aberrant type 1 immunity drives susceptibility to mucosal fungal infections. Science 2021;371.
17. Meager A, Visvalingam K, Peterson P, et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 2006;3:e289.
18. Levin M. Anti-interferon auto-antibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 2006;3:e292.
19. Meyer S, Woodward M, Hertel C, et al. AIRE-Deficient Patients Harbor Unique High-Affinity Disease-Ameliorating Autoantibodies. Cell 2016;166:582-95.
20. Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 1957;147:258-67.
21. Isaacs A, Lindenmann J, Valentine RC. Virus interference. II. Some properties of interferon. Proc R Soc Lond B Biol Sci 1957;147:268-73.
22. Gresser I. Wherefore interferon? J Leukoc Biol 1997;61:567-74.
23. Hoffmann HH, Schneider WM, Rice CM. Interferons and viruses: an evolutionary arms race of molecular interactions. Trends Immunol 2015;36:124-38.
24. Lazear HM, Schoggins JW, Diamond MS. Shared and Distinct Functions of Type I and Type III Interferons. Immunity 2019;50:907-23.
25. Jing H, Su HC. New immunodeficiency syndromes that help us understand the IFN-mediated antiviral immune response. Curr Opin Pediatr 2019;31:815-20.
26. Duncan CJA, Randall RE, Hambleton S. Genetic Lesions of Type I Interferon Signalling in Human Antiviral Immunity. Trends Genet 2021;37:46-58.
27. Duncan CJ, Mohamad SM, Young DF, et al. Human IFNAR2 deficiency: Lessons for antiviral immunity. Sci Transl Med 2015;7:307ra154.
28. Hernandez N, Bucciol G, Moens L, et al. Inherited IFNAR1 deficiency in otherwise healthy patients with adverse reaction to measles and yellow fever live vaccines. J Exp Med 2019;216:2057-70.
29. Gothe F, Hatton CF, Truong L, et al. A novel case of homozygous IFNAR1 deficiency with haemophagocytic lymphohistiocytosis. Clin Infect Dis 2020.
30. Bastard P, Manry J, Chen J, et al. Herpes simplex encephalitis in a patient with a distinctive form of inherited IFNAR1 deficiency. J Clin Invest 2020;131(1).
31. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science 2020;370.
32. Zhang Q, Bastard P, Bolze A, et al. Life-Threatening COVID-19: Defective Interferons Unleash Excessive Inflammation. Med (N Y) 2020;1:14-20.
33. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020;370.

34. Beccuti G, Ghizzoni L, Cambria V, et al. A COVID-19 pneumonia case report of autoimmune polyendocrine syndrome type 1 in Lombardy, Italy: letter to the editor. J Endocrinol Invest 2020;43:1175-7.
35. Wang EY, Mao T, Klein J, et al. Diverse Functional Autoantibodies in Patients with COVID-19. medRxiv 2020.
36. Wijst MGPvd, Vazquez SE, Hartoularos GC, et al. Longitudinal single-cell epitope and RNA-sequencing reveals the immunological impact of type 1 interferon autoantibodies in critical COVID-19. Submitted
37. Jesús Troya García, Bastard P, Planas-Serra L, et al. Neutralizing autoantibodies to type I IFNs in >10% of patients with severe COVID-19 pneumonia hospitalized in Madrid, Spain. Submitted.
38. de Prost N, Bastard P, Arrestier R, et al. Plasma Exchange to Rescue Patients with Autoantibodies Against Type I Interferons and Life-Threatening COVID-19 Pneumonia. J Clin Immunol 2021.
39. Rutger Koning Paul Bastard SdB, Amsterdam UMC Covid-19 Biobank, Jean-Laurent Casanova, Alexander P.J. Vlaar, Matthijs C. Brouwer, Diederik van de Beek. Autoantibodies against type I interferons are associated with multi-organ failure in COVID-19 patients. Intensive Care Medicine 2021;In Press.
40. Bastard P, Michailidis E, Hoffmann HH, et al. Auto-antibodies to type I IFNs can underlie adverse reactions to yellow fever live attenuated vaccine. J Exp Med 2021;218.
41. Ferre EMN, Break TJ, Burbelo PD, et al. Lymphocyte-driven regional immunopathology in pneumonitis caused by impaired central immune tolerance. Sci Transl Med 2019;11.
42. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med 2020;383:1813-26.
43. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. N Engl J Med 2021;384:229-37.
44. Weinreich DM, Sivapalasingam S, Norton T, et al. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. N Engl J Med 2021;384:238-51.
45. Simonovich VA, Burgos Pratx LD, Scibona P, et al. A Randomized Trial of Convalescent Plasma in Covid-19 Severe Pneumonia. N Engl J Med 2021;384:619-29.
46. Monk PD, Marsden RJ, Tear VJ, et al. Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Respir Med 2021;9:196-206.
47. Levy R, Bastard P, Lanternier F, Lecuit M, Zhang SY, Casanova JL. IFN-alpha2a Therapy in Two Patients with Inborn Errors of TLR3 and IRF3 Infected with SARS-CoV-2. J Clin Immunol 2021:26–7.
48. Bastard P, Levy R, Henriquez S, Bodemer C, Szwebel TA, Casanova JL. Interferon-beta Therapy in a Patient with Incontinentia Pigmenti and Autoantibodies against Type I IFNs Infected with SARS-CoV-2. J Clin Immunol 2021.
49. Consortium WHOST, Pan H, Peto R, et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med 2020.
50. Hung IF, Lung KC, Tso EY, et al. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet 2020;395:1695-704.
51. Group RC, Horby P, Lim WS, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. N Engl J Med 2020.
52. Nagafuchi S, Umene K, Yamanaka F, et al. Recurrent herpes simplex virus infection in a patient with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy

associated with L29P and IVS9-1G>C compound heterozygous autoimmune regulator gene mutations. J Intern Med 2007;261:605-10.
53. Meyts I, Casanova JL. Viral infections in humans and mice with genetic deficiencies of the type I IFN response pathway. Eur J Immunol 2021.
54. Breivik L, Oftedal BE, Boe Wolff AS, Bratland E, Orlova EM, Husebye ES. A novel cell-based assay for measuring neutralizing autoantibodies against type I interferons in patients with autoimmune polyendocrine syndrome type 1. Clin Immunol 2014;153:220-7.
55. Rice GI, Forte GM, Szynkiewicz M, et al. Assessment of interferon-related biomarkers in Aicardi-Goutieres syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. Lancet Neurol 2013;12:1159-69.

Table 1: Baseline demographic, genetic and clinical characteristics of the 22 APS-1 patients with SARS-CoV-2 infection included in the study

Patient #	Ancestry/residence	Age	Sex	*AIRE* variants	IFN-α, IFN-β, IFN-ω auto-Ab positivity	Prior non-infectious clinical manifestations	Prior infections	Treatments at the time of SARS-CoV-2 infection diagnosis
1	European/Italy	32	F	R203X/R203X	IFN-α and IFN-ω positive	AI, HP, ectodermal dystrophy, hypogonadism PA, enteropathy	CMC	Hydrocortisone, fludrocortisone, calcium, iron, magnesium, vitamin B12, folic acid, cholecalciferol, PTH, HRT, mesalasine, pancreatic enzyme replacement therapy, rifaximin
2	European/Scotland	35	F	L323SfsX51/ L323SfsX51	IFN-α and IFN-ω positive	AI, HP, hypogonadism, gastroparesis	None	Hydrocortisone, fludrocortisone, estrogen, PTH
3	European/USA	48	F	L323SfsX51/ S64TfsX71	IFN-α, IFN-β, and IFN-ω positive	AI, HP, HT, hypogonadism, Sjögren's syndrome	CMC	Hydrocortisone, fludrocortisone, calcitriol, levothyroxine, HRT
4	European/France	21	M	R257X/P539L	IFN-α and IFN-ω positive	AI, HP, enteropathy, DM, HT, asplenia, exocrine pancreatic insufficiency, myocarditis	CMC	Hydrocortisone, fludrocortisone, levothyroxine, insulin, fluconazole, trimethoprim/sulfamethoxazole, pancreatic enzymes, monthly IVIg

5	European/Russia	34	M	NT	IFN-ω positive	AI, HP, enteropathy, nail dystrophy	None	Hydrocortisone, fludrocortisone, calcitriol
6	European/Russia	13	F	R257X/ R257X	NT	AI, HP, autoimmune hepatitis, enteropathy, pancreatitis, nephritis	CMC	Hydrocortisone, fludrocortisone, calcium, amlodipine, enalapril, rituximab (treatment initiation in October 2017 with re-dosing every 6 months; last dose 8 months prior to SARS-CoV-2 infection diagnosis), monthly IVig
7	European/Russia	28	M	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, enteropathy, alopecia, ptosis, enamel dysplasia	CMC	Hydrocortisone, fludrocortisone, calcitriol, fluconazole
8	European/Russia	32	F	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, hypogonadism, enteropathy, autoimmune hepatitis, alopecia, vitiligo, asplenia, Sjögren's syndrome, PA, deep vein thrombosis, ptosis, enamel dysplasia, cataract	CMC, pneumonia	Hydrocortisone, fludrocortisone, calcium carbonate, alfacalcidol, fluconazole, rivaroxaban

9	European/Russia	14	M	R257X/R257X	IFN-α and IFN-ω positive	AI, DM, alopecia, enamel dysplasia, asthma	CMC	Hydrocortisone, fludrocortisone, fluconazole
10	European/Russia	8	F	R257X/E298X	IFN-α and IFN-ω positive	AI, HP, enteropathy, alopecia, PA, autoimmune hepatitis, autoimmune encephalitis	CMC	Hydrocortisone, fludrocortisone, calcitriol, fluconazole, monthly IVIg
11	European/Russia	28	F	R257X/R257X	IFN-α and IFN-ω positive	HP	CMC	Alfacalcidol, fluconazole
12	European/Russia	16	M	R257X/R257X	IFN-α and IFN-ω positive	enamel hypoplasia	CMC	Fluconazole
13	European/Russia	20	F	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, hypogonadism, HT	CMC	Hydrocortisone, fludrocortisone, alfacalcidol, levothyroxine, HRT
14	European/France	31	F	NT	IFN-α and IFN-ω positive	AI, HP, hypogonadism, PA, hypopituitarism, achalasia	CMC	Hydrocortisone, levothyroxine
15	European/USA	45	M	S64TfsX71/ L323SfsX51	IFN-α and IFN-ω positive	AI, HP, HT, end-stage renal disease, alopecia, PA, vitiligo, enteropathy	CMC	Hydrocortisone, fludrocortisone, calcium, ruxolitinib
16	European/France	38	F	NT	IFN-α	AI, HP,	CMC,	Hydrocortisone, fludrocortisone,

					and IFN-ω positive	myocarditis, PA, hypogonadism, cutaneous lupus	urinary tract infections	PTH, iron, magnesium, 1-0-HRT, perindopril, vitamin C, posaconazole
17	European/Sweden	8	F	P538L/P538L	IFN-α and IFN-ω positive	AI, HP, urticarial eruption, vitiligo, lupus-like systemic inflammation	None	Hydrocortisone, fludrocortisone, alfacalcidol calcium, magnesium
18	European/France	11	M	NT	IFN-α and IFN-ω positive	AI, HP, HT	CMC	Hydrocortisone, fludrocortisone, calcium, levothyroxine, tacrolimus
19	European/England	18	M	c.242T>C/C.1265delC	IFN-α and IFN-ω positive	AI, HP, urticarial eruption, DM, alopecia, hypogonadism	CMC	Hydrocortisone, fludrocortisone, calcium
20	European/France	15	F	NT	IFN-α and IFN-ω positive	AI, HP, hypogonadism, retinitis	CMC	Hydrocortisone, fludrocortisone, calcium, weekly methotrexate
21	European/Russia	10	M	R257X/R257X	IFN-α and IFN-ω positive	AI, HP, urticarial eruption, enteropathy, retinitis	CMC	Hydrocortisone, fludrocortisone, calcium, calcitriol, fluconazole
22	European/Russia	30	F	R257X/ L323SfsX51	IFN-ω positive	AI, HP, hypogonadism	None	Hydrocortisone, fludrocortisone, calcitriol

Table 2. Clinical features of 22 APS-1 patients with SARS-CoV-2 infection

Patient #	Days from symptom onset to hospital admission	COVID-19 severity (NIH ordinal scale score)	COVID-19 complications (other than hypoxemia-related)	Duration of hospital stay (days)	Hypoxemia support* (nadir SpO_2)	Intubation (duration in days)	Laboratory abnormalities	Radiographic abnormalities	Treatments	Outcomes
1	4	Critical (7)	Hypotension requiring dobutamine/ norepinephrine infusion; pneumococcal pneumonia; sepsis-induced ventricular dysfunction (EF, 30%); *Clostridium difficile* infection	37	Mechanical ventilation (N/A)	6	N/A	Bilateral, multiple GGO	High-dose hydrocortisone, LOP/RIT, ribavirin, HCQ, piperacillin/ tazobactam	Survival; low DLCO (55%) two months after discharge
2	8	Critical (7)	None	12	Mechanical ventilation (N/A)	5	↑CRP, ↓ALC, ↑AST	Bilateral, multiple GGO	High-dose hydrocortisone	Survival
3	7	Critical (7)	None	17	Mechanical ventilation (80%)	11	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑ferritin, ↑LDH, ↑D-dimer	Bilateral, multiple GGO	High-dose methyl-prednisolone, azithromycin, ceftriaxone, HCQ	Survival

4	4	Mild (4)	None	3	No	No	N/A	Bilateral GGO	None	Survival
5	10	Moderate-severe (5)	Antibiotic-associated diarrhea	10	No (93%)	No	↑CRP, ↓ALC, ↑LDH, ↑D-dimer	Bilateral, multiple GGO	High-dose prednisone, tocilizumab, vancomycin, ertapenem, levofloxacin, HCQ	Survival
6	Not hospitalized	Mild (1)	None	0	No	No	Not tested	Not performed	None	Survival
7	3	Critical (8)	Bacterial sepsis (*Acinetobacter Klebsiella*), pneumothorax (twice), acute renal failure (requiring hemodialysis)	47	Mechanical ventilation (60%)	28	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑creatinine, ↑D-dimer, ↑IL-6	Bilateral, multiple GGO	High-dose prednisone, tofacitinib, cefepime, sulbactam, polymixin B, linezolid, caspofungin	Death
8	4	Critical (8)	None	15	Mechanical ventilation (82%)	1	N/A	Bilateral, multiple GGO	High-dose dexamethasone	Death

9	7	Moderate-severe (5)	None	15	Nasal cannula (86%)	No	↑CRP, ↑LDH	Bilateral, multiple GGO	No	Survival
10	Not hospitalized	Mild (1)	None	0	No	No	Not tested	Not performed	No	Survival
11	5	Mild (4)	None	12	No	No	↓ALC	Bilateral GGO	No	Survival
12	5	Critical (7)	None	26	Mechanical ventilation (82%)	N/A	↑CRP, ↓ALC, ↑LDH, ↑D-dimer	Bilateral, multiple GGO	High-dose dexamethasone, tocilizumab, ribavirin, azithromycin, cefepime, vancomycin, voriconazole	Survival
13	3	Critical (8)	None	14	Mechanical ventilation (N/A)	5	N/A	N/A	High-dose dexamethasone, tocilizumab	Death
14	9	Critical (7)	Bacterial pneumonia, bacteremia, and sepsis	>60 days	Mechanical ventilation and ECMO (N/A)	42	↑CRP, ↑ferritin, ↓ALT, ↑AST,	Bilateral, multiple GGO	High-dose dexamethasone	Survival, tracheostomy

			(*Klebsiella*, *Serratia*, *Enterobacter*, *E. coli*), ventricular arrhythmia				↑D-dimer			
15	4	Moderate (4)	Pulmonary embolism	18	No	No	↑CRP, ↓ALC, ↑D-dimer	Bilateral GGO	High-dose hydrocortisone, remdesivir, azithromycin, ceftriaxone, apixaban	Survival
16	7	Critical (8)	Bacterial pneumonia (*Enterobacter*) pneumothorax	13	Mechanical ventilation (60%)	12	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑D-dimer	Bilateral, multiple GGO	High-dose dexamethasone	Death
17	2	Critical (7)	Transient diabetes insipidus	20	Mechanical ventilation (80%)	4	↑CRP, ↑AST, ↑IL-6	Bilateral, multiple GGO	High-dose betamethasone, plasmapheresis	Survival
18	2	Critical (7)	Hemoptysis	56	Mechanical ventilation (87%)	25	↑CRP, ↑ferritin, ↓ALC, ↑AST, ↑D-dimer	Bilateral, multiple GGO	High-dose dexamethasone, IFN-β, convalescent plasma, plasmapheresis	Survival
19	Not hospitalized	Mild (2)	None	0	No	No	Not tested	Not performed	Prolonged course of stress-dose steroids	Survival
20	5	Mild (4)	None	7	No	No	N/A	Bilateral GGO	IFN-β, convalescent plasma	Survival
21	5	Moderate-severe (5)	GI bleeding	21 days	Nasal cannula (87%)	No	↑CRP, ↓ALC, ↑LDH, ↑D-dimer,	Bilateral, multiple GGO	High-dose dexamethasone, tocilizumab, meropenem, fluconazole, IVIg	Survival
22	8	Moderate-severe (5)	None	6	Nasal cannula (89%)	No	↑CRP, ↓ALC, ↑AST, ↑ALT, ↑ferritin	Bilateral, multiple GGO	High-dose dexamethasone, tofacitinib,	Survival

favipiravir, amoxicillin-clavulanic acid, IVIg