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Contribution of Coronavirus-Specific Immunoglobulin G Responses to Complement Overactivation in Patients with Severe Coronavirus Disease 2019

Priscila M. S. Castanha,[1] Dylan J. Tuttle,[1] Georgios D. Kitsios,[2,3,4] Jana L. Jacobs,[5] Ulisses Braga-Neto,[6] Matthew Duespohl,[1] Sanjay Rathod,[7] Michelle M. Marti,[1] Sarah Wheeler,[8] Asma Naqvi,[5] Brittany Staines,[5] John Mellors,[5] Alison Morris,[2,4] Bryan J. McVerry,[2,3,4] Faraaz Shah,[3] Caitlin Schaefer,[2] Bernard J. C. Macatangay,[5] Barbara Methe,[2,4] Christian A. Fernandez,[7] Simon M. Barratt-Boyes,[1,9] Donald Burke,[10] Ernesto T. A. Marques[1]

[1]Department of Infectious Diseases and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [2]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [3]Acute Lung Injury Center of Excellence, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [4]Center for Medicine and the Microbiome, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [5]Division of Infectious Diseases, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [6]Department of Electrical and Computer Engineering, Texas A&M University, College Station, Texas, USA, [7]Center for Pharmacogenetics, Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [8]Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [9]Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA and [10]Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Background. Excessive complement activation has been implicated in the pathogenesis of coronavirus disease 2019 (COVID-19), but the mechanisms leading to this response remain unclear.

Methods. We measured plasma levels of key complement markers, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA and antibodies against SARS-CoV-2 and seasonal human common cold coronaviruses (CCCs) in hospitalized patients with COVID-19 of moderate (n = 18) and critical severity (n = 37) and in healthy controls (n = 10).

Results. We confirmed that complement activation is systemically increased in patients with COVID-19 and is associated with a worse disease outcome. We showed that plasma levels of C1q and circulating immune complexes were markedly increased in patients with severe COVID-19 and correlated with higher immunoglobulin (Ig) G titers, greater complement activation, and higher disease severity score. Additional analyses showed that the classical pathway was the main arm responsible for augmented complement activation in severe patients. In addition, we demonstrated that a rapid IgG response to SARS-CoV-2 and an anamnestic IgG response to the nucleoprotein of the CCCs were strongly correlated with circulating immune complex levels, complement activation, and disease severity.

Conclusions. These findings indicate that early, nonneutralizing IgG responses may play a key role in complement overactivation in severe COVID-19. Our work underscores the urgent need to develop therapeutic strategies to modify complement overactivation in patients with COVID-19.

Keywords. SARS-CoV-2; COVID-19; complement system; classical pathway; common cold coronaviruses; and antibodies.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in December 2019 and quickly spread throughout the globe, causing the coronavirus disease 19 (COVID-19) pandemic. Severe COVID-19 has been correlated with activation of the complement system, as indicated by the presence of low levels of C3 and high levels of the anaphylatoxin C3a, a cleavage product of C3 [1–9]. Recent studies have found deposits of complement factors (C1q, C4, C3, soluble C5-9 [sC5-9], and factor B) in lung tissue [10, 11] and in the kidneys [12] of patients with severe COVID-19. In addition, overactivation of the complement system has been strongly associated with higher mortality risk [4, 7, 8, 13]. Nevertheless, the mechanisms driving overactivation of the complement system in severe COVID-19 are poorly understood.

The complement system comprises a complex network of proteins, forming a highly regulated cascade of reactions. The system is activated through 3 pathways: the classical, the lectin, and the alternative pathway. Activation of the classical pathway is mediated by the binding of C1q to immune complexes or by binding directly to pathogen surfaces. It has been hypothesized that excessive immune complexes could be the cause of overactivation of the complement system leading to severe COVID-19 [14], but this has not yet been tested. It is well established that severe COVID-19 is correlated with faster and more intense binding and neutralizing antibody response; however,

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Correspondence: Ernesto T. A. Marques, 2131 Public Health Bldg, 130 DeSoto St, University of Pittsburgh, Pittsburgh, PA, 15261 (marques@pitt.edu).

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the nature and role of these antibodies in activating complement and causing severe disease have not been characterized. Moreover, several reports indicate that a significant fraction of plasmablasts, antibodies and $CD4^+$ T cells generated in response to SARS-CoV-2 infection are cross-reactive with the seasonal human common cold coronaviruses (CCCs) [15–20], but the impact of these anamnestic immune response on protection and disease is not well understood.

Here we report a comprehensive analysis of the complement and antibody response to SARS-CoV-2 infection in a cohort of moderately and critically ill patients hospitalized with COVID-19. We demonstrate that systemic complement activation is highly correlated with disease severity. Our findings reveal that overactivation of the complement system is mediated by the classical pathway in response to increased levels of circulating immunoglobulin (Ig) G immune complexes. Antibody responses to SARS-CoV-2 develop faster and at greater levels in patients with more severe COVID-19. Importantly, we show that antibody responses to the nucleoprotein (NP) of CCCs are boosted in patients with more severe COVID-19 and that high levels of IgG anti-NP against CCCs are associated with complement activation via the classical pathway.

METHODS

Patients and Clinical Samples

The study design and data collection for the patients with COVID-19 included in this study have been described elsewhere [21, 22]. Briefly, we enrolled patients with COVID-19 hospitalized at the University of Pittsburgh Medical Center Presbyterian and Shadyside hospitals (April–September 2020) in an observational study. We included 55 adult patients (≥18 years old) who were hospitalized either in an intensive care unit (ICU) or in dedicated hospital wards (non-ICU) for COVID-19. SARS-CoV-2 infections were documented by positive quantitative polymerase chain reaction (PCR) test results, using nasopharyngeal swab samples. Demographic and clinical variables and plasma samples were collected at day 1 after enrollment for all patients. Prepandemic plasma samples (n = 10) were obtained from healthy adults and included as controls (healthy controls [HCs]) for the complement protein measurements. We also included samples from patients enrolled in the ongoing University of Pittsburgh Acute Lung Injury Registry study [23] (n = 15; Supplementary Table 1) for comparisons of CCC antibody response. These patients were hospitalized in the ICU and met the diagnostic criteria for acute respiratory distress syndrome but tested negative for SARS-CoV-2 infection (by PCR and serology).

Laboratory Assays

Plasma levels of complement proteins C3, C3a, C5, C1q, factor H, and sC5b-9 were measured using in house quantitative enzyme-linked immunosorbent assays (ELISAs). Plasma levels of complement factor D and C5a (R&D Systems), human mannose-binding lectin (Hycult Biotech), and C1 inhibitor, Bb, and CIC-C1q (Quidel) were measured using commercially available ELISA kits, following the manufacturer's instructions. Binding IgG and IgA to SARS-COV-2, and IgG to CCCs (NL63, 229E, OC43, and HKU1) and severe acute respiratory syndrome coronavirus (SARS-CoV) were measured using indirect ELISAs. Plaque reduction neutralization test for SARS-CoV-2 was performed in the University of Pittsburgh Regional Biocontainment Laboratory biosafety level 3 facility. Laboratory procedures are described in detail in the Supplementary Materials.

Statistical Analysis

Comparisons among HCs and ICU and non-ICU patients were performed using nonparametric tests (Mann-Whitney, Kruskal-Wallis, or Fisher exact tests, as appropriate). Spearman correlation was used to measure the degree of association between 2 variables. We performed a receiver operating characteristic curve analysis for complement activation based on the C3a/C3 ratio as a predictor of COVID-19 severity (non-ICU versus ICU). The length of hospital stay was estimated using unadjusted Kaplan-Meier curve analyses. Statistical analyses were not corrected for confounders owing to the small sample size. Data were analyzed with Prism software version 9.0a (GraphPad). Differences were considered significant at $P < .05$.

We used linear discriminant analysis (LDA) to design a classifier for COVID-19 severity using complement markers and antibody levels. LDA classifiers were obtained using least-squares fitting, shrinkage, and equal priors. The classification errors were estimated using the error count on the training data and bolstered resubstitution estimates using gaussian kernels [24–27]. Log was applied to all features, and missing values were omitted from the analysis.

Study Approvals

All participants signed written informed consent forms for the study before participation. The Institutional Review Board of the University of Pittsburgh reviewed and approved all study protocols for the COVID-19 observational studies (STUDY19050099 and STUDY20040036) and for the prepandemic samples (STUDY19060213).

RESULTS

Markedly Increased Complement Activation in Patients With Severe COVID-19

To investigate the degree of systemic complement activation, we quantified plasma levels of soluble complement proteins and their cleavage products in 55 patients with COVID-19. Baseline demographic and clinical characteristics of 37 critically ill (ICU) and 18 moderately ill (non-ICU) patients are shown in Table 1. As expected, ICU patients presented with higher World

Table 1. Descriptive Characteristics of Patients with Coronavirus Disease 2019, Stratified by Disease Severity

Characteristics	All Patients (n = 55)	Moderately Ill (Non-ICU) Patients (n = 18)	Critically Ill (ICU) Patients (n = 37)	*P* Value[a]
Age, median (IQR), y	62 (53–69)	59 (52–66)	63 (57–73)	.16
Male sex, no. (%)	30 (54.5)	7 (38.9)	23 (62.1)	.15
White race, no. (%)	38 (69.1)	15 (83.3)	23 (67.6)	.33
Comorbid conditions, no. (%)				
Diabetes mellitus	20 (36.4)	5 (27.8)	15 (40.5)	.39
Active neoplasm	3 (5.4)	2 (11.1)	1 (2.7)	.25
Obesity (BMI ≥30)[b]	39 (70.9)	13 (72.2)	26 (70.3)	>.99
Congestive heart failure	4 (7.3)	0 (0.0)	4 (10.8)	.29
Chronic obstructive pulmonary disease	11 (20.0)	1 (5.6)	10 (27.0)	.08
Chronic renal failure	8 (14.5)	1 (5.6)	7 (18.9)	.25
Smoking (current or former), no (%)	27 (49.0)	6 (33.3)	21 (56.7)	.15
WHO score, median (IQR)				
Score at admission	5 (5–5)	4 (4–5)	5 (5–5.5)	<.001[c]
Worst score during hospitalization	6 (5–9)	5 (5–5)	9 (6–10)	<.001[c]
90-d Mortality, no. (%)	12 (21.8)	0 (0.0)	12 (32.4)	.005[c]
Time from symptom onset to sampling, median (IQR), d	10 (5–12)	7 (5–11)	10 (6–13)	.24

Abbreviations: BMI, body mass index; ICU, intensive care unit; IQR, interquartile range; WHO, World Health Organization.

[a]Nonparametric test comparisons were performed using Mann-Whitney and Fisher exact tests for continuous and categorical variables, respectively.

[b]BMI calculated as weight in kilograms divided by height in meters squared.

[c]Significant at $P < .05$.

Health Organization 10-point ordinal scale of severity scores at admission and during the period of hospitalization. They also had a significantly higher 90-day mortality rate than non-ICU patients (28.2% vs 0%, respectively; $P = .005$). There was no significant difference in age ($P = .16$), sex ($P = .15$), comorbid conditions, and time from symptom onset to sample collection between non-ICU and ICU patients ($P = .24$).

Plasma levels of C3 were markedly reduced in ICU patients compared with non-ICU patients and HCs, indicating increased C3 consumption in patients with severe COVID-19. C3a levels were increased in patients with COVID-19 compared with HCs and approximately 100-fold greater in patients with severe COVID-19 compared with non-ICU patients. Similarly, the ratio between C3a and C3 was markedly increased in ICU patients (Figure 1A). C5 levels were significantly reduced in ICU patients, and the levels of C5a and C5a/C5 ratio were significantly increased in ICU compared with non-ICU patients (Figures 1B). Plasma levels of sC5b-9, the activation product of the terminal complement cascade, were significantly elevated in patients with COVID-19 relative to those in HCs (Figure 1C), though no significant differences were evident based on illness severity.

We next performed a receiver operating characteristic analysis for C3a/C3 ratio as a predictor for COVID-19 severity (Figure 2A). A threshold for C3a/C3 ratio of ≥4.5 ($\log_{10}$) classified patients in high and low complement activation and provided a sensitivity and specificity for COVID-19 severity of 94.4% and 70.6%, respectively. We then analyzed the relationship between complement activation, viral RNA levels, and clinical laboratory markers of inflammation. Data on viral load was available for a total of 45 patients (16 non-ICU and 29 ICU patients) [28]. We found that plasma levels of SARS-CoV-2 RNA were highest in patients classified as having high complement activation (Figure 2B). Patients with high C3a/C3 ratios also had significantly higher plasma levels of proinflammatory cytokines IL-6 and IL-8 (Figure 2C) and procalcitonin and suppression of tumorigenicity (Supplementary Figure 1). Levels of fractalkine, pentraxin 3, tumor necrosis factor receptor 1, and receptor for advanced glycation end products were not associated with C3a/C3 ratios (Supplementary Figure 1).

We then assessed the relationship between complement activation and clinical parameters of disease severity. High levels of complement activation were significantly associated with worse 90-day survival (log-rank $P = .004$) and longer time to discharge from the hospital (log-rank $P = .002$; Figure 2D). When restricted to the group of patients with severe COVID-19, high complement activation was associated with an increased risk of intubation (odds ratio, 7.00 [95% confidence interval, 1.47–29.21; $P = .02$) and with a greater 90-day mortality risk (7.03 [1.38–34.4]; $P = .02$). Collectively, these data indicate that the complement cascade is activated in patients hospitalized with COVID-19 and that overactivation of the complement system is correlated with greater viral burden, increased systemic inflammation, and worse disease outcomes.

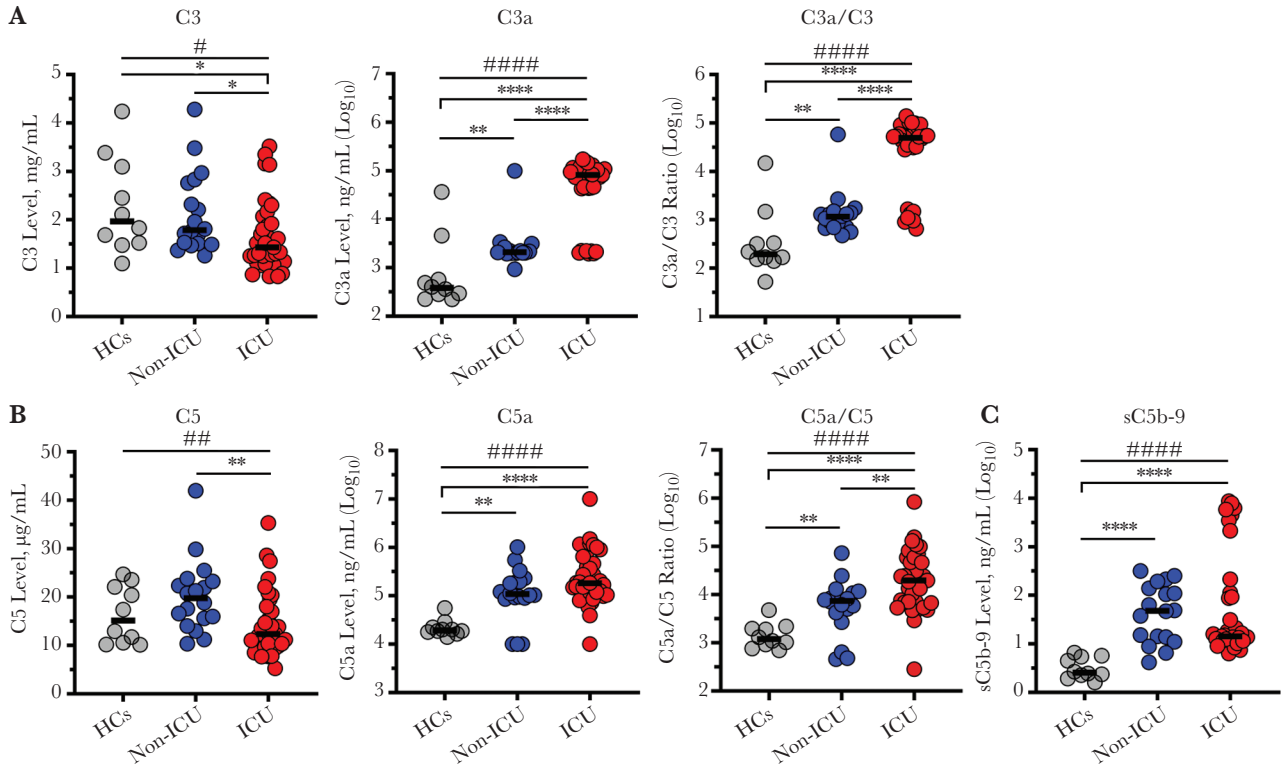

Figure 1. Markers of complement activation are increased in patients with coronavirus disease 2019 (COVID-19) and are associated with disease severity. Plasma levels of various markers of complement activation were quantified in critically ill patients hospitalized in intensive care units (ICU patients; n = 37) and moderately ill patients hospitalized in dedicated COVID-19 wards (non-ICU patients; n = 18). Plasma samples from healthy controls (HCs; n = 10) were included as a reference population for comparisons. Scatterplots show the differences between HCs and non-ICU and ICU patients in the following plasma levels and ratios: C3, C3a, and C3a/C3 (*A*); C5, C5a, and C5a/C5 ratio (*B*); and soluble C5b-9 (sC5b-9) (*C*). Each symbol in the scatterplots represents an individual patient, and horizontal lines represent medians. Statistical significance was determined using Kruskal-Wallis 1-way analysis of variance ($^{\#}P < .05$; $^{\#\#}P < .01$; $^{\#\#\#}P < .001$; $^{\#\#\#\#}P < .0001$) or the Mann-Whitney *U* test ($^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$).

High Levels of Classical Pathway Activation Correlated With COVID-19 Severity

We next sought to determine which arms of the system contribute to the observed overactivation of complement in patients with severe COVID-19. We first quantified markers that are specific to the initial activation steps of the alternative pathway. We observed no differences in the plasma levels of factors D and Bb, a byproduct of factor B cleavage, between patients with COVID-19 and HCs (Figure 3A). Subsequently, we found that plasma levels of factor H, a regulatory protein of the alternative pathway, were increased approximately 2-fold in ICU compared with non-ICU patients. Factor H–factor D ratios, which act in antagonistic ways to influence the activation/deactivation of the alternative pathway, were higher in both non-ICU and ICU patients compared with HCs, suggesting that the alternative pathway is being suppressed in patients with COVID-19. We also found that plasma levels of functional mannose-binding lectin, the main recognition molecule of the lectin pathway, were similar among HCs and ICU and non-ICU patients (Figure 3B), indicating that this arm of the complement system is not being disproportionally activated in patients with COVID-19.

We next assessed whether complement system initiation through the classical pathway accounted for the increased complement activation observed in patients with COVID-19. We observed an increase in plasma levels of total C1q in patients with COVID-19, which were significantly higher in ICU than in non-ICU patients (Figure 3C). In addition, we found that levels of circulating immune complexes (CICs) with the ability to bind to functional C1q (CIC-C1q) were markedly increased in patients with COVID-19 and were greater in ICU than in non-ICU patients. Furthermore, levels of CIC-C1q were 2-fold greater in patients with high complement activation (Figure 3D). We found no differences in levels of functional C1 inhibitor, a plasma protease that regulates activation of the classical pathway, between patients with COVID-19 and HCs (Figure 3C). Collectively, these findings suggest that the classical pathway is the main factor leading to the overactivation of the complement system in patients with severe COVID-19.

Increased Antibody Titers Against SARS-CoV-2 in Patients With Severe COVID-19 and Association With Greater Complement Activation

We subsequently analyzed the interactions between the antibody response to SARS-CoV-2 and complement activation. We

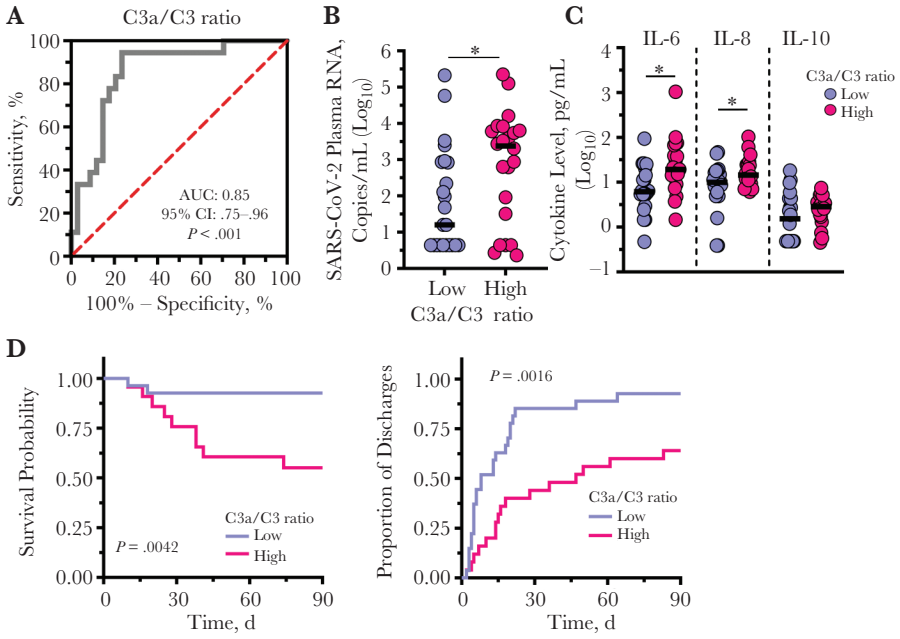

Figure 2. Relationship between complement activation, viral RNA levels, and clinical laboratory markers of inflammation. *A,* Receiver operating characteristic curve (ROC) analysis for C3a/C3 ratio as a predictor for coronavirus disease 2019 (COVID-19) severity. A C3a/C3 ratio threshold ≥4.5 $\log_{10}$ provided a sensitivity of 94.4% and a specificity of 7.06%. Abbreviations: AUC area under the ROC curve; CI, confidence interval. *B, C,* Scatterplots of the differences in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) plasma RNA (*B*) and inflammatory cytokine (*C*) levels between patients classified as having low (C3a/C3 ratio, <4.5 $\log_{10}$) or high (C3a/C3 ratio, ≥4.5 $\log_{10}$) complement activation. Each symbol in the scatterplots represents an individual patient, and horizontal lines represent medians. Abbreviations: IL-6, IL-8, and IL-10, interleukin 6, 8, and 10. **P* < .05 (Mann-Whitney *U* test). *D,* Kaplan-Meier curves for 90-day survival and time to discharge from hospital admission, stratified by low versus high complement activation.

first developed ELISAs to estimate titers of binding IgG and IgA against SARS-CoV-2 structural antigens (Supplementary Table 2 and Supplementary Figures 2 and 3). Similar to previous reports [29–31], we observed that patients with severe COVID-19 mounted a faster and stronger binding IgG and IgA response against the spike protein and NP (Figure 4A) and neutralizing antibody response (Figure 4D) against SARS-CoV-2, compared with patients who had moderate disease. Notably, IgG, IgA, and neutralizing antibody titers (Figure 4B–4D) trended higher in patients with severe COVID-19 even relatively soon after the onset of illness. These findings point to an early and robust antibody response as one determinant of COVID-19 severity.

We next investigated the degree of association between the antibody response and complement system activation. Levels of CIC-C1q were positively correlated with binding titers of IgG against the spike protein and NP and with neutralizing antibody titers to SARS-CoV-2 (Figure 5A), particularly in the ICU patients. Levels of CIC-C1q were not correlated with binding IgA titers, as this immunoglobulin class does not activate complement via the classical pathway. In line with this, the IgG/IgA binding titer ratio was greater in ICU than in non-ICU patients (Figure 5B). Substantiating our hypotheses of complement overactivation by the classical pathway, we also found that IgG titers were markedly increased in patients with COVID-19 classified as having high levels of complement activation, while levels of IgA did not differ between groups (Figure 5C). Finally, a 2-dimensional LDA classifier using C3a/C3 ratio and CIC-C1q levels distinguished non-ICU and ICU patients with an error <0.2 and with an estimated sensitivity and specificity of 73.5% and 94.4%, respectively (Figure 5D). Collectively, these data provided strong evidence that COVID-19 severity is associated with a predominant IgG response and with high levels of CIC-C1q that ultimately lead to overactivation of the complement system.

High IgG Titers Against the NP of CCCs and Complement Overactivation in Patients With COVID-19

To obtain insights into the apparent rapid increase in antibody response to SARS-CoV-2 in patients with severe COVID-19, we analyzed the IgG responses to the antigenically related CCCs. We developed binding assays to estimate IgG titers toward a range of spike and NP antigens of CCCs and the spike protein of SARS-CoV. We validate our binding assays using a panel of remnants samples from a pediatric seroprevalence study (in patients aged 1–16 years) living in the same region as the adult patients with COVID-19 [32] (Supplementary Tables 3 and 4 and Supplementary Figure 4). The frequency of binding IgG against all CCCs increased with age (Supplementary Table 5), which is consistent with endemic circulation of these viruses in the United States.

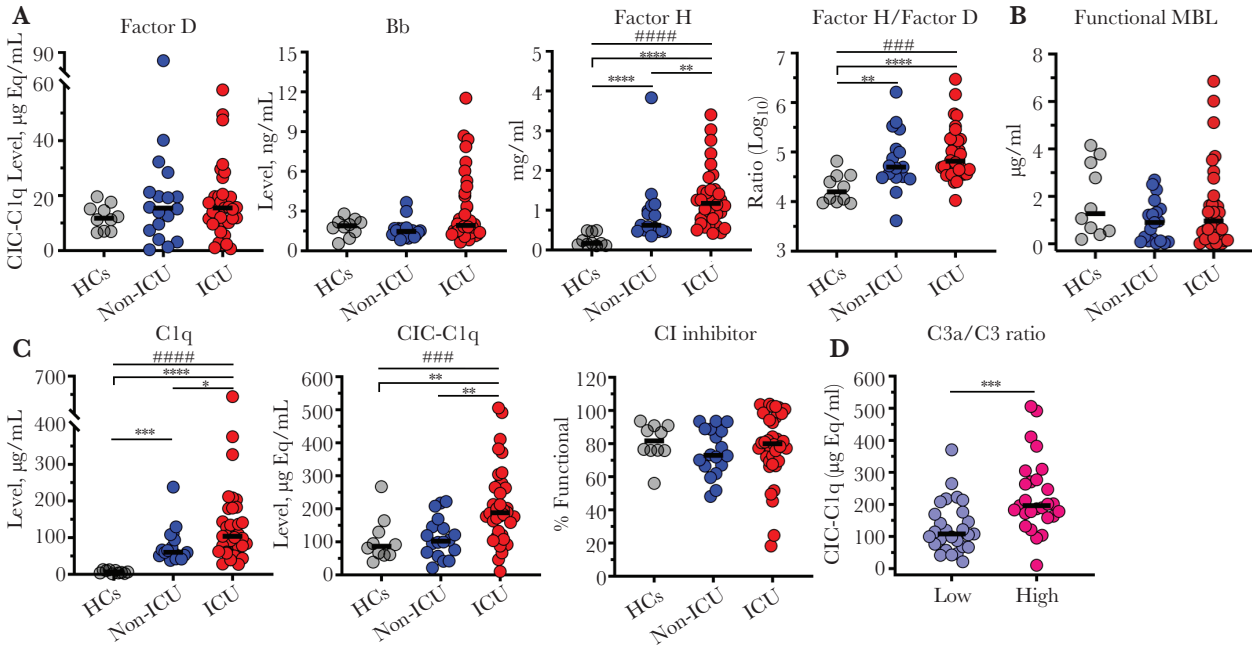

Figure 3. Markers of complement activation by the classical pathway are significantly increased in patients with severe coronavirus disease 2019 (COVID-19). Plasma levels of markers of complement initiation and regulatory proteins unique to each individual arm (alternative, lectin, and classical pathways) of the complement system were quantified in critically ill patients hospitalized in intensive care units (ICU patients; n = 37) and moderately ill patients hospitalized in dedicated COVID-19 wards (non-ICU patients; n = 18). Plasma samples from healthy controls (HCs; n = 10) were included as a reference population for comparisons. *A–C*, Scatterplots of the differences in plasma levels of markers of the alternative pathway, including factor D, Bb, factor H, and factor H–factor D ratio (*A*); functional mannose-binding lectin (MBL) of the lectin pathway (*B*); and markers of the classical pathway, (C) including total human C1q, circulating immune complexes (CICs) binding to C1q (CIC-C1q), and C1 inhibitor (*C*). *D*, Scatterplots of the differences in CIC-C1q between patients classified as having low (C3a/C3 ratio, <4.5 $\log_{10}$) or high (C3a/C3 ratio, ≥4.5 $\log_{10}$) complement activation. Each symbol in the scatterplots represents an individual patient, and horizontal lines represent medians. Statistical significance was determined using Kruskal-Wallis 1-way analysis of variance ($^{###}P < .001$; $^{####}P < .0001$) or the Mann-Whitney *U* test ($^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$).

We then quantified IgG titers against CCC antigens in our patients with COVID-19. All patients with COVID-19 exhibited substantial IgG reactivity to ≥1 of the CCC antigens (Supplementary Table 6). Notably, the increase in IgG titer to CCC antigens was more prominent in ICU than in non-ICU patients and most striking against NP antigens (Figure 6A). We also noted an increase in IgG titers against SARS-CoV spike protein in patients with COVID-19, especially in the ICU patients. This likely reflects cross-reactive antibody responses, given the structural similarities between SARS-CoV and SARS-CoV-2 [33].

We next evaluated the relationship between antibody binding to CCCs and complement activation and found that IgG titers to NP antigens (Figure 6C) but not to the spike (Figure 6B) were greater among patients classified as having high complement activation. In addition, patients with COVID-19 displayed a significant positive correlation between levels of CIC-C1q and IgG titers against NP antigens for all CCCs (Figure 6D). This correlation was not significant when stratifying patients by disease severity. Collectively, these findings reveal a cross-reactive anamnestic response to CCCs in patients with COVID-19 that is correlated with increased complement activation.

DISCUSSION

We show that IgG responses against SARS-CoV-2 and CCCs are associated with complement activation in patients with severe COVID-19. In line with earlier reports [1, 6–9], we confirm that markers of systemic complement activation are significantly increased in patients with severe COVID-19 and distinguish those with worse disease outcomes. More importantly, our in-depth analysis of pathway-specific components delineate the role of the classical pathway in the enhanced complement activation observed in patients with severe COVID-19 and suggests a previously unrecognized mechanism of antibody-mediated activation driven by an overexuberant IgG response against SARS-CoV-2 and CCC antigens. These data indicate that an immune dysregulation of both the innate and adaptive immune systems is a primary feature of COVID-19 severity.

Our data showed that high complement activation is associated with some established markers of inflammation commonly elevated in patients with severe COVID-19 [34, 35]. This is consistent with a hyperinflammatory milieu characteristic of SARS-CoV-2 pathophysiology, which is likely influenced by the enhanced levels of the potent anaphylatoxins C3a and C5a, particularly in ICU patients [6, 8]. In contrast with a prior report [1], our findings indicate that complement overactivation is

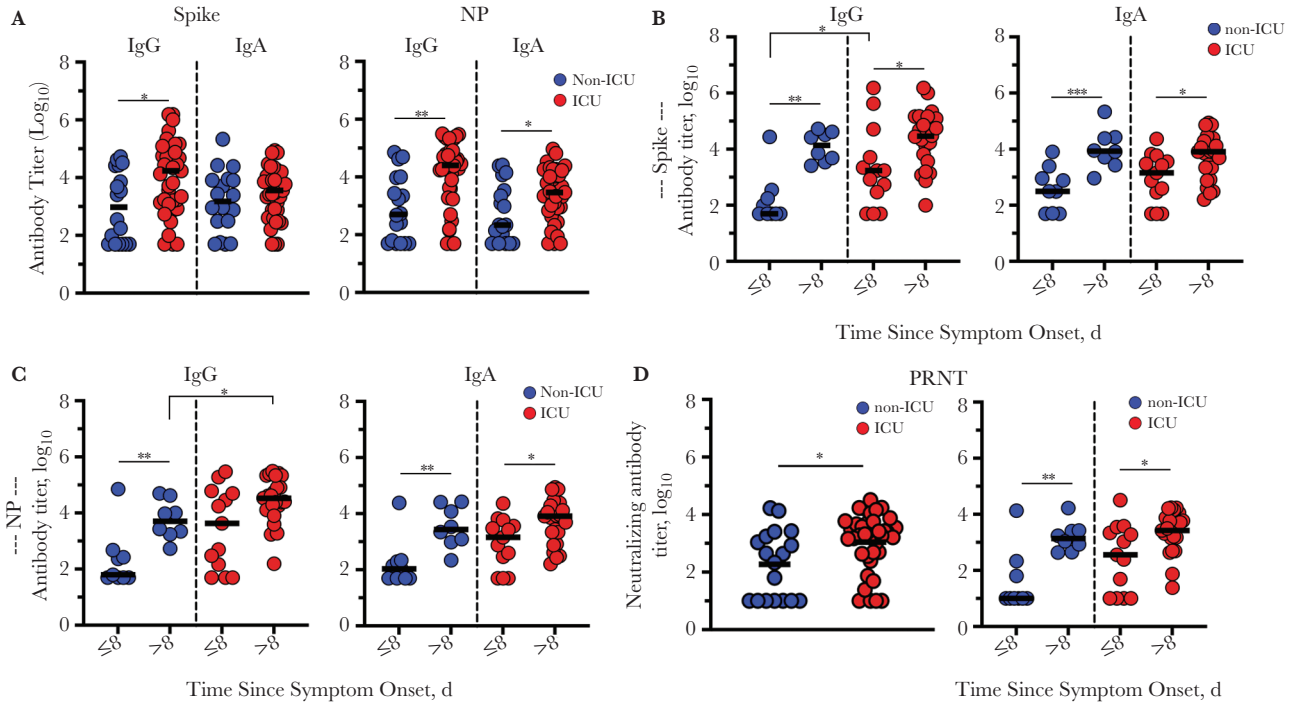

Figure 4. Binding immunoglobulin (Ig) G and IgA, and neutralizing antibody titers against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are markedly increased in patients with severe coronavirus disease 2019. *A–C,* Scatterplots of the differences in plasma titers of binding IgG and IgA against the spike protein and the nucleoprotein (NP) of SARS-CoV-2 between intensive care unit (ICU) and non-ICU patients, regardless of time from illness onset (*A*) and stratified by days after symptom onset (*B, C*). *D,* Titers of neutralizing antibodies estimated by plaque reduction neutralization test (PRNT), regardless of time from illness onset and stratified by days after symptom onset. Each symbol in the scatterplots represents an individual patient, and horizontal lines represent medians. $*P < .05$; $**P < .01$; $***P < .001$ (Mann-Whitney *U* test).

correlated with levels of SARS-CoV-2 RNA in the plasma. This discrepancy between studies might be related to the sensitivity of the assays used for detection of viral RNA. SARS-CoV-2 RNA in our patients was measured using an ultrasensitive quantitative reverse-transcription PCR method, resulting in a greater proportion of RNAemia-positive ICU patients than reported elsewhere [28].

While earlier reports indicate that overactivation of the complement system plays a critical role in COVID-19 immunopathology [1, 5–10], our detailed analysis of several pathway-specific markers extend these findings by revealing that the classical pathway and the humoral response are associated with unfavorable COVID-19 outcomes. The high levels of CIC-C1q seen in our patients are likely due to the presence of greater amounts of antigens and antibodies present in the circulation, reduced CIC clearance, or both. Earlier reports have shown extensive deposition of C1q and C4d, a marker of complement activation by CICs, in postmortem lung sections of patients with COVID-19 [10, 36]. Complement activation by CICs has also been associated with the pathophysiology of other respiratory viruses [37, 38]. Interestingly, a prior in vitro study reported no C1q binding when mixing SARS-CoV-2 proteins with nonimmune human serum, indicating that the classical pathway is not activated in the absence of specific antibodies [39]. Of note, the assay used in our study measures CICs with the ability to bind to functional C1q and thus activate the complement system—a feature that is further confirmed by the association between levels of CIC-C1q and C3a/C3 ratio seen in our patients. In addition, it is important to mention that CICs can also induce a complement-independent dysregulated inflammatory milieu via CIC engagement to FcγR, leading to excessive activation of alveolar macrophages that likely contributes to amplification of the proinflammatory cytokine release seen in patients with severe COVID-19 [40, 41].

Consistent with earlier reports [29–31], our analysis of the magnitude of the antibody response to SARS-CoV-2 suggest that ICU patients seroconvert earlier and developed higher titers of binding IgG and IgA and neutralizing antibodies than patients with moderate disease. Interestingly, patients with higher IgG/IgA ratios had more severe disease, which is consistent with the greater capacity of IgG to mediate complement activation. Patients with severe COVID-19 also displayed a strong rise in the levels of IgG against the NP of CCCs, in agreement with 2021 reports showing that IgG antibodies and B cell clones targeting structural antigens of CCCs are boosted on SARS-CoV-2 infection [20, 42, 43]. Extending on these findings, our data reveal a marked correlation between enhanced complement activation by CIC-C1q and IgG levels to SARS-CoV-2 and CCCs. These findings suggest that overactivation of the complement system by CICs at the pulmonary and systemic levels

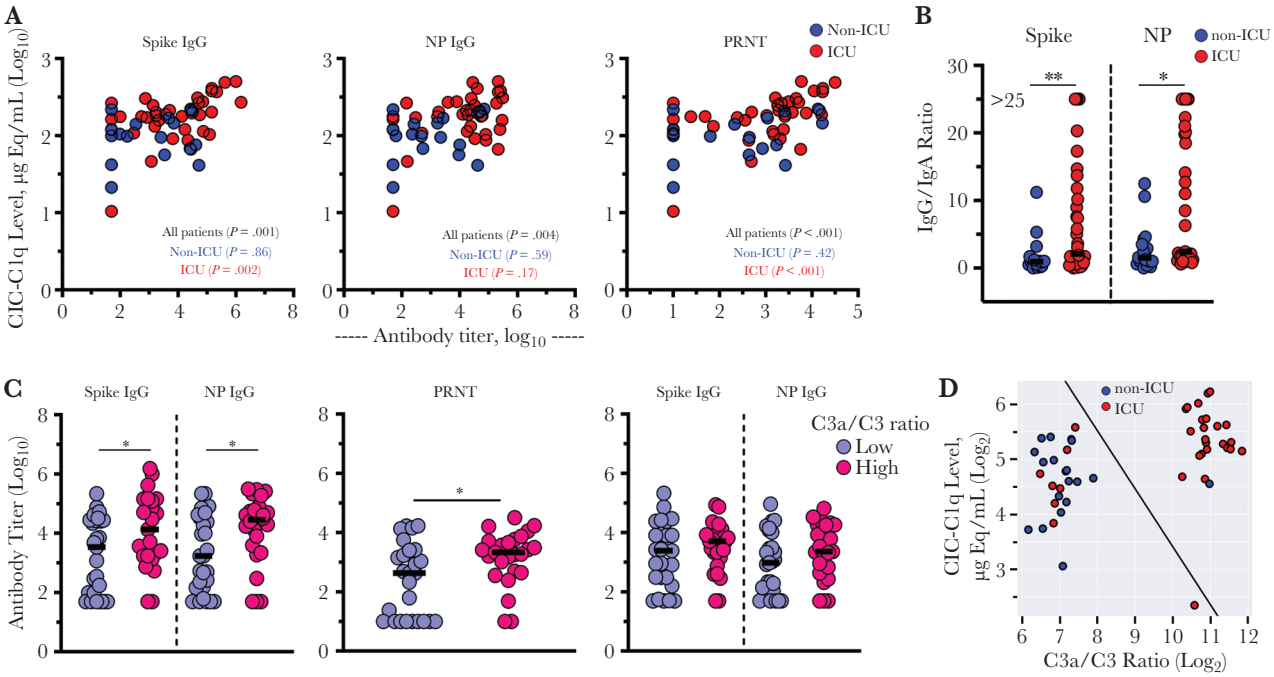

Figure 5. Binding immunoglobulin (Ig) G and IgA, and neutralizing antibody titers against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are associated with complement activation in patients with severe coronavirus disease 2019 (COVID-19). *A,* Degree of correlation between plasma levels of circulating immune complexes (CIC-C1q) and binding IgG against the spike and nucleoprotein (NP) and neutralizing antibody titers, assessed using the Spearman rank correlation coefficient test. *B,* Scatterplots of the differences in IgG/IgA ratios between intensive care unit (ICU) and non-ICU patients with COVID-19. Abbreviation: PRNT, plaque reduction neutralization test. *C,* Scatterplots of binding titers of IgG against spike protein and NP, neutralizing antibody titers, and binding titers of IgA against spike protein and NP, stratified by the C3a/C3 ratio as low (C3a/C3 ratio, <4.5 $\log_{10}$) or high (C3a/C3 ratio, ≥4.5 $\log_{10}$) complement activation. Each symbol in the scatterplots represents an individual patient, and horizontal lines represent medians. **P* < .05; ***P* < .01 (Mann-Whitney *U* test). *D,* Linear discriminant analysis using all measurements for complement and SARS-CoV-2 antibody, showing a 2-dimensional discriminator using CIC-C1q and the C3a/C3 ratio.

may contribute to the secretion of proinflammatory cytokines, immune cell recruitment, and complement deposition within the lung, in a mechanism similar to the antibody-mediated enhanced respiratory disease observed in other respiratory viruses [38, 44].

Because activation markers of the alternative and the lectin pathways were present at similar levels, we were unable to ascribe their role in the observed complement overactivation seen in our patients with COVID-19. However, this does not exclude the possibility that these arms of the complement system also contribute to COVID-19 severity. Earlier reports have shown the ability of mannose-binding lectin to bind SARS-CoV-2 proteins and activate complement [39, 45], suggesting that the lectin pathway is possibly involved in complement activation in COVID-19. Activation of the alternative pathway has also been implicated in COVID-19 pathogenesis using in vitro systems and in clinical studies [9, 46, 47]. Interestingly, recent reports have suggested that activation of the alternative pathway in SARS-CoV-2–infected patients is factor D independent [9, 46]. This is to some extent consistent with our data showing no differences in factor D levels between patients with COVID-19 and HCs. Notably, negative regulatory mediators of the alternative pathway (eg, factor H) were markedly up-regulated in our patients, suggesting that this pathway is being suppressed rather than synergistically amplifying complement activation in COVID-19. This variability among studies may result from differences in timing of sample collection, type of sample tested (plasma EDTA, citrate, and serum), and sensitivity of the methods used for complement measurements. However, based on available data, it is reasonable to hypothesize that SARS-CoV-2 infection triggers activation of the complement cascade via all 3 pathways and that the classical pathway likely contributes the most to overactivation of the system and hence disease severity.

We acknowledge that the number of patients included in our analysis was relatively small, which might underpower some of the conclusions. In addition, all patients enrolled in our cohort were hospitalized, and complement activation and antibody response in asymptomatic or mild COVID-19 cases could not be assessed in this study. More extensive studies are needed to clearly elucidate the mechanistic basis by which SARS-CoV-2 mediates excessive activation of the complement system, ideally with longitudinal sampling throughout the course of the disease, including samples from patients with mild COVID-19 and from those who develop moderate symptoms and later progress to severe disease.

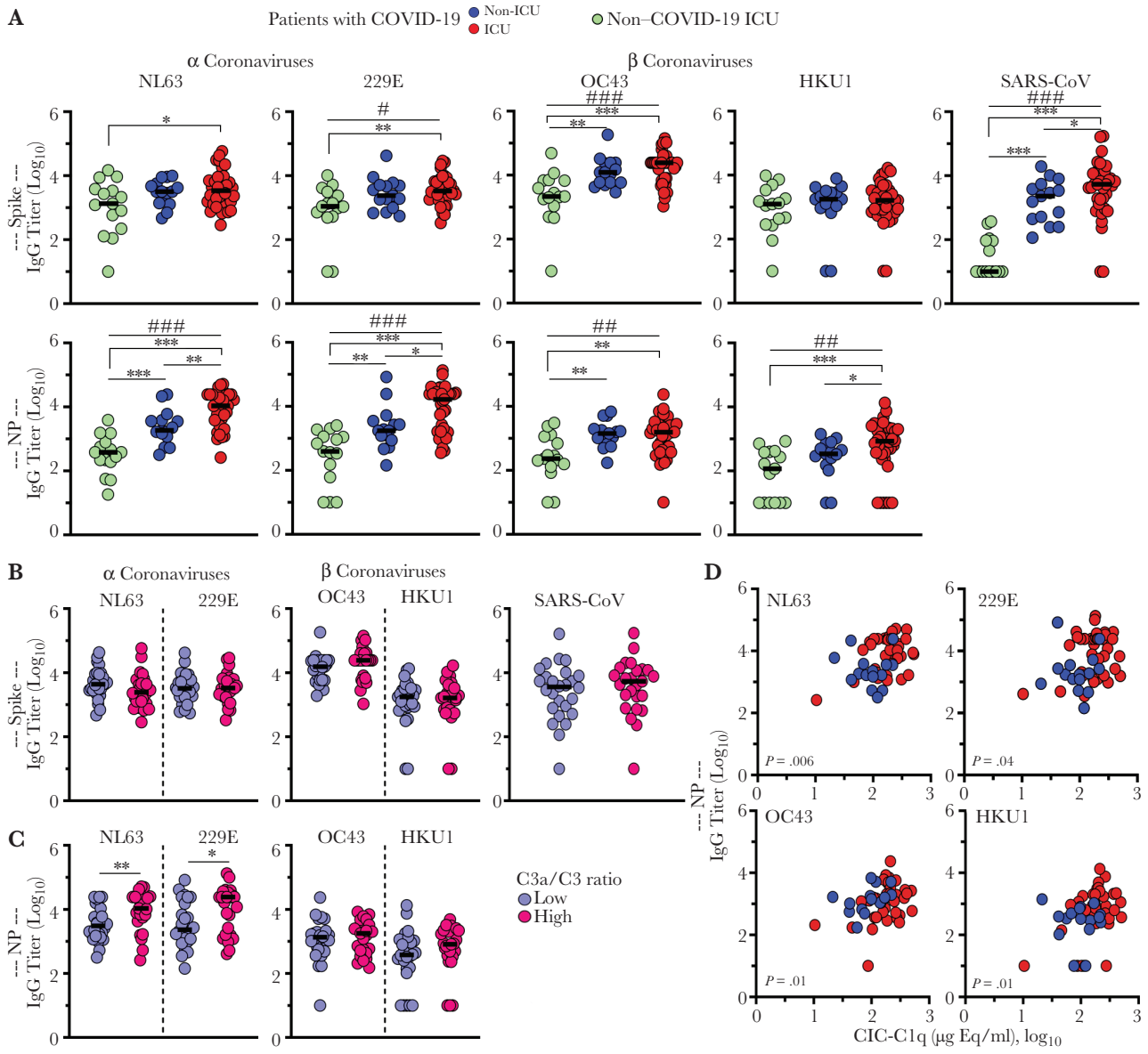

Figure 6. Binding immunoglobulin (Ig) G titers against various antigens of the common cold coronaviruses (CCCs) are higher in patients with severe coronavirus disease 2019 (COVID-19) and are also associated with complement activation. *A,* Scatterplots of the differences in plasma titers of binding IgG against the spike protein and the nucleoprotein (NP) of various CCCs (NL63, 229E, OC43, and HKU1) and the spike protein of severe acute respiratory syndrome coronavirus (SARS-CoV) between intensive care unit (ICU) and non-ICU patients. Plasma samples from patients who were hospitalized in ICU wards with respiratory illness not related to severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection were included as a group for comparisons (non–COVID 19 ICU; n = 20). Scatterplots of the differences in plasma titers of binding IgG against the spike protein of CCCs and of SARS-CoV (*B*) and against the NP of the CCCs (*C*), stratified by the C3a/C3 ratio as low (C3a/C3 ratio, <4.5 $\log_{10}$) or high (C3a/C3 ratio, ≥4.5 $\log_{10}$) complement activation. Each symbol in the scatterplots represents an individual patient, and horizontal lines represent medians. Statistical significance was determined using Kruskal-Wallis 1-way analysis of variance ($^{\#}P < .05$; $^{\#\#}P < .01$; $^{\#\#\#}P < .001$) and the Mann-Whitney *U* test ($^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$). *D,* Degree of correlation between plasma levels of circulating immune complexes (CIC-C1q) and IgG titers to CCC NP, assessed using the Spearman rank correlation coefficient test.

In summary, our findings highlight the role of the classical pathway in mediating complement overactivation in patients with severe COVID-19 and reveal that the interplay between IgG responses to SARS-CoV-2 and to CCCs in SARS-CoV-2–infected patients may be associated with adverse outcomes. Although the viral and immunoglobulin content of the CICs needs to be determined, our findings provide new insights into the immunopathogenesis of SARS-CoV-2 infection and indicate that IgG-mediated effector functions may contribute to COVID-19 severity. Importantly, such identification of immune parameters might help in accurately selecting interventional strategies, including inhibition of the C4bC2a and C3bBb convertases, blockers of C3a and C5a receptors, and a variety of others reviewed elsewhere [48].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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