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Type I IFN immunoprofiling in COVID-19 patients

To the Editor:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes coronavirus disease 2019 (COVID-19), is characterized by a wide spectrum of disease encompassing asymptomatic carriage, mild to severe upper respiratory tract illness that can evolve into respiratory failure, or rapidly progressing severe viral pneumonia with acute respiratory distress syndrome. Disease severity depends on viral strain, and host risk factors have been identified such as age and male sex. In addition, an excessive immune response has been identified in patients showing a cytokine storm associated with acute respiratory distress syndrome. Various immunosuppressive drugs, including IL-6 blockers or Janus kinases (JAK)-signal transducer and activator of transcription signaling inhibitors, have been suggested for the treatment of SARS-CoV-2 infection, whereas additional clinical trials are evaluating the use of recombinant IFN to foster host antiviral response (clinical trials NCT04315948 and NCT04293887). Type 1 IFNs (IFN-I) are major components of the innate immune system and represent critical antiviral molecules. To date, IFN-I response has not been evaluated in patients with COVID-19 and its contribution to the viral control and inflammation is unknown.

In this study, we assessed the kinetics of plasma IFN-I in patients with COVID-19 with a spectrum of severity degree. This study was approved by an ethical committee for biomedical research (Comité de Protection des Personnes HCL) (see text and this article’s Methods section in the Online Repository at www.jacionline.org).

First, we explored 3 patients issued from the first COVID cluster diagnosed in France (Les Contamines, Haute Savoie, France) in February 2020. We took advantage of the new digital ELISA technology single-molecule arrays (Simoa) and analyzed the kinetics of plasma inflammatory cytokines, IL-6, C-reactive protein (CRP), and IFN-γ-induced protein 10 (IP-10) were elevated in the 2 symptomatic patients (patients 1 and 3) (see Fig E1 in this article’s Online Repository at www.jacionline.org). Strikingly, no IFN-α2 was detectable in these 2 patients. In contrast, IL-6, CRP, and IP-10 remained low during the hospital isolation stay for the asymptomatic individual and a significant elevation in plasmatic IFN-α2 was observed. Viral loads were low, with no obvious quantitative difference between all 3 patients.

We further explored a larger cohort of 26 critically ill patients with COVID from 1 of the intensive care unit at Hospices Civils de Lyon (Lyon, France). Of note, all the patients were treated with standard of care and none received antiviral or immunotherapies. Considering the first 28 days of infection, more than half of critically ill patients required invasive mechanical ventilation (14 of 26). We observed that patients demonstrated a peak in IFN-α2 at day 8 to 10 of symptom onset corresponding to the viral replication phase, which decreased overtime to low but still detectable IFN-α2 concentrations. Conversely, a subset of patients (n = 5 [19%]) presented with sustained abrogation of IFN-I production (Fig 1, A). Simoa IFN-α2 measurement demonstrated a positive correlation with IFN-stimulated genes (see Fig E2, A, in this article’s Online Repository at www.jacionline.org) as already shown in viral infections. We noticed a strong proinflammatory response in all cases (CRP, IL-6, or IP-10), which started early and remained positive, whereas IFN-I response decreased after day 10 of infection (Fig 1, B-D). Patients with no IFN-α production presented poorer outcome, all of them requiring invasive ventilation (n = 5 of 5) and showing a longer intensive care unit stay (Table I). The viral load tended to be higher in IFN-negative patients with COVID-19 at disease diagnosis. IFN-β and IFN-λ were undetectable, whereas low amount of IFN-γ was detected in all patients with no evident link with IFN-α2 level (see Fig E2, B-D).

Taken together, our data demonstrate a heterogeneous pattern of IFN-α response in patients with COVID-19, with IFN-I response being impaired in about 1 of 5 of critically ill patients. This defective innate immune response may be associated with a poor outcome. In murine models of SARS-CoV-1 infection, delayed IFN-I production is associated with lung lesions and fatal outcome whereas early administration of IFN-I prevents lung lesions. SARS-CoV-2 displays a better sensitivity to IFN-I in vitro compared with SARS-CoV-1 in infected cell lines. Therefore, early administration of IFN-α2 might be promising for patients with COVID-19, especially in those who demonstrate a defective IFN response. The timing of IFN exposition may be critical to control the virus and avoid immunopathogenesis. Channappanavar et al have shown that delayed IFN-I expression can be detrimental in mice in the context of SARS-CoV-1 infection. Our data suggest that screening patients for IFN production is instrumental to select those who could benefit from early intervention with IFN. Following day 10, IL-6 remains increased whereas IFN-α tapered. This kinetics highlight that cytokine inhibitors could be helpful at the second phase of the disease following IFN-I decrease. Viral characteristic or individual genetic susceptibility should be explored to understand the defect of IFN-α production in some patients with COVID. Some IFN-α2-positive patients also experienced fatal outcome, highlighting the multifactorial causes of disease severity. We acknowledge limitations of this study, related to the small number of included patients and the technical limitation for the measurement of IFN-β and IFN-λ, in this proof-of-concept study.

Here, we provide new arguments for an early intervention with recombinant IFN-α2 and we also highlight the window of opportunity for immunosuppressors at the second phase of the disease, opening new avenues in COVID-19 therapies.
FIG 1. Plasma IFN-α, IL-6, CRP, and IP-10 concentrations in COVID-19 critically ill patient cohort (n = 26). A, Plasma IFN-α concentrations (fg/mL) were determined by single-molecule array (Simoa). Fit Loess curve represents local polynomial regression performed with Loess method. CI at 95% was indicated (orange area). B-D, CRP (μg/mL), IL-6, and IP-10 (pg/mL) concentrations were measured using a multiplexed assay with the Ella platform. Normal values for healthy volunteers were indicated by grey area. Vertical bar indicates the median delay between symptom onset and intensive care unit admission.

TABLE I. Clinical characteristics of patients with COVID-19 in intensive care unit

| Clinical feature                                   | IFN-negative (n = 5) | IFN-positive (n = 21) | P value |
|----------------------------------------------------|----------------------|-----------------------|---------|
| Age (y), median (min-max)                          | 81 (63-83)           | 74 (28-91)            | .696    |
| Sex (male), n (%)                                  | 5 (100)              | 18 (86)               | 1.000   |
| Delay between symptom onset and ICU admission (d)  | 7 (1-11)             | 7 (0-15)              | .769    |
| Bacterial coinfection during ICU stay, n (%)       | 3 (60)               | 7 (33)                | .3402   |
| Diabetes, n (%)                                    | 1 (20)               | 3 (14)                | .5043   |
| Chronic obstructive pulmonary disease, n (%)       | 0 (0)                | 3 (14)                | 1.000   |
| Cardiovascular disease, n (%)                      | 2 (40)               | 9 (43)                | 1.000   |
| Hypertension, n (%)                                | 3 (60)               | 7 (33)                | .3402   |
| Cancer, n (%)                                       | 1 (20)               | 3 (14)                | 1.000   |
| Active smokers, n (%)                              | 0 (0)                | 1 (5)                 | 1.000   |
| BMI >30 kg/m², n (%)                               | 3 (60)               | 8 (38)                | .620    |
| Biological feature, median (min-max)               | 20.9 (18-28.2)       | 25.1 (16.1-38.0)      | .172    |
| Viral load at diagnosis (Ct), median (min-max)     |                      |                       |         |
| Standard oxygen therapy only                       | 0 (0)                | 5 (24)                | .5451   |
| High flow oxygen therapy only                      | 0 (0)                | 7 (33)                | .278    |
| Invasive ventilation at any time during ICU stay   | 5 (100)              | 9 (42)                | .0425   |
| ICU length of stay, median (min-max)               | 20 (7-30)            | 5 (0-35)              | .0503   |
| Mortality at day 28 after symptom onset, n (%)     | 2 (40)               | 8 (38)                | 1.000   |

*P* values were calculated using Mann-Whitney test for quantitative values and using Fisher-exact test for qualitative ones.

Statistical significance is defined by *P* < .05 (boldface).

*BMI*, Body mass index; *ICU*, intensive care unit.
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ACE2, TMPRSS2, and furin gene expression in the airways of people with asthma—implications for COVID-19

To the Editor:

Coronavirus disease 2019 (COVID-19) is caused by a novel zoonotic coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has been identified as a pandemic by the World Health Organization. Several risk factors have been identified for severe COVID-19–associated pneumonia including increased age and the presence of comorbidities, in particular diabetes, cardiovascular disease, and tobacco smoking. However, a number of reports have failed to identify excess risk in patients with respiratory airway diseases such as asthma.2

SARS-CoV-2 infects people by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, a transmembrane endopeptidase that cleaves both angiotensin 1 and 2, and which is expressed by epithelial cells in several organs including the airways. Cofactors facilitating SARS-CoV-2 infectivity are the transmembrane peptidase serine 2 (TMPRSS2), which cleaves the SARS-CoV-2 spike protein, and possibly the protease furin.3 Understanding the expression of ACE2, TMPRSS2, and furin in the airways of people with asthma may help determine whether asthma itself or treatment with inhaled or oral corticosteroids may alter susceptibility to SARS-CoV-2 infection and potentially related disease severity. We have therefore explored the RNA expression of ACE2, TMPRSS2, and furin in human bronchial brushes and biopsies from previously described cohorts of people with asthma of varying corticosteroid treatment intensity (as an index of severity) and healthy controls.

Airway brushes and biopsies were collected at bronchoscopy with written informed consent and ethical approvals. Airway brushes were placed into RNAProtect and airway biopsies from the second- to fifth-generation airways were placed into RNA later. Bronchial brush ACE2 expression data were available from 356 patients (88 healthy volunteers and 268 patients with asthma [mild to moderate asthma, 125; severe asthma, 143]), across 5 asthma/healthy volunteer cohorts, Leicester, UK (n = 34).4 the multicenter Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (n = 54),5 the

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METHODS
Clinical characteristics of nonsevere patients with COVID
We explored the first 3 SARS-COV-2–positive patients diagnosed in France (Les Contamines, France) in February 2020.
Patient 1: One week before the first positive test result, a 46-year-old man developed mild signs and symptoms (fever, dry cough, asthenia, chills). Monitoring of viral load from consecutive nasopharyngeal swabs was performed (Fig E1, E). He had a benign evolution.
Patient 2: A 56-year-old woman was asymptomatic and did not develop any sign or symptom despite the detection of SARS-CoV-2 from nasopharyngeal swabs during several consecutive days (Fig E1, E).
Patient 3: A high-risk contact (a 54-year-old man) initially negative for SARS-CoV-2 developed fever and cough with respiratory crackles at auscultation on the fifth day of hospital isolation. A bilateral interstitial syndrome at the computed tomography scan with bilateral ground-glass opacification was predominant on the left. SARS-CoV-2 was detected from endotracheal aspirates (Fig E1, E).
For these 3 patients, no other respiratory pathogens were detected. These patients did not need oxygenation, nor antibiotics, steroids, or antiviral agents.

Patients
Plasma samples and Paxgene tubes were collected from patients with COVID-19 hospitalized in the University Hospital of Lyon (Hospices Civils de Lyon, France). Diagnosis of COVID-19 was confirmed in all patients by RT-qPCR.
All critically ill patients, admitted to intensive care unit, were included in the MIR-COVID study. This study was registered to the French National Data Protection Agency under the number 20-097 and was approved by an ethical committee for biomedical research (Comité de Protection des Personnes HCL) under the number N°20-41. In agreement with the General Data Protection Regulation (Regulation (EU) 2016/679 and Directive 95/46/EC) and the French data protection law (Law n°78-17 on 06/01/1978 and Décret n°2019-536 on 29/05/2019), we obtained consent from each patient or his next of kin.

Plasma protein quantification
Plasma IFN-α concentrations (fg/mL) were determined by single-molecule array (Simoa) using a commercial kit for IFN-α2 quantification (Quanterix, Lexington, Mass) on plasma samples of patients with COVID-19. The assay was based on a 3-step protocol using an HD-1 Analyzer (Quanterix). IL-6, CRP, and IP-10 concentrations were measured using a multiplexed assay with the Ella platform (Protein simple, San Jose, Calif), according to manufacturer’s instructions.
Plasma IFN-γ and IFN-β have been quantified using the Ella platform (Protein simple), according to manufacturer’s instructions. Plasma IL28A/B and IL-29 (type III IFN) have been quantified by ELISA (PBL Laboratories, Piscataway, NJ).

IFN score assessment
RNA was extracted from whole blood contained in Paxgene tubes (Kit PreAnalytix, Qiagen, Hilden, Germany) and quantified by spectrophotometric assay (Nanodrop 2000, Thermo Scientific, Waltham, Mass). RNA integrity was then evaluated by Agilent RNA microarray (Agilent Technologies, Santa Clara, Calif). mRNA quantification of 6 IFN-stimulated genes (IFN-α–inducible protein 27, IFN-induced protein 44 like, IFN-Induced Protein With Tetra-tripeptide Repeats 1, ISG15 Ubiquitin Like Modifier, Radical S-Adenosyl Methionine Domain Containing 2, and Sialic Acid Binding Ig Like Lectin 1 and 3 housekeeping genes—Actin Beta, Hypoxanthine Phosphoribosyltransferase 1, and RNA Polymerase II Subunit A) was performed using nanostring technology (Nanostring Technologies, Seattle, Wash). Data standardization was obtained using the geometric mean of internal control and housekeeping genes count number. IFN score was calculated as previously described.

Virus quantification load
Viral load was quantified from nasopharyngeal swabs or endotracheal aspirates. RNA extraction was performed by the automated NucliSENS easyMAG (BioMérieux, Marcy l’Etoile, France) using manufacturer’s instructions. A 25-μL reaction contained 5 μL of RNA, 12.5 μL of 2× reaction buffer provided with the Superscript III 1-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each dNTPs and 3 mM magnesium sulfate), 1 μL of reverse transcriptase/Taq mixture from the kit, and 0.4 μL of a 50 mM magnesium sulfate solution (Invitrogen). Thermal cycling was performed at 55°C for 20 minutes for reverse transcription, followed by 95°C for 3 minutes and then 50 cycles of 95°C for 15 seconds, 58°C for 30 seconds, on the QuantStudio 5 rT-PCR Systems (Thermo Fisher Scientific, Waltham, Mass). Primer and probe used are based on the Institut Pasteur protocol (Paris, France).

Statistical analysis
P values were calculated using Mann-Whitney test for quantitative values and using Fisher-exact test for qualitative ones.

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FIG E1. Plasma cytokine levels and viral load in 3 SARS-COV-2–positive patients diagnosed in France. A, Plasma IFN-α concentrations (fg/mL) were determined by single-molecule array (Simoa). B–D, IL-6, CRP, and IP-10 concentrations were measured using a multiplexed assay with the Ella platform. E, Viral load is represented as cycle threshold of IP2 RT-quantitative PCR using assay designed by Pasteur Institut in Paris.