Clinical Infectious Diseases

MAJOR ARTICLE

Differential Cytokine Signatures of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Influenza Infection Highlight Key Differences in Pathobiology

Andrew H. Karaba,[1,a] Weiqiang Zhou,[2,a] Leon L. Hsieh,[1] Alexis Figueroa,[1] Guido Massaccesi,[1] Richard E. Rothman,[3] Katherine Z. J. Fenstermacher,[3] Lauren Sauer,[3] Kathryn Shaw-Saliba,[3] Paul W. Blair,[1] Matthew L. Robinson,[1] Sherry Leung,[4] Russell Wesson,[4] Nada Alachkar,[1] Ramy El-Diwany,[4] Hongkai Ji,[2] and Andrea L. Cox[1,5,6]

[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [2]Department of Biostatistics, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA; [3]Department of Emergency Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [4]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [5]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA; and [6]Bloomberg Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Background. Several inflammatory cytokines are upregulated in severe coronavirus disease 2019 (COVID-19). We compared cytokines in COVID-19 versus influenza to define differentiating features of the inflammatory response to these pathogens and their association with severe disease. Because elevated body mass index (BMI) is a known risk factor for severe COVID-19, we examined the relationship of BMI to cytokines associated with severe disease.

Methods. Thirty-seven cytokines and chemokines were measured in plasma from 135 patients with COVID-19, 57 patients with influenza, and 30 healthy controls. Controlling for BMI, age, and sex, differences in cytokines between groups were determined by linear regression and random forest prediction was used to determine the cytokines most important in distinguishing severe COVID-19 and influenza. Mediation analysis was used to identify cytokines that mediate the effect of BMI and age on disease severity.

Results. Interleukin-18 (IL-18), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) were significantly increased in COVID-19 versus influenza patients, whereas granulocyte macrophage colony-stimulating factor, interferon-γ (IFN-γ), IFN-λ1, IL-10, IL-15, and monocyte chemoattractant protein 2 were significantly elevated in the influenza group. In subgroup analysis based on disease severity, IL-18, IL-6, and TNF-α were elevated in severe COVID-19, but not in severe influenza. Random forest analysis identified high IL-6 and low IFN-λ1 levels as the most distinct between severe COVID-19 and severe influenza. Finally, IL-1RA was identified as a potential mediator of the effects of BMI on COVID-19 severity.

Conclusions. These findings point to activation of fundamentally different innate immune pathways in severe acute respiratory syndrome coronavirus 2 and influenza infection, and emphasize drivers of severe COVID-19 to focus both mechanistic and therapeutic investigations.

Keywords. COVID-19; Influenza; Cytokines; SARS-CoV-2; Obesity.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to more than 2 million deaths worldwide in 2020 [1]. Coronavirus disease 2019 (COVID-19), the disease caused by SARS-CoV-2, spans mild disease to multiorgan failure and death [2, 3]. One hallmark of severe disease is immune dysregulation characterized by elevated proinflammatory markers and cytokines [4–9] including interleukin (IL)-6, IL-10, interferon-γ-induced protein 10 (IP-10), IL-1RA, and monocyte chemoattractant protein 1 (MCP-1 [8, 10–15]. Studies have challenged the uniqueness of the inflammatory cytokine profile of COVID-19 by highlighting similarities to sepsis or acute respiratory distress syndrome from other causes [16–18].

Influenza is another respiratory viral cause of severe pneumonia and pandemics [19]. The case fatality rate for influenza is lower than that of COVID-19, but many of the cytokines upregulated in COVID-19 are also increased in severe influenza infection [15, 20, 21]. Thus, it is unclear what unique cytokine upregulation in SARS-CoV-2 infection leads to more severe disease than influenza [22]. Several clinical factors correlate with severe COVID-19, including advanced age and elevated body mass index (BMI) [23, 24]. Obese patients are at increased risk for hospitalization and death, particularly at younger ages [25,

Received 22 January 2021; editorial decision 22 April 2021; published online 20 May 2021.

[a]A.H.K. and W. Z. contributed equally to this work.

Correspondence: A. L. Cox, John Hopkins School of Medicine, 855 N. Wolfe St, Baltimore, MD 21205, USA (acox@jhmi.edu).

Clinical Infectious Diseases® 2022;74(2):254–62

https://doi.org/10.1093/cid/ciab376

26]. Obesity leads to chronic inflammation, and elevated BMI is associated with increases in IL-10, IL-6, tumor necrosis factor-α (TNF-α), and IL-1RA [27–29]. Yet, few studies examining these cytokines in COVID-19 have incorporated this in their analyses.

To determine how cytokines produced during COVID-19 and influenza differ and to understand the increased pathogenicity of SARS-CoV-2, we measured 37 cytokines and chemokines in patients hospitalized with either influenza or COVID-19 and compared cytokine levels based on disease severity. We also performed mediation analysis to identify cytokines that mediate the effect of BMI and age on disease severity. We found that severe COVID-19 induces a macrophage proinflammatory cytokine profile, whereas severe influenza leads to interferon induction. We found that although multiple cytokines mediate the effects of advanced age, IL-1RA is the primary mediator underlying the relationship between obesity and severe COVID-19. These findings highlight that disparate immune pathways are activated in these potentially life-threatening respiratory viral infections.

METHODS

Study Participants and Samples

All studies were approved by the Johns Hopkins institutional review board (IRB). Hospitalized patients diagnosed with COVID-19 by positive SARS-CoV-2 RNA testing in the Johns Hopkins Healthcare System were enrolled in a prospective consented protocol to investigate research questions specific to the clinical course of COVID-19 (IRB 00245545). Demographic information, clinical laboratory test results, International Classification of Diseases, 10th revision, coded diagnoses (comorbidities), BMI, and other clinical parameters were linked to data for COVID-19 patients in the study. Those who received tocilizumab before cytokine measurement were excluded. Participants were categorized by maximum COVID-19 disease severity score based on the World Health Organization severity scale [30]. Those with a score <4 were categorized as having mild/moderate disease and those with a score ≥5 were considered severe. Blood was obtained as close to admission as feasible and centrifuged to separate cells from plasma in Basics of Biosafety Level 2+ laboratory conditions.

Healthy control (HC) plasma was obtained from human immunodeficiency virus/hepatitis C virus-antibody seronegative participants enrolled before 2020 in the Baltimore Before and After Acute Study of Hepatitis study (IRB NA_00046368), an ongoing prospective, community-recruited, observational cohort study of people who inject drugs, as previously described [31].

Plasma from hospitalized patients infected with influenza between 2017 and 2019 was obtained as previously described for comparison to hospitalized COVID-19 patients in this study [31, 32] (IRB 00091667). Patients hospitalized with influenza requiring no more than nasal cannula and those that required higher levels of oxygen support were classified as having mild/moderate disease or severe disease, respectively, which approximates the World Health Organization COVID-19 severity score [30].

All plasma samples were frozen at -80°C until thawed for cytokine measurement as described in the following section.

Cytokine Measurement

Plasma cytokines and chemokines (interferon-α2a [IFN-α2a], IFN-β, IL-18, IL-1RA, IL-23, IFN-λ1, IL-2Ra, MCP-2, granulocyte macrophage colony-stimulating factor [GM-CSF], IL-23p40, IL-15, IL-16, IL-17A, IL-1α, IL-5, IL-7, TNF-β, vascular endothelial growth factor [VEGF], Eotaxin, Eotaxin-3, induced protein-10 [IP-10], MCP-1, MCP-4, macrophage derived chemokine (MDC), macrophage inflammatory protein-1α [MIP-1α], MIP-1β, thymus- and activation-regulated chemokine (TARC), IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α) were measured using a custom multiplex kit from Meso Scale Diagnostics (Rockville, MD) according to the manufacturer's protocol, and data were acquired on a MESO QuickPlex SQ 120. Each sample was measured on first thaw and in duplicate. If an analyte signal was below background, it was set to 0; if detectable, but below the manufacturer's lower limit of quantification, it was set to the lower limit of detection.

Statistical Analysis

Data were analyzed using the statistical computing software R version 3.6.3 [33]. The cytokine/chemokine (i.e., analyte) signals were first $\log_2$ transformed after adding a pseudocount of 1. To compare the analytes between patient groups, a linear regression analysis, which is equivalent to a 2-tailed *t* test after adjusting for covariates, was applied. For instance, a linear regression model was fitted for each analyte to test the difference between every pair of patient groups (e.g., COVID-19 vs influenza) after adjusting for covariates (age, gender, and BMI). *P* values of the coefficient for the patient group from the model were obtained and converted to false discovery rates (FDRs) using the Benjamini-Hochberg procedure [34]. An FDR of 0.25 was considered significant. Random forest and mediation analysis were performed as described in the Supplementary Methods [35–37].

RESULTS

Cohort Characteristics

A total of 135 participants with SARS-CoV-2, 57 participants with influenza, and 30 HCs were studied. Based on final infection outcome, we categorized influenza and COVID-19 subgroups as mild/moderate or severe, as described in Materials and Methods. Thirteen of 57 (23%) participants in the influenza cohort and 80 of 135 (59%) patients with COVID-19 had severe disease (Table 1). The influenza and COVID-19 cohorts were not significantly different in gender, non-White

Table 1. Characteristics of Study Participants

Subject Group	COVID-19	Influenza		Healthy Controls
Demographics				
Male, N (%)	70 (52)	26 (46)	*P* = .53	19 (63)
Female, N (%)	65 (48)	31 (54)	…	11 (37)
Mean age (range), y	56.2 (20–90)	48.2 (19–89)	*P* = .006	31.2 (20–45)
Mean BMI (range)	32.8 (12–70)	30.2 (18–60)	*P* = .06	NA
Race and ethnicity				
Race	N (%)	…	…	
Black	62 (46)	40 (70)	…	11 (37)
White	26 (19)	10 (18)	…	19 (63)
Other[a]	38 (28)	5 (9)	…	0 (0)
Asian	9 (7)	2 (3)	…	0 (0)
White vs non-White			*P* = .84	
Ethnicity				
Hispanic/Latinx	N (%)	…	…	
Yes	33 (24)	NA	…	0 (0)
No	102 (76)	NA	…	30 (100)
Maximum disease severity[b], N (%)				
Mild/moderate	55 (41)	44 (77)	…	NA
Severe	80 (59)	13 (23)	…	NA
Comorbidities	N (%)			
Diabetes mellitus	56 (41)	NA	…	0 (0)
HIV infection	4 (3)	7 (12)	…	0 (0)

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; NA, data not available.

[a]Most self-identified as Hispanic/Latino.

[b]Maximum disease severity indicates the most severe COVID-19 disease class for the patient when under observation: Mild/moderate = no or low flow oxygen required, or high flow oxygen or noninvasive positive-pressure ventilation required; severe = patient required intubation or patient died (ventilated or not). *P* values were calculated as described in the Supplementary Methods.

race, or BMI. The influenza cohort was younger than the COVID-19 cohort on average (mean age, 48.2 vs 56.2 years). The interval between admission and cytokine measurement was not significantly different between the mild/moderate and severe disease subgroups (Supplementary Figure 1*A*). Principal component analysis of the 2 cohorts revealed clustering of the severe subgroups together in the principal component space, suggesting a similar level of overall inflammation between the 2 subgroups (Supplementary Figure 1*B*).

Cytokine Elevations in COVID-19 and Influenza Compared With Healthy Controls

To determine which cytokines and chemokines are upregulated in influenza and COVID-19, we compared cytokines/chemokines in HCs to those with influenza and COVID-19. We selected potentially important markers of disease severity for our custom panel based on prior publications in SARS-CoV-1, SARS-CoV-2, and influenza [8, 15, 38–40]. We found that GM-CSF, IFN-β, IFN-γ, IFN-λ1, IL-10, IL-15, IL-18, IL-1RA, IL-6, IL-8, IP-10, MCP-1, MCP-2, and TNF-α were significantly increased in both influenza and COVID-19 compared with HCs (Figure 1). In contrast, IL-12p70, IL-13, eotaxin-3, MDC, and TARC were significantly decreased in COVID-19 and influenza compared with HCs. Seven analytes were not significantly elevated in either virus group: IL-1α, IL-23, IL-4, IL-5, MCP-4, MIP-1α, and MIP-1β (Supplementary Figure 1*C*). IL-1β, IL-2, IL-23p40, IL-2Ra, IL-7, and VEGF were elevated in COVID-19 exclusively, not influenza, compared with HCs. Conversely, IFN-α2a, IL-16, and IL-17A were significantly elevated solely in the influenza cohort, with no difference between COVID-19 participants and HCs.

Differences in Cytokines and Chemokines Between Influenza and COVID-19 Reveal a Proinflammatory Macrophage Signature in COVID-19

Focusing on analytes elevated in 1 or both viral cohorts, we found that IL-18, IL-1β, IL-6, IL-7, TNF-α, and VEGF were higher in COVID-19, whereas GM-CSF, IFN-α2a, IFN-γ, IFN-λ1, IL-10, IL-15, IL-16, IL-17A, and MCP-2 were higher in influenza (Figure 1) (Supplementary Table 1 and 2). The cytokines most elevated in the COVID-19 group are produced primarily by macrophages and characterize macrophage activation syndrome [41, 42]. Elevated levels of IL-18 and IL-1β suggest prominent inflammasome activation in COVID-19 relative to influenza [43]. Macrophages are major sources of inflammasome cytokines in other viral infections [44–46]. Conversely, IFN-λ1 was nearly 2-fold higher in influenza compared with COVID-19, consistent with a small study demonstrating lower interferon production in COVID-19 versus influenza [47] and an in vitro study demonstrating limited induction of IFN-λ1, IFN-α2a, and IFN-β by SARS-CoV-2

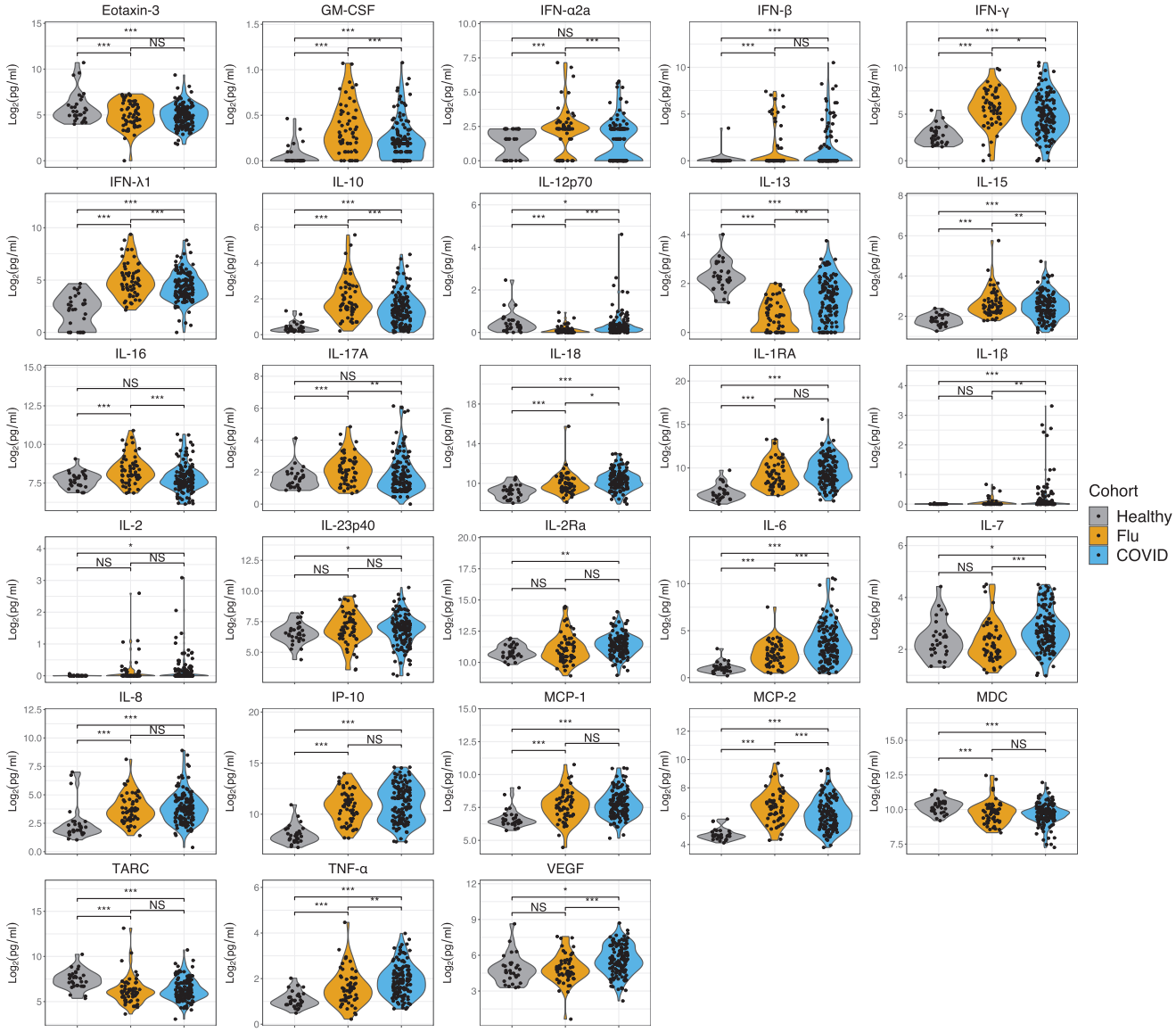

Figure 1. Cytokines and chemokines in influenza and COVID-19 compared with healthy controls. Differences between the COVID-19 cohort (blue) or influenza cohort (orange) and healthy controls (gray) were determined by 2-tailed *t* test after adjusting for sex and age. Differences between the COVID-19 cohort and influenza cohort for each analyte were determined by 2-tailed *t* test after adjusting for sex, age, and BMI. FDR was obtained using the Benjamini-Hochberg procedure. Statistical significance is indicated by NS, *, **, or *** above the brackets indicating FDR >0.25, <0.25, <0.1, or <0.05, respectively. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; FDR, false discovery rate; NS, not significant.

[39]. Though IL-10 was implicated in COVID-19 pathogenesis [15, 48, 49], IL-10 levels were actually higher in moderate influenza compared with COVID-19.

Plotting the correlation of each analyte with every other analyte in correlation matrices by disease revealed that many of the cytokines/chemokines elevated in influenza relative to COVID-19 strongly correlate, including IL-10, IFN-λ1, MCP-2, and IFN-γ. Similarly, those increased in COVID-19 relative to influenza positively correlate including IL-18, TNF-α, and IL-6 (Supplementary Figure 2*A* and 2*B*). A similar pattern emerged after generating a heatmap grouped by disease subgroup (Supplementary Figure 2*C*). These findings suggest that distinct inflammatory pathways are activated in these respiratory viral infections.

When we compared elevated analytes statistically by severity subgroups, we found minimal overlap in the cytokines/chemokines that distinguished severe from mild/moderate influenza and those that distinguished severe from mild/moderate COVID-19 (Figure 2A and 2B). IL-1RA, IL-1β, IL-2, IL-7, MCP-1, MCP-2, and VEGF were elevated in both severe diseases compared with their mild/moderate counterpart. Only IFN-β and IFN-λ1 were elevated in severe influenza, but not in severe COVID-19 (Figure 2 and Supplementary Table 1), consistent with low interferon responses in COVID-19. Cytokines

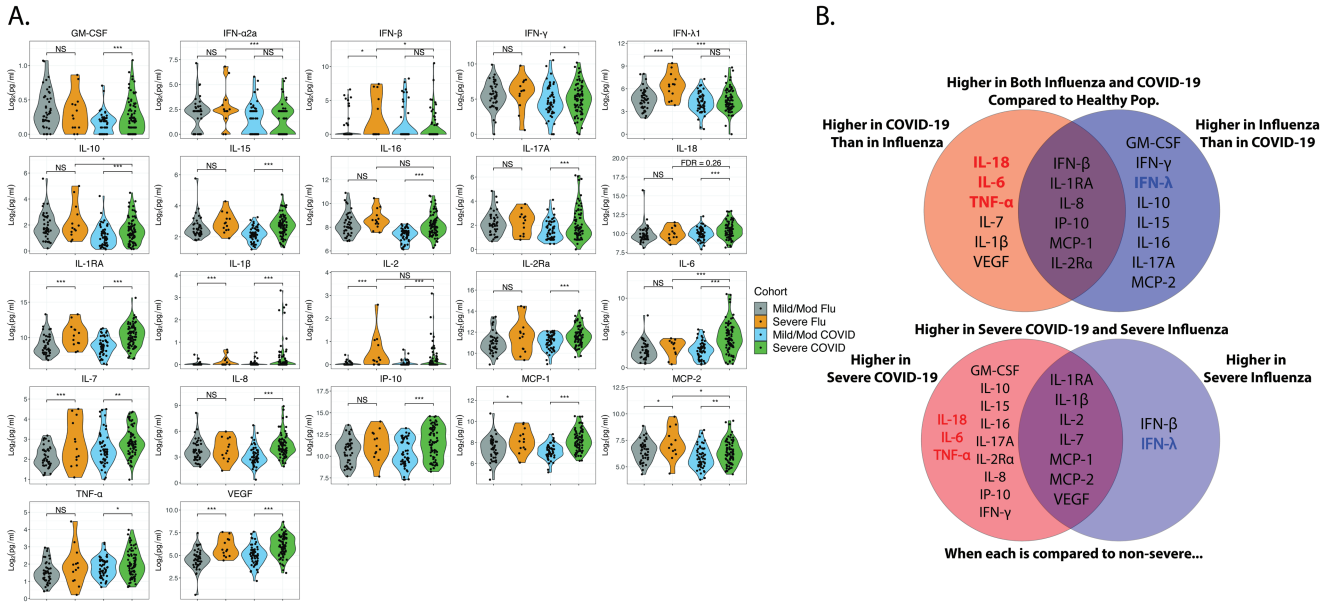

Figure 2. Cytokines and chemokines elevated in severe disease compared with mild/moderate disease and according to infection. *A*, Differences between disease severity subgroups were determined by 2-tailed *t* test after adjusting for sex, age, and BMI. Participants who received steroids before cytokine measurement were excluded. Differences between the severe COVID-19 cohort (green) and severe influenza cohort (orange) for each analyte were determined by 2-tailed *t* test after adjusting for sex, age, and BMI. False discovery rate (FDR) was obtained using the Benjamini-Hochberg procedure. Statistical significance is indicated by NS, *, **, or *** above the brackets indicating FDR >0.25, <0.25, <0.1, or <0.05 respectively. *B*, Top: Cytokines/chemokines higher in the COVID-19 cohort compared with influenza (left), influenza compared with COVID-19 (right) both COVID-19 and influenza compared with healthy controls, but not significantly different between COVID-19 and influenza (overlap center). Bottom: Cytokines/chemokines elevated in severe COVID-19 relative to mild/mod COVID-19 (left side), those elevated in severe influenza relative to mild/mod influenza (right side), and those that are elevated in severe forms of both diseases (overlap center). Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; NS, not significant.

elevated in severe COVID-19, but not severe influenza, include GM-CSF, IL-10, IL-15, IL-16, IL-17A, IL-18, IL-2Ra, IL-6, IL-8, and IP-10. Zero influenza and 22 COVID-19 participants received steroids before cytokine measurement. When excluding these, IFN-γ and TNF-α were also significantly elevated in severe COVID-19 (Figure 2A and 2B, Supplementary Figure 3, and Supplementary Table 3). Of the cytokines elevated in severe COVID-19, but not severe influenza, only IL-18, IL-6, and TNF-α were also elevated in the whole COVID-19 cohort compared with the whole influenza cohort (Figures 1 and 2). These 3 cytokines are highly associated with proinflammatory macrophages [50].

When comparing severe COVID-19 to severe influenza directly, only IL-6 was significantly higher in severe COVID-19, whether those who received steroids were included or not (Figure 2 and Supplementary Tables 1 and 3). When excluding steroid recipients, IL-18 narrowly missed our predetermined FDR cutoff for significance of 0.25 (FDR = 0.26) (Supplementary Tables 1 and 2). Elevated analytes higher in severe influenza compared with severe COVID-19 included IFN-λ1, IFN-α2a, IFN-β, IL-10, and MCP-2 (Figure 2 and Supplementary Table 5).

IL-6 and IFN-λ1 Are the Most Important Cytokines in Distinguishing Severe COVID-19 From Severe Influenza

To further characterize differences in the inflammatory pathways activated, we performed a multivariate analysis based on random forest using all the analytes and basic demographic information to compare severe COVID-19 and severe influenza. IL-6 and IFN-λ1 emerged as the most important factors distinguishing these 2 diseases in this analysis (Figure 3), with the highest fold changes between the severe subgroups. The importance of IL-6 and IFN-λ1 were confirmed when removing participants treated with steroids and when using a univariate analysis (Supplementary Figure 4 and Supplementary Methods). These findings underscore differences in the innate immune programs activated by these viruses; inflammatory macrophage activation pathways in COVID-19 and interferon pathways in influenza.

IL-1RA Is a Potential Mediator of the Effect of BMI on COVID-19 Severity

Previous studies demonstrated an association between BMI and elevation of multiple cytokines increased in severe COVID-19, including IL-6, IL-1β, and IL-1RA [27, 29, 51, 52]. Plotting BMI versus cytokine concentration demonstrated a positive association between IL-1RA, IL-23p40, MDC, IL-17A, and MCP-2 (Figure 4A and Supplementary Figure 5). With mediation analysis and after adjusting for multiple testing, diabetes, and heart disease, only IL-1RA had a significant mediation effect. Although the total effect of BMI on COVID-19 severity was 0.0078 (FDR = 0.13), most of the effect was indirectly through IL-1RA. Indeed, the direct effect of BMI on COVID-19 severity

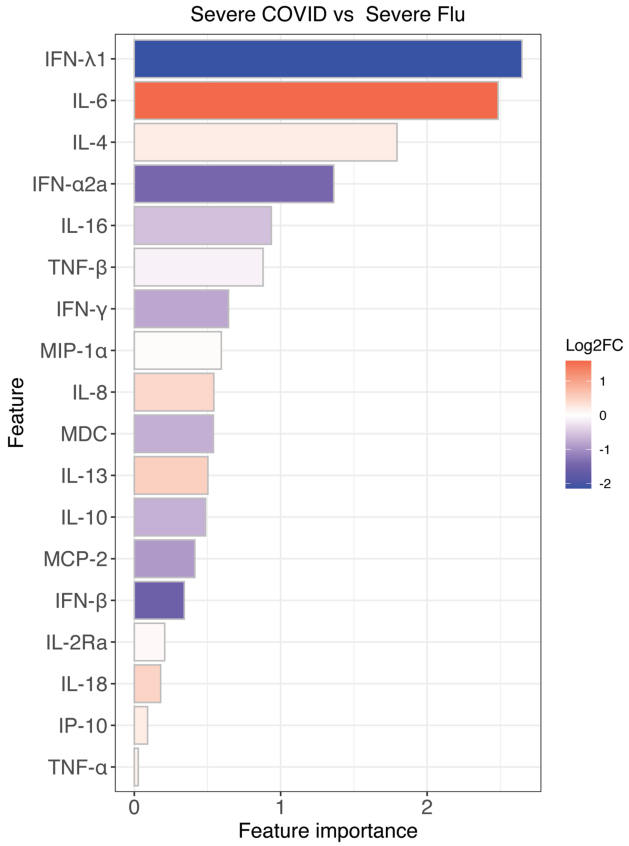

Figure 3. Multivariable analysis based on random forest revealed the most important variables in distinguishing severe COVID-19 and severe influenza. Feature importance was obtained from the random forest model for predicting severe COVID-19 versus severe influenza. The color indicates the $\log_2$ fold change of the analyte signal between severe COVID-19 and severe influenza. Red color indicates a higher value in COVID-19 and blue color indicates a higher value in influenza. Abbreviation: COVID-19, coronavirus disease 2019.

was minimal (0.0007, FDR = 0.88) compared with the indirect effect of BMI on severity through IL-1RA (0.0071, FDR <0.05). This suggests that the effect of increased BMI on the likelihood of severe COVID-19 may be mediated by IL-1RA (Figure 4B, Supplementary Table 6). Cardiovascular disease and diabetes are associated with elevated BMI, but we compared the mild/moderate and severe COVID-19 subgroups while adjusting for these conditions and found no differences in the cytokines that were significant between severe and mild/moderate COVID-19 (Supplementary Table 4). Analysis of the influenza cohort did not reveal any cytokines/chemokines mediating BMI and disease severity (Supplementary Table 7).

Given that advanced age is a risk factor for severe COVID-19, we also performed mediation analysis using age as the independent variable. Unlike BMI, numerous cytokines are potential mediators of the effect of age on severity including IL-10, IL-1RA, IL-2Ra, and MCP-1 (Supplementary Figure 6 and Supplementary Table 8).

DISCUSSION

Our analysis of cytokines and chemokines elevated in COVID-19 compared with influenza reveals distinct cytokine profiles of these respiratory diseases. We found that several cytokines previously reported to be elevated in COVID-19 were not different from or were higher in influenza than in COVID-19, including IFN-γ, IL-10, and IL-15 [15]. Some cytokines (GM-CSF, IL-10, IL-15, IL-16, and IL-17A) that were higher in influenza than COVID-19 overall and distinguished severe COVID-19 from moderate COVID-19 did not differ between severe and moderate influenza. Influenza infection generally induces high

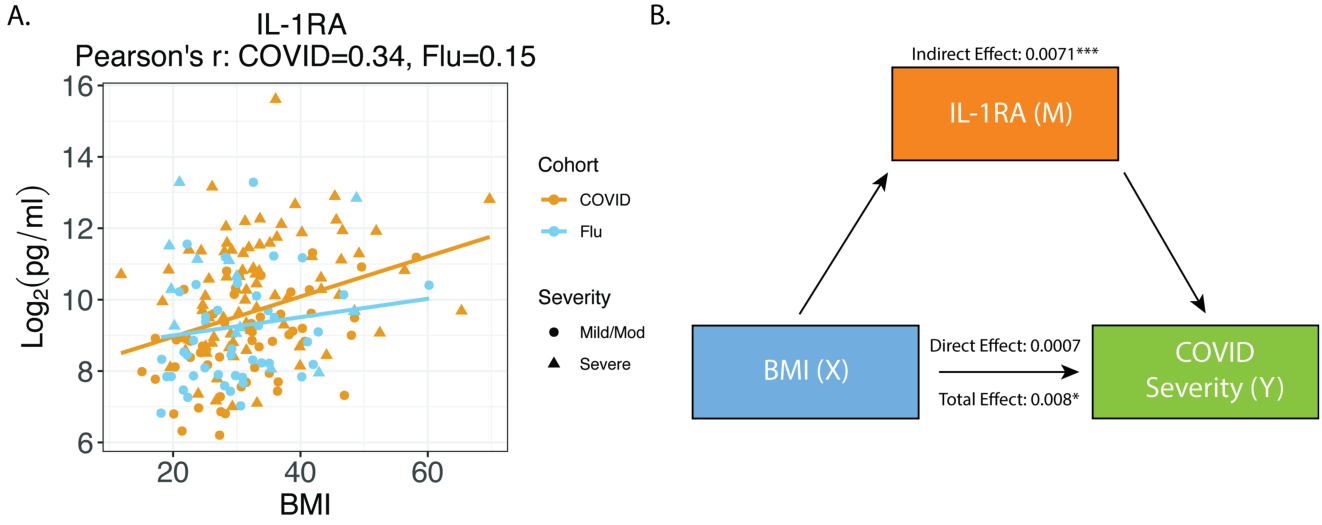

Figure 4. Mediation analysis of BMI and IL-1RA on COVID-19 severity. *A*, BMI (X axis) was plotted versus IL-1RA level (Y axis). The yellow line is a linear regression line for the COVID-19 cohort and the blue line is a linear regression line for the influenza cohort. The Pearson's correlation coefficient between IL-1RA and BMI is 0.34 for the COVID-19 cohort and 0.15 for the influenza cohort. *B*, Diagram of the mediation analysis of BMI and IL-1RA on COVID-19 severity while controlling for diabetes and heart disease. The indirect, direct, and total effects were showed in the diagram. Statistical significance is indicated by *, **, or *** representing FDR <0.25, <0.1, or <0.05, respectively. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; FDR, false discovery rate; IL, interleukin.

levels of these cytokines and severe influenza is not marked by additional elevations.

Another study also found that many cytokines were either not different or were significantly less elevated in COVID-19 versus influenza [53]. Consistent with their results, we found that IFN-γ and IL-17A were elevated in influenza. They found significant increases in IL-1RA, IL-2, and MIP-1α in their influenza cohort relative to COVID-19, whereas we did not. These differences may be due to our adjustment for BMI, our larger cohort size, different platforms used to measure cytokines, or that a higher percentage of their influenza patients had severe disease. They observed a trend toward increased IL-6 in the COVID-19 group compared with influenza and we found high IL-6 to be one of the most distinguishing cytokines in COVID-19. They did not measure IL-18 or IFN-λ1, important in distinguishing severe COVID-19 and severe influenza in our analysis. Although the "cytokine storm" hypothesis was proposed to explain the pathology observed in COVID-19, our study and others demonstrate lower overall cytokine levels in COVID-19 than those observed in other inflammatory diseases [17, 18, 53, 54].

Both influenza and SARS-CoV-2 are respiratory RNA viruses, but our study emphasizes that they induce distinct inflammatory pathways. We found that severe COVID-19 leads to upregulation of cytokines associated with a proinflammatory macrophage phenotype [55] characterized by high levels of IL-6, TNF-α, and IL-18, whereas interferons and cytokines involved in T-cell activation (IL-15, IL-16, and IFN-γ) are upregulated in influenza. IL-18 and IL-1β, which is difficult to detect in blood, are released from macrophages upon activation of a component of the innate immune system called the inflammasome [43, 45, 56]. Emerging evidence suggests inflammasome activation is central to the SARS-CoV-2 pathogenesis and marks severe disease [8, 12, 57, 58]. Our study provides additional evidence that the inflammasome is activated in SARS-CoV-2 infection. High IL-6 and low IFN-λ1 were the most distinct features of severe COVID-19 compared with severe influenza, consistent with results of another study of COVID-19 and influenza patients [47]. We do not know if these cytokine disparities mediate pathology differences or are merely correlates of other distinct immune responses. Although steroids have proven beneficial in later stages of the disease in patients with COVID-19 requiring oxygen, early immunosuppression is not beneficial [59]. Given the association we observed with severe COVID-19 and a proinflammatory macrophage phenotype, targeting of the cytokines mediating macrophage activation syndrome, singly or in combination, might provide a more specific approach to immune modulation. Both targeted anti-IL-6 therapy and IL-1 antagonists are associated with benefit in some studies. [60–71].

Another strength of our analysis is the focus on BMI as a contributor to severe disease. Although this association has been widely described, few analyses of inflammatory cytokines have taken this variable into account [72]. We found that IL-1RA is a potential mediator of the effect of BMI on COVID-19 disease severity. This novel observation points to a possible mechanism linking BMI to severe COVID-19. IL-1RA is an acute phase reactant produced by adipocytes, macrophages, and the liver in response to inflammatory cytokines and pathogens through pathways that upregulate IL-6 and TNF-α [73, 74]. This was an unexpected finding given the anti-inflammatory nature of this cytokine and the role of IL-1RA in pathogenesis warrants additional investigation. Inflammation is an important component of aging [75]. In contrast to IL-1RA being the sole potential cytokine mediator of the effect of BMI on disease severity, we identified numerous potential cytokine mediators of the effect of age on disease severity, highlighting the specificity of our association between IL-1RA, BMI, and COVID-19 severity.

Limitations to our study include that outpatients with milder COVID-19 were not included. However, we would predict that differences between those with and without severe disease in our study would be more significant if milder disease were included. In addition, our study participants with influenza also required hospitalization, making them an appropriate comparator. An additional limitation is that we examined a single timepoint, and that the time from admission to sampling was not identical, but principal component analysis revealed similar inflammatory states. It is possible that dynamic changes in these cytokines during the course of hospitalization would make the patterns more or less distinct from influenza. However, as patients remain hospitalized, complications from critical illness arise that could obfuscate this comparison. Finally, although the influenza cohort was, on average, younger than the COVID-19 cohort, we adjusted for age in our analysis.

This study provides insight into pathways activated by SARS-CoV-2 and influenza, demonstrating that some inflammatory cytokines elevated in COVID-19 likely reflect common pathways activated in respiratory tract inflammation, whereas others are more specific to COVID-19 pathogenesis. In summary, this study demonstrates activation of a proinflammatory cytokine macrophage pathway and a role for IL-1RA in the effect of BMI on severe COVID-19, highlighting potential therapeutic targets.

Notes

Acknowledgments. We acknowledge the contribution of the Johns Hopkins IVAR team and assistance for clinical data coordination and retrieval from the Core for Clinical Research Data Acquisition. The specimens from COVID-19 patients used for this study were part of the Johns Hopkins Biospecimen Repository, which is based on the contribution of many patients, their families, research teams, and clinicians. We thank members of the Viral Hepatitis Center at Johns Hopkins for advice and discussion and blood processing. We owe additional thanks to Sherry Kelly Gibson, Muhammad Munir, Tingtin Niu, Nikitta Dhillon, Dan Warren, Robin Avery, and Niraj Desai for assistance with the study.

Financial support. This work was supported by the Johns Hopkins COVID-19 Research Response Program, a Johns Hopkins University Provost

Research Grant, The Bill and Melinda Gates Foundation (134582), National Cancer Institute (U54CA260491), and National Institutes of Health Centers of Excellence in Influenza Research and Surveillance (HHSN272201400007C). The study was supported in part by a cooperative agreement between Johns Hopkins University and the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, the US Department of Health and Human Services Biomedical Advanced Research and Development Authority (agreement number IDSEP160031-01-00), the National Institute of Allergy and Infectious Diseases U19AI088791 and R01AI108403, and National Institute of Allergy and Infectious Diseases contract HHSN272201400007C awarded to Johns Hopkins Center of Excellence in Influenza Research and Surveillance. H. J. and W. Z. were supported in part by National Institute of Health grant R01HG009518. A. H. K. was supported by the National Institute of Health T32 AI007291-27. A. F. was supported, in part, by grant D18HP29037 from the US Health Resources and Services Administration, Bureau of Health Workforce, Health Careers Opportunity Program. R. E. R. was supported in part by the National Institute of Allergy and Infectious Diseases contract HHSN272201400007C awarded to the Johns Hopkins Center of Excellence in Influenza Research and Surveillance at the Johns Hopkins University and US Department of Health and Human Services Biomedical Advanced Research and Development Authority (agreement number IDSEP160031-01-00).

Potential conflicts of interest. None of the authors has any relevant conflict of interests to disclose.

References

1. COVID-19 map. Available at: https://coronavirus.jhu.edu/map.html. Accessed 15 June 2020.
2. Argenziano MG, Bruce SL, Slater CL, et al. Characterization and clinical course of 1000 patients with coronavirus disease 2019 in New York: retrospective case series. BMJ **2020**; 369:m1996.
3. Chau NVV, Thanh Lam V, Thanh Dung N, et al. The natural history and transmission potential of asymptomatic SARS-CoV-2 infection. Clin Infect Dis. **2020**; 71:2679–87.
4. Zhang X, Tan Y, Ling Y, et al. Viral and host factors related to the clinical outcome of COVID-19. Nature **2020**; 583:437–40.
5. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host & Microbe **2020**; 0. Available at: https://www.cell.com/cell-host-microbe/abstract/S1931-3128(20)30236-5. Accessed 23 April 2020.
6. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet **2020**; 395:497–506.
7. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest **2020**; 130:2620–9.
8. Lucas C, Wong P, Klein J, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature **2020**; 584:463–9.
9. Leisman DE, Ronner L, Pinotti R, et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. Lancet Respir Med **2020**; 8: 1233–44.
10. Silvin A, Chapuis N, Dunsmore G, et al. Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. Cell **2020**; 182:1401–1418.e18.
11. Mandel M, Harari G, Gurevich M, Achiron A. Cytokine prediction of mortality in COVID19 patients. Cytokine **2020**; 134:155190.
12. Young BE, Ong SWX, Ng LFP, et al. Viral dynamics and immune correlates of coronavirus disease 2019 (COVID-19) severity. Clin Infect Dis **2021**; 73:e2932–42.
13. Del Valle DM, Kim-Schulze S, Huang H-H, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med **2020**; 26:1636–43.
14. Han H, Ma Q, Li C, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg Microbes Infect **2020**; 9:1123–30.
15. Zhao Y, Qin L, Zhang P, et al. Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. JCI Insight **2020**; 5. Available at: https://doi.org/10.1172/jci.insight.139834. Accessed 9 July 2020.
16. Wilson JG, Simpson LJ, Ferreira A-M, et al. Cytokine profile in plasma of severe COVID-19 does not differ from ARDS and sepsis. JCI Insight **2020**; 5. Available at: https://doi.org/10.1172/jci.insight.140289. Accessed 10 August 2020.
17. Kox M, Waalders NJB, Kooistra EJ, Gerretsen J, Pickkers P. Cytokine levels in critically ill patients with COVID-19 and other conditions. JAMA **2020**; 324:1565–7.
18. Sinha P, Matthay MA, Calfee CS. Is a "cytokine storm" relevant to COVID-19? JAMA Intern Med **2020**; 180:1152–4.
19. Krammer F, Smith GJD, Fouchier RAM, et al. Influenza. Nat Rev Dis Primers **2018**; 4:1–21.
20. Betakova T, Kostrabova A, Lachova V, Turianova L. Cytokines induced during influenza virus infection. Curr Pharm Des **2017**; 23:2616–22.
21. Bradley-Stewart A, Jolly L, Adamson W, et al. Cytokine responses in patients with mild or severe influenza A(H1N1)pdm09. J Clin Virol **2013**; 58:100–7.
22. Short KR, Kroeze EJBV, Fouchier RAM, Kuiken T. Pathogenesis of influenza-induced acute respiratory distress syndrome. Lancet Infect Dis **2014**; 14:57–69.
23. Garibaldi BT, Fiksel J, Muschelli J, et al. Patient trajectories among persons hospitalized for COVID-19. Ann Intern Med **2021**; 174:33–41.
24. Goyal P, Choi JJ, Pinheiro LC, et al. Clinical characteristics of Covid-19 in New York City. New Engl J Med **2020**; 382:2372–4.
25. Hendren NS, de Lemos JA, Ayers C, et al. Association of body mass index and age with morbidity and mortality in patients hospitalized with COVID-19. Circulation **2021**; 143:135–44.
26. Garibaldi BT, Wang K, Robinson ML, et al. Comparison of time to clinical improvement with vs without remdesivir treatment in hospitalized patients with COVID-19. JAMA Netw Open **2021**; 4:e213071.
27. Charles BA, Doumatey A, Huang H, et al. The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. J Clin Endocrinol Metab **2011**; 96:E2018–22.
28. Schmidt FM, Weschenfelder J, Sander C, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. PLoS One **2015**; 10:e0121971.
29. Juge-Aubry CE, Somm E, Giusti V, et al. Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. Diabetes **2003**; 52:1104–10.
30. World Health Organization. R&D Blueprint: novel coronavirus: COVID-19 therapeutic trial synopsis. **2020**.
31. Cox AL, Netski DM, Mosbruger T, et al. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. Clin Infect Dis **2005**; 40:951–8.
32. Dugas AF, Hsieh YH, LoVecchio F, et al; Emergency Department National Influenza Network Investigators. Derivation and validation of a clinical decision guideline for influenza testing in 4 US emergency departments. Clin Infect Dis **2020**; 70:49–58.
33. R: The R Project for Statistical Computing. Available at: https://www.r-project.org/. Accessed 4 January 2021.
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc Series B (Methodological) **1995**; 57:289–300.
35. Kuhn M, Wing J, Weston S, et al. caret: classification and regression training. **2020**. Available at: https://CRAN.R-project.org/package=caret. Accessed 4 January 2021.
36. Liaw A, Wiener M. Classification and regression by randomForest. R News **2002**; 2:5.
37. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. mediation: R Package for causal mediation analysis. Journal of Statistical Software **2014**; 59:1–38.
38. Chattergoon MA, Levine JS, Latanich R, Osburn WO, Thomas DL, Cox AL. High plasma interleukin-18 levels mark the acute phase of hepatitis C virus infection. J Infect Dis **2011**; 204:1730–40.
39. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell **2020**; 181:1036–1045.e9.
40. Broggi A, Ghosh S, Sposito B, et al. Type III interferons disrupt the lung epithelial barrier upon viral recognition. Science **2020**; 369:706–12.
41. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. Front Immunol **2014**; 5:491.
42. Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine-directed therapies. Annu Rev Med **2015**; 66:145–59.
43. Evavold CL, Kagan JC. Inflammasomes: threat-assessment organelles of the innate immune system. Immunity **2019**; 51:609–24.
44. Mantovani A, Dinarello CA, Molgora M, Garlanda C. Interleukin-1 and related cytokines in the regulation of inflammation and immunity. Immunity **2019**; 50:778–95.
45. Kuriakose T, Kanneganti TD. Regulation and functions of NLRP3 inflammasome during influenza virus infection. Mol Immunol **2017**; 86:56–64.
46. Chattergoon MA, Latanich R, Quinn J, et al. HIV and HCV activate the inflammasome in monocytes and macrophages via endosomal Toll-like receptors without induction of type 1 interferon. PLoS Pathog **2014**; 10:e1004082.
47. Galani IE, Rovina N, Lampropoulou V, et al. Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. Nat Immunol **2021**; 22:32–40.
48. Lu L, Zhang H, Dauphars DJ, He Y-W. A potential role of interleukin-10 in COVID-19 pathogenesis. Trends Immunol **2021**; 42:3–5.

49. Laing AG, Lorenc A, Del Molino Del Barrio I, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. Nat Med **2020**; 26:1623–35.
50. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun Rev **2020**; 19:102537.
51. Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM. IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? J Clin Endocrinol Metab **2002**; 87: 1184–8.
52. Stienstra R, Tack CJ, Kanneganti TD, Joosten LA, Netea MG. The inflammasome puts obesity in the danger zone. Cell Metab **2012**; 15:10–8.
53. Mudd PA, Crawford JC, Turner JS, et al. Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm. Sci Adv **2020**; 6:eabe3024.
54. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet **2020**; 395:1033–4.
55. Liao M, Liu Y, Yuan J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med **2020**; 26:842–4.
56. Yap JKY, Moriyama M, Iwasaki A. Inflammasomes and pyroptosis as therapeutic targets for COVID-19. J Immunol **2020**; 205:307.
57. Lee JS, Park S, Jeong HW, et al. Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. Sci Immunol **2020**; 5:eabd1554.
58. Rodrigues TS, de Sá KSG, Ishimoto AY, et al. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. J Exp Med **2021**; 218. Available at: https://doi.org/10.1084/jem.20201707. Accessed 28 July 2021.
59. The RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med **2021**; 384:693–704.
60. Gupta S, Wang W, Hayek SS, et al. Association between early treatment with tocilizumab and mortality among critically ill patients with COVID-19. JAMA Intern Med **2021**; 181:41–51.
61. Salama C, Han J, Yau L, et al. Tocilizumab in patients hospitalized with Covid-19 pneumonia. N Engl J Med **2021**; 384:20–30.
62. Ignatius EH, Wang K, Karaba A, et al. Tocilizumab for the treatment of COVID-19 among hospitalized patients: a matched retrospective cohort analysis. Open Forum Infectious Diseases **2021**; 8. Available at: https://doi.org/10.1093/ofid/ofaa598. Accessed 28 July 2021.
63. Cauchois R, Koubi M, Delarbre D, et al. Early IL-1 receptor blockade in severe inflammatory respiratory failure complicating COVID-19. Proc Natl Acad Sci U S A **2020**; 117:18951–3.
64. Dimopoulos G, Mast Q de, Markou N, et al. Favorable anakinra responses in severe Covid-19 patients with secondary hemophagocytic lymphohistiocytosis. Cell Host & Microbe **2020**; 28:117–123.e1.
65. Huet T, Beaussier H, Voisin O, et al. Anakinra for severe forms of COVID-19: a cohort study. Lancet Rheumatol **2020**; 2:e393–400.
66. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. The Lancet Rheumatology **2020**; 2:e474–84.
67. Morena V, Milazzo L, Oreni L, et al. Off-label use of tocilizumab for the treatment of SARS-CoV-2 pneumonia in Milan, Italy. Eur J Intern Med **2020**; 76:36–42.
68. Hossen MdS, Barek MA, Jahan N, Safiqul Islam M. A review on current repurposing drugs for the treatment of COVID-19: reality and challenges. SN Compr Clin Med **2020**; 2:1777–89.
69. Salvarani C, Dolci G, Massari M, et al. Effect of tocilizumab vs standard care on clinical worsening in patients hospitalized with COVID-19 pneumonia: a randomized clinical trial. JAMA Intern Med **2021**; 181:24–31.
70. Hermine O, Mariette X, Tharaux P-L, et al. Effect of tocilizumab vs usual care in adults hospitalized with COVID-19 and moderate or severe pneumonia: a randomized clinical trial. JAMA Intern Med **2021**; 181:32–40.
71. Stone JH, Frigault MJ, Serling-Boyd NJ, et al; BACC Bay Tocilizumab Trial Investigators. Efficacy of tocilizumab in patients hospitalized with Covid-19. N Engl J Med **2020**; 383:2333–44.
72. Popkin BM, Du S, Green WD, et al. Individuals with obesity and COVID-19: a global perspective on the epidemiology and biological relationships. Obes Rev **2020**; 21:e13128.
73. Rehani K, Wang H, Garcia CA, Kinane DF, Martin M. Toll-like receptor-mediated production of IL-1Ra is negatively regulated by GSK3 via the MAPK ERK1/2. J Immunol **2009**; 182:547–53.
74. Gabay C, Smith MF, Eidlen D, Arend WP. Interleukin 1 receptor antagonist (IL-1Ra) is an acute-phase protein. J Clin Invest **1997**; 99:2930–40.
75. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nature Reviews Cardiology **2018**; 15:505–522.