The Journal of Infectious Diseases

BRIEF REPORT

Type I, II, and III Interferon Signatures Correspond to Coronavirus Disease 2019 Severity

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We analyzed plasma levels of interferons (IFNs) and cytokines, and expression of IFN-stimulated genes in peripheral blood mononuclear cells in patients with coronavirus disease 2019 of varying disease severity. Patients hospitalized with mild disease exhibited transient type I IFN responses, while intensive care unit patients had prolonged type I IFN responses. Type II IFN responses were compromised in intensive care unit patients. Type III IFN responses were induced in the early phase of infection, even in convalescent patients. These results highlight the importance of early type I and III IFN responses in controlling coronavirus disease 2019 progression.

Keywords. SARS-CoV-2; interferon-stimulated genes; IRF-1.

Infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results in diverse clinical outcome of coronavirus disease 2019 (COVID-19). Most patients with COVID-19 experience a mild clinical course, but approximately 5% of SARS-CoV-2–positive patients experience severe disease [1], acute respiratory distress syndrome, which necessitates supplemental oxygen therapy or intensive care unit (ICU) care. Patients with mild cases, who do not need either hospitalization or ICU care, recover within about 14 days after symptom onset with viral clearance [2]. However, patients who need ICU care experience mild to moderate symptoms followed by a secondary respiratory worsening with prolonged viral load. Although vaccination against SARS-CoV-2 has been feasible, therapeutic options for patients with COVID-19 are still limited, including SARS-CoV-2 neutralizing antibodies, antiviral agents, and immunosuppressive agents.

Interferon (IFN) responses constitute the major defense against SARS-CoV-2 infection. Virus recognition by innate immune sensors of host cell induces production type I and type III IFN. Type I and III IFNs induce the expression of IFN-stimulated genes (ISGs), which have antiviral capacity [3]. Type I IFN, not type III IFN, induces proinflammatory gene expression by selective induction of the transcription factor IFN regulatory factor 1 (IRF-1) [4]. In contrast to virally induced types I and III, type II IFN is produced predominantly by T and natural killer cells on stimulation with antigens and cytokines. Type II IFN stimulates antigen-specific adaptive immunity and activates innate immunity, particularly through the activation of macrophages.

However, SARS-CoV-2 evades the IFN responses of host by escaping immune recognition, suppressing the functions of IFNs and ISGs, and interfering with the antigen presentation process [3]. IFN responses modulated by both viral and host factors determine the clinical outcome in patients with COVID-19. Several studies have reported impaired type I and II IFN responses in patients with severe COVID-19 during the early phase of infection [5, 6]; however, the dynamic IFN responses during SARS-CoV-2 infection need to be defined. In the current study, we comprehensively investigated type I, II, and III IFN signatures in COVID-19 with different disease severity. We analyzed the plasma levels of IFNs and IRF-1–regulated cytokines and chemokines, and the expression of ISGs in peripheral blood mononuclear cells (PBMCs).

METHODS

Subjects and Specimen Collection

Patients were recruited between March and June 2020 at Massachusetts General Hospital by the Massachusetts Consortium on Pathogen Readiness. Patients had COVID-19 diagnosed by means of reverse-transcription polymerase chain reaction testing for SARS-CoV-2 in nasopharyngeal swab samples. The severity of COVID-19 was classified based on the National Institutes of Health COVID-19 treatment guidelines [7]. The study conforms to the principles outlined in the Declaration of Helsinki and received approval by the ethics committees of Massachusetts General Hospital, Boston, Massachusetts

Received 10 March 2021; editorial decision 18 May 2021; accepted 21 May 2021; published online May 24, 2021.

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[a]See Supplementary Acknowledgments for MGH COVID-19 Collection & Processing Team details.

The Journal of Infectious Diseases® 2021;XX:1–6

DOI: 10.1093/infdis/jiab288

(approval no. 2020P000804) All participants provided informed consent.

Plasma Cytokine and Chemokine Analysis

Plasma levels of IFN-α (41110–1; R&D Systems) and IFN-λ1/3 (DY1598B-05; R&D Systems) were measured by means of enzyme-linked immunosorbent assay. Plasma levels of IFN-γ, interleukin 6, interleukin 12 (IL-12) (p70), interleukin 18, tumor necrosis factor (TNF) α, TNF-related apoptosis-inducing ligand (TRAIL), CCL2, CCL4, CCL7, CCL8, CXCL8, CXCL9, and CXCL10 were measured using the MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A (HCYTA-60K, Merck Millipore) and Panel II (HCYP2MAG-62K, Merck Millipore).

ISG Analysis

ISGs, previously reported to be relevant in viral infection [4], were selected for analyzing messenger RNA expression of ISGs. Messenger RNA expression of ISGs was quantified with the NanoString platform (NanoString Technologies) and reverse-transcription polymerase chain reaction using PowerUP SYBR Green Master Mix (Thermo Fisher Scientific) (Supplementary Table 1).

Statistical Analysis

Grouped data are generally presented as medians with interquartile range, with groups compared by means of the Kruskal-Wallis test and Dunn multiple comparisons test for nonparametric data, using Prism 9.0 software (GraphPad)

RESULTS

Patients and Sample Collection

Patients confirmed positive for SARS-CoV-2 were subdivided into 3 groups based on disease severity during their clinical encounters: outpatients (n = 23), patients hospitalized under non-ICU conditions (mild disease; n = 21), and patients hospitalized in the ICU (n = 23). Convalescent patients, who were recovered from COVID-19 and confirmed negative for SARS-CoV-2, were included as a control group (n = 19). The blood samples were typically collected 5–44 days after symptom onset (Supplementary Table 2 and Supplementary Figure 1*A*).

Plasma Levels of Type I, II, and III IFNs in Patients With COVID-19

The plasma levels of the type I IFN, IFN-α, were highest in ICU patients, followed by patients hospitalized with mild disease and outpatients. However, when evaluated by duration after symptom onset, some of the outpatients and patients with mild disease at <14 days after symptom onset exhibited higher levels of IFN-α than ICU patients. In contrast, at 15–26 days after symptom onset, most patients with mild disease and outpatients had decreased levels of IFN-α, while ICU patients still had increased levels of IFN-α (Figure 1A). Plasma levels of IFN-γ, a type II IFN, were significantly reduced in ICU patients regardless of sample collection date (Figure 1B and Supplementary Table 3). Plasma levels of IFN-λ1/3, a type III IFN, were comparable among patients. IFN-λ1/3 appeared to be preferentially induced during the early phase of infection in patients hospitalized with mild disease and ICU patients (Figure 1C).

Plasma Levels of IRF-1–Regulated Cytokines and Chemokines

Levels of most of the IRF-1–regulated cytokines and chemokines, including interleukin 6 and 18, TNF-α, CCL2, CCL4, CCL8, CXCL8, CXCL9, and CXCL10, were highest in the ICU patients, followed by the patients hospitalized with mild disease and outpatients, in a similar pattern to that for IFN-α levels (Supplementary Figure 2*A* and Supplementary Table 3). The levels of IL-12, CCL7, and TRAIL were higher in outpatients and patients with mild disease, as with IFN-γ (Supplementary Figure 2*B*). Heat map clustering of plasma cytokines and chemokines yielded 2 major clusters: a cluster consisting of IFN-γ, IL-12, CCL7, and TNF-α (IFN-γ cluster) and another consisting of IRF-1–regulated cytokines and chemokines (IRF-1 cluster). The IFN-γ cluster was up-regulated in outpatients and patients with mild disease, but not in the ICU patients (Figure 1D).

Expression of ISGs in PBMCs

IRF-1–regulated genes in PBMCs were up-regulated only in the ICU patients, while antiviral genes were up-regulated in both ICU patients and those hospitalized with mild disease (Figure 2A and 2B and Supplementary Figure 3). The expression of antiviral genes and IRF-1–regulated genes was positively correlated with plasma IFN-α levels but was not associated with type II or type III IFN levels (Figure 2C and Supplementary Figure 4). The IFN-γ–stimulated genes, including *HLA-A, HLA-B, β2M, HLA-DPA1, HLA-DRA,* and *CIITA*, were down-regulated in the patients with mild disease but especially in ICU patients, compared with outpatients and convalescent patients (Figure 2D).

DISCUSSION

It is known that older adults and men with diagnosed COVID-19 are at higher risk of hospitalization and death, compared with younger persons and women. Our study further affirmed this finding since the patients hospitalized with mild disease and ICU patients were older than the outpatients (Supplementary Figure 1*B*). However, patients with mild disease and ICU patients displayed differences in IFN signatures despite being comparable in age. We performed a multivariable regression analysis with each of 15 cytokines and chemokines as dependent variables and age, sex, race/ethnicity, and sample collection day as independent variables (Supplementary Table 3). Based on this analysis, we confirmed that our findings were not significantly affected

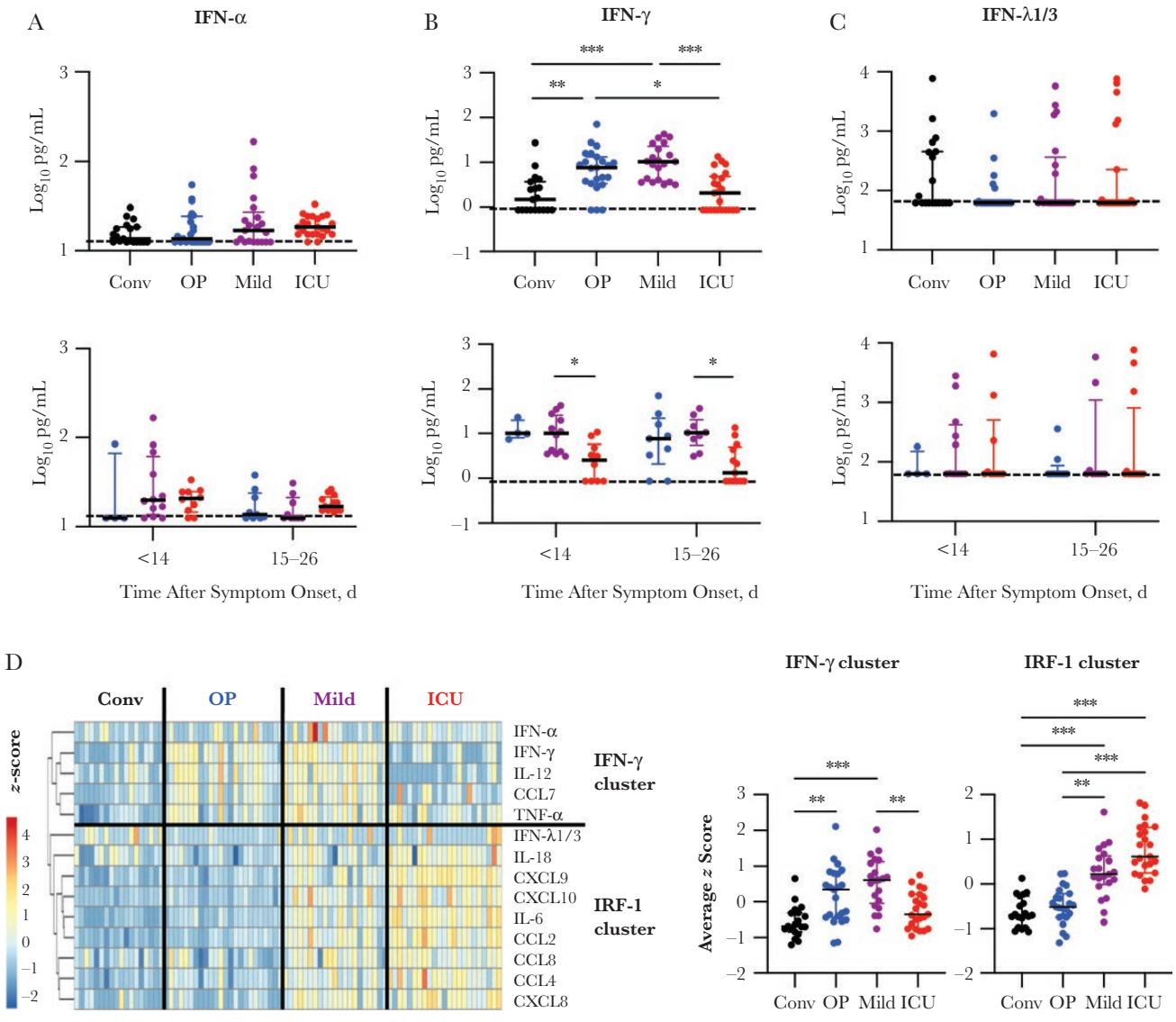

Figure 1. Plasma levels of type I, II, and III interferons (IFNs) in patients with coronavirus disease 2019 (COVID-19). *A–C*, Plasma concentrations of IFN-α (*A*), IFN-γ (*B*), and IFN-λ1/3 (*C*) by disease severity, including convalescent patients (Conv; n = 19), outpatients (OP; n = 23), patients with mild disease, hospitalized under non–intensive care unit (ICU) conditions (Mild; n = 23), and patients hospitalized in the ICU (n = 23). The levels of IFN-α, IFN-γ, and IFN-λ1/3 were subdivided into <14 and 15–26 days after symptom onset, with detection limits indicated by dotted lines. *D,* Heat map clustering of the plasma levels of factor IFN regulatory factor 1 (IRF-1)–related cytokines and chemokines. The values of concentrations were transformed into *z* scores for heat map analysis. Heat map clustering of plasma cytokines and chemokines yielded 2 major clusters, the IFN-γ and the IRF-1 cluster. The *z* scores of cytokines and chemokines belonging to the IFN-γ or the IRF-1 cluster were averaged in each patient and then compared by disease severity. Significance was determined using the Kruskal-Wallis test with Dunn multiple comparisons test. **P* < .05; ***P* < .01; ****P* < .001. Abbreviations: IL-6, IL-12, and IL-18, interleukin 6, 12, and 18, respectively; TNF, tumor necrosis factor.

by the independent variables. Therefore, we concluded that our observations were not explained by age, sex, or race/ethnicity.

Several studies have reported that type I and III IFN responses in patients with severe COVID-19 are suppressed during the early phase of infection [5, 8]. However, other studies have shown that patients with severe COVID-19 have robust type I IFN responses [6, 9]. In our study, we observed the induction of rapid and transient type I IFN responses in outpatients and patients with mild disease but prolonged type I IFN responses in ICU patients. Furthermore, lower viral loads in outpatients and patients with mild disease and shorter hospitalizations in those with mild disease suggest rapid viral clearance, while ICU patients had prolonged hospitalizations and higher viral loads (Supplementary Figure 1*C* and 1*D*).

Type I IFN activates transcription of proinflammatory genes by inducing the transcription factor IRF-1 [3, 4], which up-regulates cytokines that contribute to hyperinflammation in COVID-19. In our study, the ICU patients had higher plasma IFN-α levels during the later phase of infection. The expression

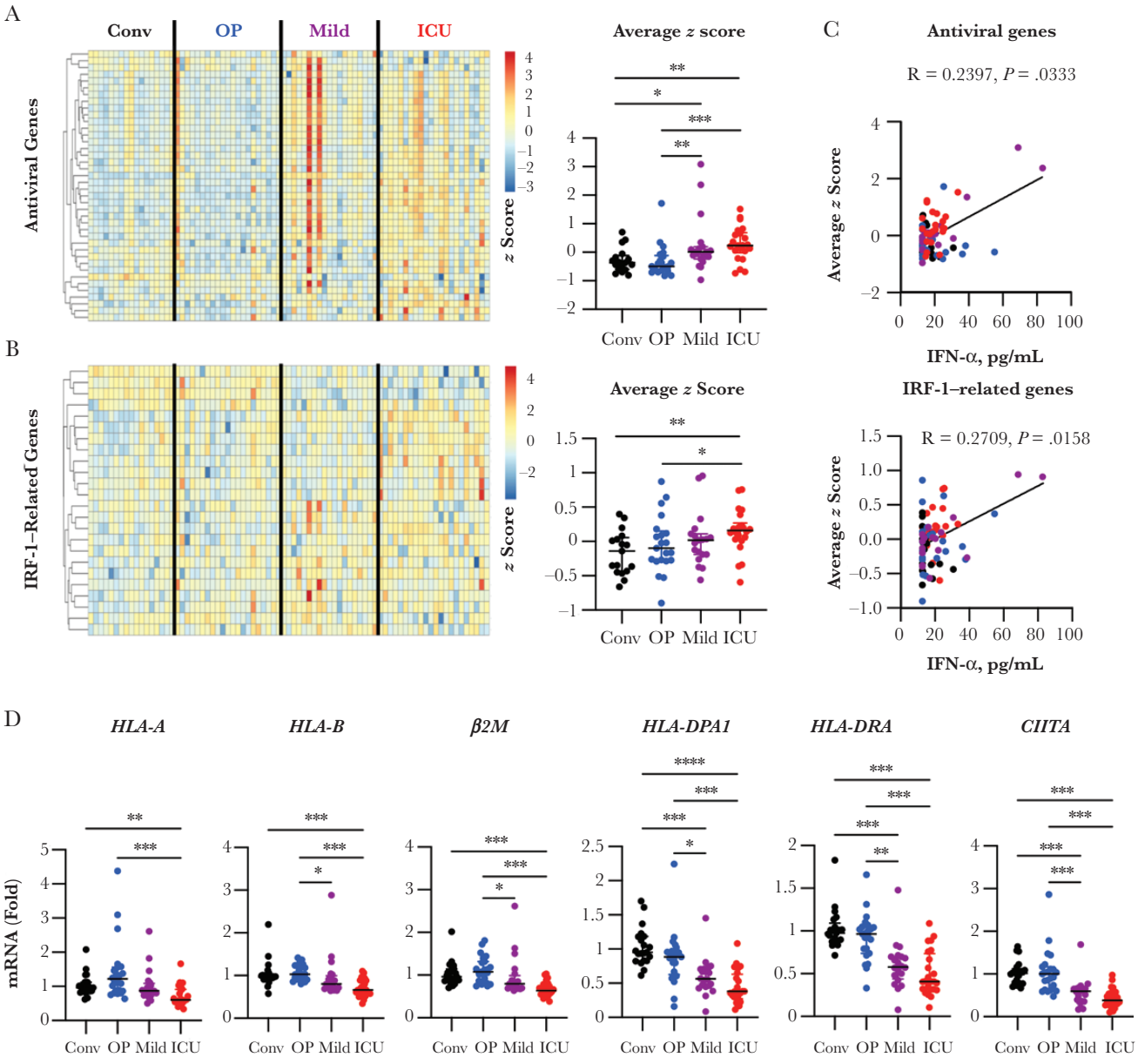

Figure 2. The expression of interferon (IFN)–stimulated genes in peripheral blood mononuclear (PBMCs). *A, B,* Normalized messenger RNA (mRNA) expression levels of antiviral genes (*A*) and factor IFN regulatory factor 1 (IRF-1)–related genes (*B*) in PBMCs were transformed into *z* scores and visualized in a heat map. Average *z* scores of antiviral genes or IRF-1–related genes were compared by disease severity, including convalescent patients (Conv), outpatients (OP), patients with mild disease, hospitalized under non–intensive care unit (ICU) conditions (Mild), and patients hospitalized in the ICU. *C,* Correlation between IFN-α levels and the average *z* scores of antiviral or IRF-1–related genes. Spearman rank test was used for correlations. Solid lines represent regression lines. *D,* mRNA expression levels of IFN-γ–stimulated genes. Significance was determined using Kruskal-Wallis test with Dunn multiple comparisons test. **P* < .05; ***P* < .01; ****P* < .001.

of IRF-1–regulated genes in PBMCs was up-regulated only in the ICU patients and positively correlated with plasma IFN-α levels. These results demonstrate that the hyperinflammation in ICU patients can be traced to prolonged type I IFN responses.

Several studies have reported the reduction of the plasma type II IFN in patients with severe COVID-19 similar to our findings [5]. A series of analyses on the immune cells have suggested that IFN-γ–producing $CD4^+$ T, $CD8^+$ T, and natural killer cells are exhausted and depleted in patients with severe COVID-19 [10, 11], which could plausibly explain the decreased plasma IFN-γ levels in ICU patients. However, there are conflicting data suggesting that PD-1–expressing SARS-CoV-2 specific $CD8^+$ T cells are not truly exhausted in patients with COVID-19 [12].

The levels of IFN-γ–stimulated genes were somewhat diminished in patients hospitalized with mild disease compared with outpatients and convalescent patients, but they were still substantially up-regulated compared with ICU patients. In this regard, these findings were similar to the pattern observed in

plasma IFN-γ levels. Viruses including coronaviruses, Middle Eastern respiratory syndrome coronavirus, and H5N1 influenza virus, interfere with the antigen presentation process through major histocompatibility complex (MHC) molecules. The open reading frame 8 protein of SARS-CoV-2 down-regulates MHC class I molecules [13], although evidence is still lacking for SARS-CoV-2 interference with antigen presentation. Several studies have demonstrated down-regulation of MHC class I and II molecules in antigen-presenting cells of patients with COVID-19, regardless of disease severity [14, 15]. Thus, decreased expression of MHC molecules in PBMCs and reduced plasma IFN-γ could synergistically subvert adaptive immunity in ICU patients.

Type III IFN is induced earlier than type I IFN in virus infection, and it suppresses initial viral spread without activating inflammation. Type I IFN response is triggered later to enhance antiviral activity and induce IRF-1–mediated inflammatory responses [3]. Interestingly, some of the convalescent patients showed increased IFN-λ1/3 levels (Figure 1C) and up-regulated antiviral genes, without induction of IRF-1–related genes (Figure 2A and 2B), even though they were confirmed negative for SARS-CoV-2. While it is remotely possible that these patients may have been reinfected with SARS-CoV-2 without development of detectable viral RNA, it seems much more likely that the virus was rapidly cleared by type III IFN responses before engagement of type I IFN responses.

Type III interferon therapy could be a novel therapeutic strategy against COVID-19. Early use of type I IFNs has benefits in virus clearance and clinical outcomes in patients with COVID-19. However, later use of type I IFNs could potentially delay recovery and increase mortality rates. This could be attributed to IRF-1–related hyperinflammation. While the expression of type I IFN receptors is ubiquitous, the expression of type III IFN receptors is limited to epithelial cells [3]. Thus, type III interferon therapy could be an effective alternative to type I interferon therapy since it can promote virus clearance without inducing IRF-1–related inflammation. Several clinical trials by our group and others are ongoing to confirm the validity of interferon λ therapy in patients with mild to severe COVID-19 (NCT04343976, NCT04354259, NCT04388709, and NCT04344600). A clinical trial testing interferon λ therapy in outpatients with COVID-19 indicates an antiviral effect in ambulatory patients with COVID-19 with high levels of virus [16].

Overall, our analyses provide a much clearer picture of the dynamic signatures of type I, II, and III IFN during COVID-19. Type I and III IFN responses during the early phase of infection appear to be important in controlling viral spread and disease progression. Failure to limit viral spread during the early phase of infection can lead to susceptibility to hyperinflammation mediated by prolonged type I IFN– and IRF-1–mediated responses, the exhaustion and depletion of IFN-γ–producing cells, and the down-regulation of antigen presentation through MHC class I and class II. Analogously, enhanced antigen presentation and type II IFN responses appear to be associated with milder clinical illness. Finally, these findings provide rationale for use of type III interferon therapy to maximize antiviral activity without IRF-1–mediated proinflammatory responses relatively early in COVID-19 illness.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This work was supported by the National Institutes of Health (grant U19 AI082630) and the Massachusetts General Hospital (MGH) Research Scholars Program. The MGH/Massachusetts Consortium on Pathogen Readiness coronavirus disease 2019 biorepository was supported by a gift from Enid Schwartz and by the Mark and Lisa Schwartz Foundation, the Massachusetts Consortium for Pathogen Readiness, and the Ragon Institute of MGH, MIT and Harvard.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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