ORIGINAL ARTICLE

# Pulmonary Surfactant Proteins Are Inhibited by Immunoglobulin A Autoantibodies in Severe COVID-19

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## Abstract

**Rationale:** Coronavirus disease 2019 (COVID-19) can lead to acute respiratory distress syndrome with fatal outcomes. Evidence suggests that dysregulated immune responses, including autoimmunity, are key pathogenic factors.

**Objectives:** To assess whether IgA autoantibodies target lung-specific proteins and contribute to disease severity.

**Methods:** We collected 147 blood, 9 lung tissue, and 36 BAL fluid samples from three tertiary hospitals in Switzerland and one in Germany. Severe COVID-19 was defined by the need to administer oxygen. We investigated the presence of IgA autoantibodies and their effects on pulmonary surfactant in COVID-19 using the following methods: immunofluorescence on tissue samples, immunoprecipitations followed by mass spectrometry on BAL fluid samples, enzyme-linked immunosorbent assays on blood samples, and surface tension measurements with medical surfactant.

**Measurements and Main Results:** IgA autoantibodies targeting pulmonary surfactant proteins B and C were elevated in patients with severe COVID-19 but not in patients with influenza or bacterial pneumonia. Notably, pulmonary surfactant failed to reduce surface tension after incubation with either plasma or purified IgA from patients with severe COVID-19.

**Conclusions:** Our data suggest that patients with severe COVID-19 harbor IgA autoantibodies against pulmonary surfactant proteins B and C and that these autoantibodies block the function of lung surfactant, potentially contributing to alveolar collapse and poor oxygenation.

**Keywords:** COVID-19; autoimmunity; immunoglobulin A; pulmonary surfactant; pulmonary-associated surfactant protein

Coronavirus disease 2019 (COVID-19) is caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has rapidly evolved into a global pandemic (1, 2). Although the introduction of effective vaccines against SARS-CoV-2 has had a major impact on fighting the disease (3–6), it remains a burden on healthcare systems as high vaccination coverage has yet to be achieved in most countries (7). Dysfunctional immune responses and early alveolar damage are thought to play a determining role in COVID-19 progression (8, 9). In the lungs, severe COVID-19 is characterized by the following features: cell pyroptosis that occurs because of viral replication and cytokine release, and edema and protein deposition, which is induced by the strong inflammatory response and further impair alveolar function. Elevated angiotensin II concentrations may then lead to increased vascular permeability and promote microthrombus formation (8, 10, 11). Postmortem studies have shown that the lungs of affected patients resemble those of patients without SARS-CoV-2 with acute respiratory distress syndrome (ARDS) (12), and a subset of patients with COVID-19 display areas of atelectasis (13, 14).

Systemic treatment with dexamethasone can improve the outcome of severe COVID-19 (15), and the use of inhaled budesonide in early COVID-19 may reduce disease progression (16). Thus, understanding the mechanisms underlying the defective or dysregulated immune responses that occur in patients with severe COVID-19 is critical for combating the disease and for developing treatments for the most severely affected patients.

Recent studies have shown that SARS-CoV-2 infection induces autoantibodies against various cytokines and autoimmune disease-related proteins in patients with severe disease (17–19). Although these reports generally focus on IgG, rapid and sustained production of IgA has been shown to occur during the early immune response to SARS-CoV-2 infection and in patients with severe COVID-19 (20, 21). These previously

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published observations provide a rationale for studying the role of IgA-driven autoreactivity in COVID-19 pathogenesis.

Kanduc and colleagues recently demonstrated similarities between the amino acid sequences of the SARS-CoV-2 spike protein and some human lung surfactant proteins (22), raising the possibility of autoreactivity to pulmonary surfactant proteins because of antigen mimicry (23, 24). Surfactant proteins are essential for maintaining respiratory physiology by promoting alveolar stability. Therefore, these results prompted us to ask whether patients with severe COVID-19 develop autoreactive IgA directed against pulmonary proteins, possibly affecting lung function and oxygenation status.

Some of the results of these studies have been previously reported in the form of a preprint (bioRxiv, 7 February 2021 https://doi.org/10.1101/2021.02.02.21250940) (25).

## Methods

### Study Design and Sample Collection

We established a multicenter cross-sectional study at three Swiss hospitals and one German tertiary hospital. For all patients, SARS-CoV-2 infection was detected by reverse-transcription polymerase chain reaction or rapid antigen testing, and COVID-19 was clinically confirmed by the supervising physician (26). Severe



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This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

## At a Glance Commentary

**Scientific Knowledge on the Subject:** Severe coronavirus disease 2019 (COVID-19) can lead to fatal acute respiratory distress syndrome. Although it has been shown that COVID-19 can induce autoimmunity, there are limited data on autoantibodies in the lungs and their contribution to COVID-19 severity.

**What This Study Adds to the Field:** This multicenter cross-sectional study shows that patients with COVID-19 with severe disease harbor IgA autoantibodies targeting pulmonary surfactant proteins B and C. Furthermore, the presence of these IgA autoantibodies in pulmonary surfactant interferes with its capability to reduce surface tension. These results suggest that IgA autoantibodies directed against surfactant proteins contribute to COVID-19 severity.

COVID-19 was defined as respiratory insufficiency with oxygen supplementation because of SARS-CoV-2, whereas mild COVID-19 describes oligosymptomatic patients without the need for additional oxygen (27). We collected clinical data, blood, and lung tissue samples from patients with severe COVID-19 and patients with mild COVID-19 from April 2020 through April 2021. We further included preexisting clinical data and biological material from noninfected healthy control patients, patients with bacterial pneumonia, and patients with influenza that had been collected between October 2017 and June 2020. In addition, we obtained BAL fluid (BALF) samples from patients with severe COVID-19 and patients without COVID-19, collected from March through November 2020. BALF collection and storage were performed as previously described (28).

Plasma and serum samples were isolated from whole blood collected into heparin-containing tubes (BD Vacutainer CPT tubes; Becton Dickinson) or serum tubes after centrifugation at 1,650 × g for 20 minutes. The undiluted plasma or serum samples were then aliquoted and stored at −80°C. Postmortem lung tissue samples were collected and processed according to the local standard protocols.

We designated the first cohort with severe COVID-19 from the Cantonal Hospital St. Gallen as the derivation cohort (dCOVID) and a second independent cohort from the University Hospital Basel as the validation cohort (vCOVID). Sample characterization, including type, quantities, and centers of the collection, is outlined in Table E1 in the online supplement.

All patients gave informed consent for study participation, and the study was performed in accordance with the Declaration of Helsinki guidelines. The study was approved by the responsible ethical committee at each study site (Swiss ethics protocol numbers 2020–01006, 2020–00566, 2020–00629, and 2020–00646, and Ethical Commission Tübingen number 259/2022BO2).

### Identification of IgA-bound Proteins in BALF

To identify auto-IgA targeting pulmonary proteins, BALF samples were fixed and subsequently analyzed by liquid chromatography–tandem mass spectrometry. Preparation and analysis protocols are detailed in the METHODS section of the online supplement.

### Histology

To assess the presence of auto-IgA against SP-B (surfactant protein B) and SP-C (surfactant protein C) in lung tissue, double immunofluorescence stainings for IgA, SP-B and SP-C were performed. The staining strategy can be found in the METHODS section of the online supplement.

### Enzyme-linked Immunosorbent Assay (ELISA) Measurements

To identify auto-IgA against pulmonary surfactant proteins, we performed ELISAs using recombinant proteins and poractant alfa (Curosurf; Chiesi Farmaceutici), a therapeutic porcine surfactant replacement. Our approach is described in the METHODS section of the online supplement.

### Surface Tension Measurement through Capillary Rise

To determine whether plasma or IgA from patients with severe COVID-19 impairs surfactant function *in vitro*, we performed capillary rise measurements using poractant alfa. Details can be found in the METHODS section of the online supplement.

### Statistical Analyses

Data were analyzed using GraphPad Prism version 9.3.1. For two-group comparisons, the Mann-Whitney test was used, whereas, for multiple-group comparisons, the Kruskal-Wallis test with Dunn's correction was applied. The Wilson/Brown method was used to perform area under the curve (AUC) receiver operating characteristics analyses (29).

## Results

### Patient Characteristics

For blood sampling, we enrolled a total of 201 patients, of which 137 had severe COVID-19: 77 in the dCOVID cohort and 60 in the vCOVID cohort. Twelve patients had mild COVID-19; 30 patients had bacterial pneumonia and tested negative for SARS-CoV-2; and 22 individuals were healthy control subjects without SARS-CoV-2 infection. Clinical patient characteristics and demographics are listed in Table 1. The median age of dCOVID was 66 years, 70 years for vCOVID, and 69 years for bacterial pneumonia, whereas patients with mild COVID-19 were younger (median, 34 yr). The average duration of hospitalization in the dCOVID and vCOVID cohorts was 15 days. In addition, we received BALF from patients with severe COVID-19 ($n = 18$) and patients without COVID-19 ($n = 18$), and we collected formalin-fixed paraffin-embedded postmortem lung samples from four patients with severe COVID-19 and 5 patients who did not have COVID-19.

### Patients with Severe COVID-19 Harbor IgA Against SP-B and SP-C in Their Lungs

In line with previous reports, we found that ARDS in COVID-19 was frequently associated with alveolar collapse in histology (Figure E1). A major cause of alveolar collapse may be the dysfunction of the pulmonary surfactant. We hypothesized that autoantibodies disturb surfactant function and first investigated whether IgA targeting any pulmonary-associated proteins was detectable in BALF from severely ill patients. Using peptide-M agarose IgA purification followed by liquid chromatography–tandem mass spectrometry analysis, we identified proteins bound to IgA in BALF (Figures 1A and B). IgA was bound to SP-B in 9 out of 18 (50%) patients, and SP-B was among the 30 most frequent proteins that were

**Table 1.** Clinical Characteristics of Patients

| Characteristic | dCOVID (*n* = 77) | vCOVID (*n* = 60) | Mild COVID-19 (*n* = 12) | Bacterial Pneumonia (*n* = 30) |
|---|---|---|---|---|
| Sex (male), *n*/total (%) | 55/77 (71) | 46/60 (77) | 4/12 (33) | 18/30 (60) |
| Median age (IQR), yr | 66 (60–73) | 70 (62–79) | 34 (32–48) | 69 (59–78) |
| Median d of hospitalization (IQR) | 15 (10–25) | 15 (11–23) | — | — |
| Oxygen supplementation, *n*/total (%) | 77/77 (100) | 60/60 (100) | 0/12 (0) | 17/30 (57) |
| Intubation | 20/77 (26) | 53/60 (88) | 0 (0) | — |
| ECMO | 10/77 (13) | 2/60 (3) | 0 (0) | — |
| Other (nasal, mask) | 47/77 (61) | 5/60 (8) | 0 (0) | — |
| Comorbidities,* *n*/total (%) | 71/77 (92) | 54/60 (90) | 3/12 (25) | 23/30 (77) |
| Hypertension | 50/77 (65) | 37/60 (62) | 2/12 (17) | 15/30 (50) |
| Cardiovascular disease | 14/77 (18) | 24/60 (40) | 0/12 (0) | 15/30 (50) |
| Diabetes | 29/77 (38) | 30/60 (50) | 1/12 (8) | 2/30 (7) |
| Obesity† | 52/77 (68) | 28/60 (47) | 1/12 (8) | 8/30 (27) |
| Autoimmune disease | 9/77 (12) | 6/60 (10) | 1/12 (8) | — |
| Cancer | 8/77 (10) | 6/60 (10) | 2/12 (17) | — |
| Hematological disorder | 6/77 (8) | 12/60 (20) | 2/12 (17) | — |
| Immunosuppression,‡ *n*/total (%) | 76/77 (99) | — | 1/12 (8) | — |
| Laboratory parameters§ | | | | |
| Median CRP concentration, mg/L (IQR) | 194 (107–254) | 125 (68–173) | — | — |
| Patients with data, *n* | 77 | 60 | — | — |
| Median D-dimer concentration, mg/L (IQR) | 2.2 (1.1–9.9) | 1.3 (0.9–2.6) | — | — |
| Patients with data, *n* | 68 | 53 | — | — |
| Median leukocyte count, $10^9$/l (IQR) | 13.9 (10.5–19.6) | 9.1 (5.4–13.5) | — | — |
| Patients with data, *n* | 76 | 60 | — | — |

*Definition of abbreviations*: COVID-19 = coronavirus disease 2019; CRP = C-reactive protein; dCOVID-19 = severe COVID-19 derivation cohort; ECMO = extracorporeal membrane oxygenation; IQR = interquartile range; vCOVID = severe COVID-19 validation cohort.
*Only comorbidities considered relevant for COVID-19 outcome are included and listed.
†Obesity is defined as a body mass index of 25 or higher.
‡Immunosuppression is defined as any of the following: systemic prednisone ⩾7.5 mg/day (or equivalent), IL inhibitors (such as tocilizumab), other systemic immunosuppressive drugs (e.g., calcineurin inhibitors), or chemotherapy. All therapies had been established before the treatment of COVID-19.
§Laboratory parameters are included when available. The number of patients with available values is provided in the consecutive row. For each patient and parameter, the highest value measured during hospitalization was used.

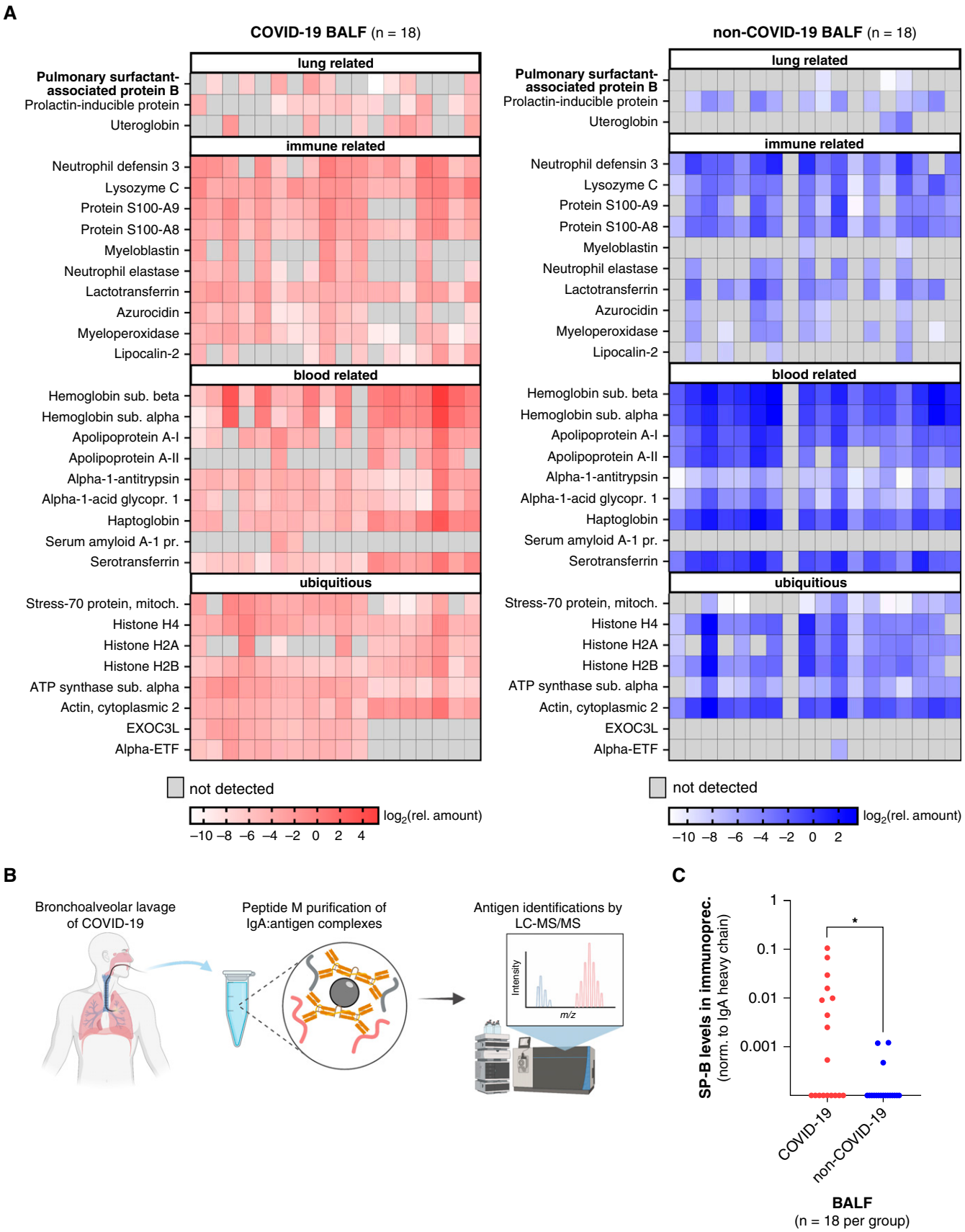
immunoprecipitated by our antigen detection approach.

In comparison, SP-B was only detected in BALF from 3 out of 18 (17%) patients without COVID-19 and at lower overall concentrations (Figure 1A). The difference in IgA-bound BALF SP-B concentrations between patients with and without COVID-19 was statistically significant (*$P = 0.03$, Mann-Whitney test) (Figure 1C). We next sought to determine whether these IgA were also present and colocalized with SP-B in the lungs of patients with COVID-19. To that end, we performed immunofluorescence staining on postmortem formalin-fixed paraffin-embedded lung sections from patients in the dCOVID cohort and compared them to lung tissue from patients without COVID-19 with influenza or without viral infection (Figure 2A). All lung samples from the dCOVID cohort displayed the presence of IgA colocalized with SP-B within the alveoli (Figure 2B). In contrast, we did not detect any IgA in lung samples from patients with influenza (Figure 2C) or without viral infection (Figure 2D).

In its secreted form (sIgA), IgA exists as a dimer consisting of two monomers linked by a J-chain. A predominance of monomeric IgA in the lungs of patients with severe COVID-19 could be the result of vascular damage, whereas sIgA would suggest a targeted immune reaction. Therefore, we performed an additional stain for the J-chain protein in these samples and found a strong signal only in the lungs of patients with COVID-19. This protein colocalized with SP-B and IgA, confirming the prominent presence of sIgA in these samples (Figure E2). Because lung SP-C is another key protein for lung surfactant function, we repeated the same staining with an SP-C–specific antibody and were able to detect IgA colocalizing with SP-C in lung tissue from patients with COVID-19 (Figure 3A). As with SP-B, no IgA was detectable in lung tissue from influenza or uninfected patients (Figures 3B and 3C). Thus, COVID-19 pneumonitis was associated with the presence of autoreactive IgA against SP-B and SP-C in the lung.

### IgA against SP-B and -C Are Elevated in Blood Samples from Patients with Severe COVID-19

To determine whether autoantibodies against SP-B and other pulmonary surfactant proteins are also present in the blood of patients with COVID-19, we examined samples from dCOVID, mild COVID-19, and SARS-CoV-2–negative cohorts. First, we transduced HEK293 (human embryonic kidney) cells with lentiviral vectors encoding the human surfactant proteins SP-A1, -A2, -B, -C, or -D and confirmed their expressions by Western blot (Figure E3A). Using pooled plasma from patients within the dCOVID ($n = 15$) or mild COVID-19 ($n = 5$) cohorts, we then performed indirect immunofluorescence staining for IgA on the transduced cells. We observed that patients


A
COVID-19 BALF (n = 18)
non-COVID-19 BALF (n = 18)
lung related
Pulmonary surfactant-associated protein B
Prolactin-inducible protein
Uteroglobin
immune related
Neutrophil defensin 3
Lysozyme C
Protein S100-A9
Protein S100-A8
Myeloblastin
Neutrophil elastase
Lactotransferrin
Azurocidin
Myeloperoxidase
Lipocalin-2
blood related
Hemoglobin sub. beta
Hemoglobin sub. alpha
Apolipoprotein A-I
Apolipoprotein A-II
Alpha-1-antitrypsin
Alpha-1-acid glycopr. 1
Haptoglobin
Serum amyloid A-1 pr.
Serotransferrin
ubiquitious
Stress-70 protein, mitoch.
Histone H4
Histone H2A
Histone H2B
ATP synthase sub. alpha
Actin, cytoplasmic 2
EXOC3L
Alpha-ETF
not detected
log2(rel. amount)
−10 −8 −6 −4 −2 0 2 4
B
Bronchoalveolar lavage of COVID-19
Peptide M purification of IgA:antigen complexes
Antigen identifications by LC-MS/MS
Intensity
m/z
C
SP-B levels in immunoprec. (norm. to IgA heavy chain)
1
0.1
0.01
0.001
*
COVID-19
non-COVID-19
BALF
(n = 18 per group)

with severe COVID-19 harbored much higher concentrations of anti-SP-B and -C IgA compared with those with mild COVID-19. Furthermore, IgA from both groups showed minimal to no binding to the other surfactant proteins (i.e., SP-A1, -A2, or -D) (Figure E3B). These data demonstrate that the severity of COVID-19 correlates with the concentrations of anti–SP-B and SP-C IgA. We repeated this experiment with A549 lung adenocarcinoma cells, comparing plasma from the dCOVID ($n = 15$) and noninfected control ($n = 5$) cohorts. Here, we observed the same IgA staining pattern seen in HEK293 cells (Figure E3C). Analysis of the gene expression repository Lung Cell Atlas also revealed particularly high expression of SP-C (SFTPC) and SP-B (SFTPB) in alveolar epithelial cells (30). Interestingly, SP-B and SP-C are both predominantly produced by type 2 pneumocytes, which are a key target cell type for SARS-CoV-2 (31).

Next, we assessed the concentrations of antisurfactant IgA in patients with mild and severe COVID-19 via ELISAs using recombinant SP-B and SP-C (Figure 4A). We observed significantly higher concentrations of anti–SP-B and anti–SP-C IgA in the plasma of patients with severe disease (dCOVID, $n = 77$) compared with patients with mild COVID-19 ($n = 12$) and healthy control subjects ($n = 12$) (Figure 4B). To confirm our observation with a clinically applied surfactant product, we coated our ELISA with poractant alfa (Curosurf; Chiesi Farmaceutici), a porcine lung surfactant used for intratracheal rescue therapy in preterm neonates with ARDS. As with the recombinant proteins, plasma from patients with severe COVID-19 presented significantly higher concentrations of surfactant-targeting IgA compared with plasma from patients who were not infected. We then asked whether the concentrations of these IgA correlated with the clinical need for supplemental oxygen. Using receiver operating characteristics curves, we tested for thresholds that classified patients into severe versus mild COVID-19. Anti–SP-B– (AUC = 0.779), anti–SP-C– (AUC = 0.751), and anti-poractant alfa-IgA (AUC = 0.834) all showed AUC values significantly above 0.5 (all $^{***}P < .001$). An absorption threshold at 0.054 for SP-B yielded a sensitivity of 79.2% and a specificity of 72.7%, a threshold at 0.056 for SP-C resulted in 79.2% sensitivity and 68.5% specificity, and a threshold for poractant alfa at 0.034 gave 70.8% sensitivity and 76.6% specificity (Figure 4C). Next, we sought to validate our auto-IgA findings in an independent vCOVID cohort and, in addition, investigated whether these IgA were COVID-19–specific by including a cohort of patients without COVID-19 with severe bacterial pneumonia. IgA against surfactant proteins was significantly elevated in the vCOVID cohort compared with patients with bacterial pneumonia and uninfected control subjects. No significant difference was found between the IgA concentrations of patients with bacterial pneumonia and uninfected control subjects in any of the analyses (Figure 4D). These data strongly suggest that elevated IgA targeting surfactant proteins are specific for severe COVID-19.

To confirm that auto-IgAs were specifically targeting SP-B and SP-C, as shown with HEK293 and A549 cells (Figures E3B and C) rather than other surfactant proteins, we repeated the ELISAs for IgA reactivity against SP-A and SP-D (Figure E4). Although we did observe a significant difference between plasma samples from dCOVID and patients with mild COVID-19, there was no difference when comparing dCOVID to healthy control subjects, indicating that the elevated signal seen in severe COVID-19 against SP-A and SP-D is not relevant (Figure E4A and B). When measuring IgA in vCOVID, patients with bacterial pneumonia, and non-COVID-19 control subjects, no difference among the three groups was observed (Figure E4C).

Like other immunoglobulins, IgA can exhibit canonical (i.e., Fab-dependent) and noncanonical binding to antigens or target receptors (32). To examine whether the binding of IgA to surfactant proteins was canonical, we isolated IgA from a plasma pool of dCOVID patients ($n = 15$) and performed antibody fragmentation with papain (33). ELISA with SP-B and SP-C using an Fc-specific horseradish peroxidase-conjugated secondary anti-human IgA antibody revealed significantly less signal compared with nonfragmented IgA. This supports the notion of a canonical binding of IgA to these proteins (Figures E5A and B). To explore whether the SP-B– and -C–binding IgA from blood are virus-specific and crosstarget surfactant proteins because of molecular mimicry, we performed a competition assay. Virus-specific antibodies were blocked by preincubation of plasma with SARS-CoV-2 lysate at a 20-fold excess compared with ELISA coating. Indeed, the absorbance signal was significantly reduced by an average of 22% for SP-B and 25% for SP-C after three replicates of ELISA (Figure E5C). Although the signal reduction is modest, it is, nevertheless, likely caused by mimicry between viral and surfactant proteins.

### Pulmonary Surfactant Function Is Inhibited by Plasma and Isolated IgA from Patients with Severe COVID-19

We then assessed whether plasma and auto-IgA from patients with severe COVID-19 impaired the function of pulmonary surfactant, potentially contributing to alveolar collapse. First, we investigated if plasma from patients with severe COVID-19 affected the surface tension of pulmonary surfactant using capillary rise measurements. Poractant alfa was incubated with pooled plasma from either dCOVID ($n = 15$), mild COVID ($n = 5$), or uninfected healthy control subjects ($n = 5$). Incubation with plasma from patients with severe COVID-19 significantly increased surface tension compared with plasma from patients with mild COVID-19 or uninfected control subjects (Figure 5A). In contrast, there was no notable difference in surface tension

**Figure 1.** Surfactant protein B (SP-B) IgA are found in BAL fluid (BALF) of patients with severe COVID-19. (*A*) The top 30 IgA-bound proteins in BALF of patients with severe COVID-19 ($n = 18$) are presented in the left heatmap (red) and grouped by their respective physiological compartments. Each column represents a patient. The right heatmap (blue) shows results of IgA:protein precipitates of BALF from patients without COVID-19. Protein concentrations were normalized to the IgA heavy chain signals, and $\log_2$ transformed. (*B*) Schematic workflow of the experimental setup for the identification of IgA-bound antigens. BALF was processed with peptide M pulldown columns, and IgA-bound proteins were measured and identified via liquid chromatography–tandem mass spectrometry. (*C*) The quantitative comparison revealed a significantly higher concentration of IgA:SP-B complexes in the severe COVID-19 versus non-COVID-19 BALF ($^{*}P = 0.03$, Mann-Whitney test). Alpha-ETF = electron transfer flavoprotein subunit alpha; EXOC3L = exocyst complex component 3-like protein; glycopr. = glycoprotein; immunoprec. = immunoprecipitate; LC-MS/MS = liquid chromatography with tandem mass spectrometry; norm. = normalized; pr. = protein; sub. = subunit.

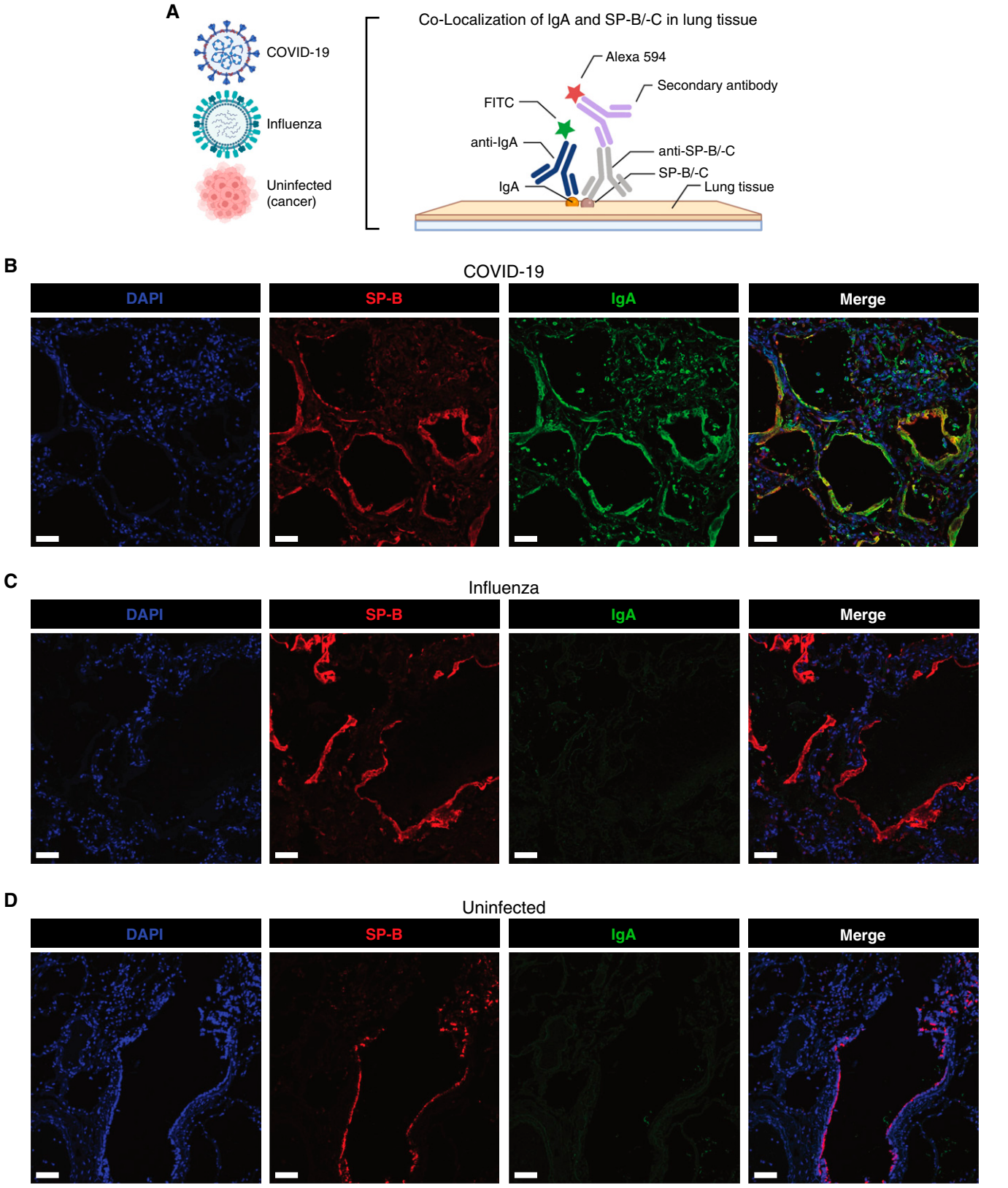



**Figure 2.** Lungs of patients with severe coronavirus disease (COVID-19) harbor colocalized surfactant protein B (SP-B) and IgA. (*A*) Schematic of the staining strategy used to detect colocalization (merge = yellow) of IgA (fluorescein isothiocyanate [FITC] = green) with SP-B (Alexa 594 = red) in lung tissue from fatal COVID-19, influenza, or patients with cancer, by immunofluorescence. (*B*) Double immunofluorescence

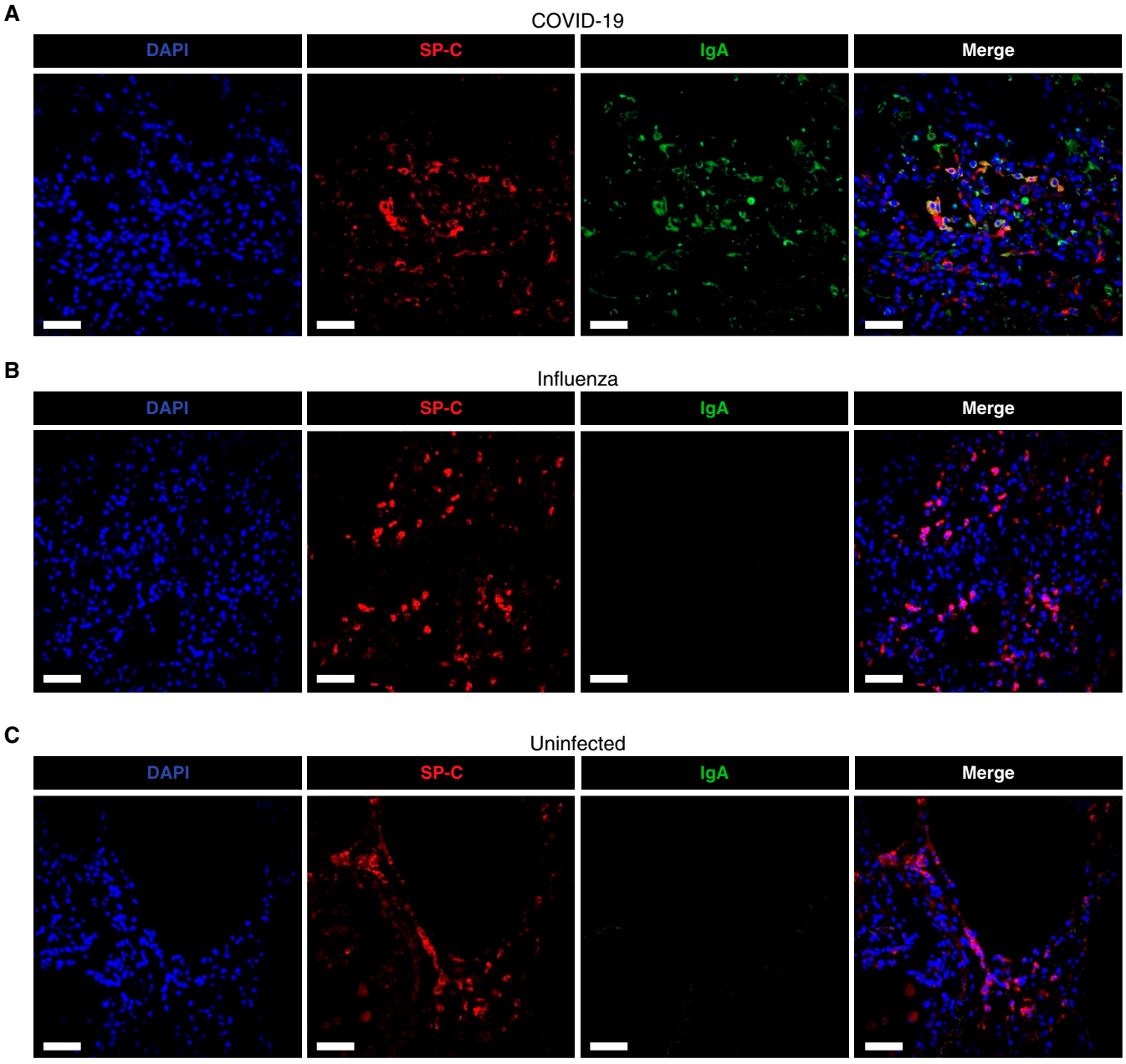



**Figure 3.** Lungs of patients with severe coronavirus disease (COVID-19) harbor colocalized surfactant protein C (SP-C) and IgA. (*A*) Double immunofluorescence staining for SP-C (Alexa 594 = red) and IgA (fluorescein isothiocyanate [FITC] = green) in formalin-fixed paraffin-embedded sections from lung tissue of patients with COVID-19 ($n = 4$), (*B*) in biopsy specimens from patients with influenza ($n = 2$), and (*C*) in uninfected patients with metastatic melanoma ($n = 3$). Colocalization of SP-C with IgA was consistently seen only in samples from deceased patients who had COVID-19 and not in the lungs of the other two groups. Scale bars, 100 μm.

when comparing incubation with plasma from patients with mild COVID-19 or uninfected control subjects. To investigate whether IgA was the cause of this functional loss, we repeated the experiment with purified IgA from the dCOVID cohort ($n = 5$). Concurringly, incubation with IgA resulted in significantly increased surface tension (Figure 5B). Taken together, these results strongly suggest that auto-IgA impairs pulmonary surfactant function in patients with severe COVID-19 (Figure 5C).

**Figure 2.** (*Continued*). staining for SP-B and IgA in formalin-fixed paraffin-embedded sections from lung tissue of patients with COVID-19 ($n = 4$), (*C*) in biopsy specimens from patients with influenza ($n = 2$), and (*D*) in uninfected patients with metastatic melanoma ($n = 3$). Consistently, colocalization of SP-B with IgA was only observed in samples from deceased patients who had COVID-19 and not in the lungs of the other two groups. Scale bars, 100 μm.

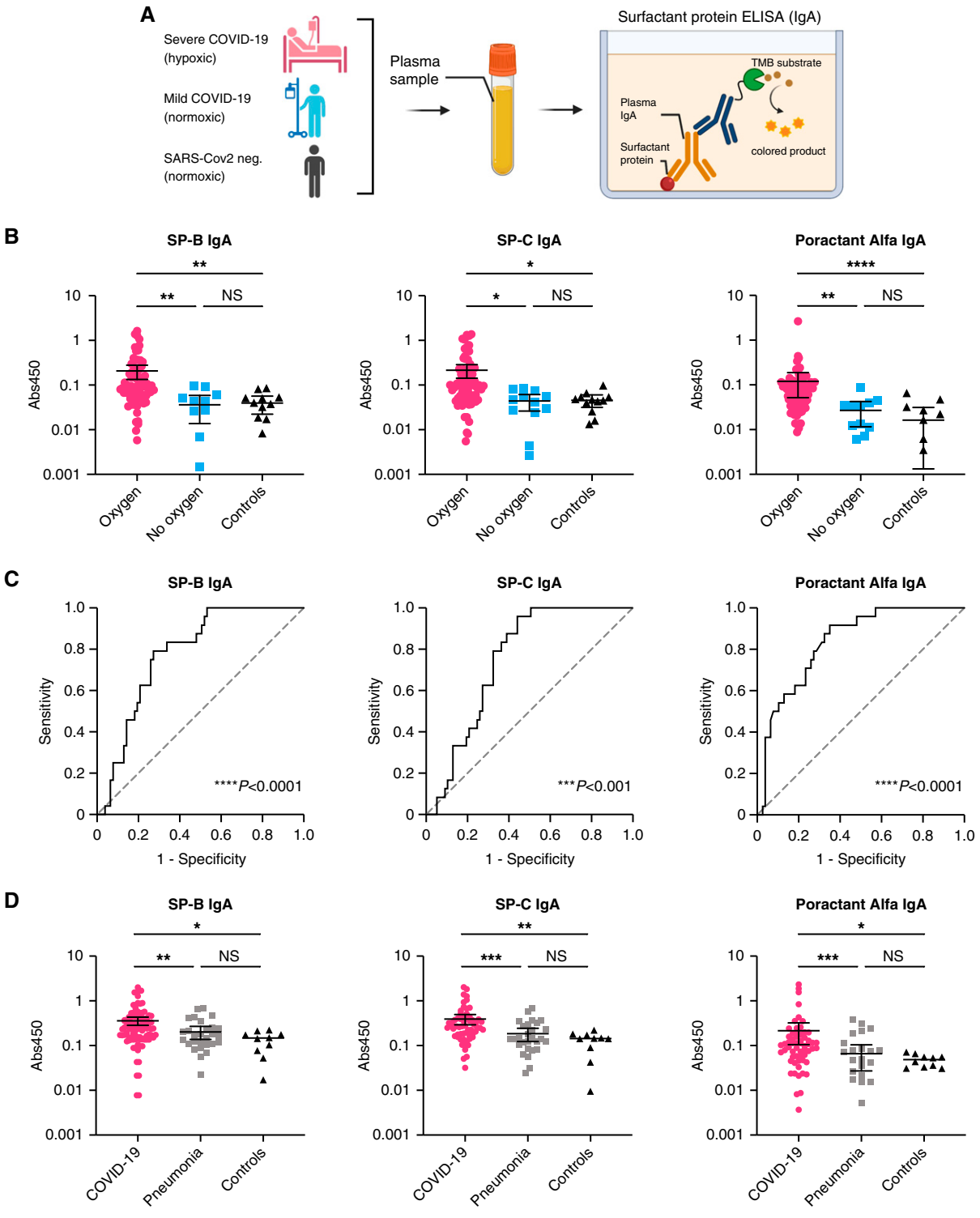



**Figure 4.** IgA targeting surfactant proteins B and C (SP-B and -C) are elevated in blood samples from patients with severe coronavirus disease (COVID-19). (*A*) Schematic representation of the IgA enzyme-linked immunosorbent assay (ELISA) experiment using recombinant proteins as coating and diluted plasma from patients as the antibody source. (*B*) SP-B (left), SP-C (center), or poractant alfa (right) ELISAs revealed significantly more IgA against the target proteins in plasma from patients with severe COVID-19 ($n = 77$) compared with those with mild COVID-19 ($n = 12$) or healthy control subjects ($n = 12$). (*C*) Receiver operating characteristics analysis demonstrated that the presence of IgA against either SP-B (left), SP-C (center), or poractant alfa (right) was significantly associated with the requirement of the patient for oxygen supplementation. (*D*) Comparison of the surfactant-specific IgA concentrations in serum from patients with COVID in the validation cohort ($n = 60$), patients with bacterial pneumonia and without severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection ($n = 30$), and noninfected control

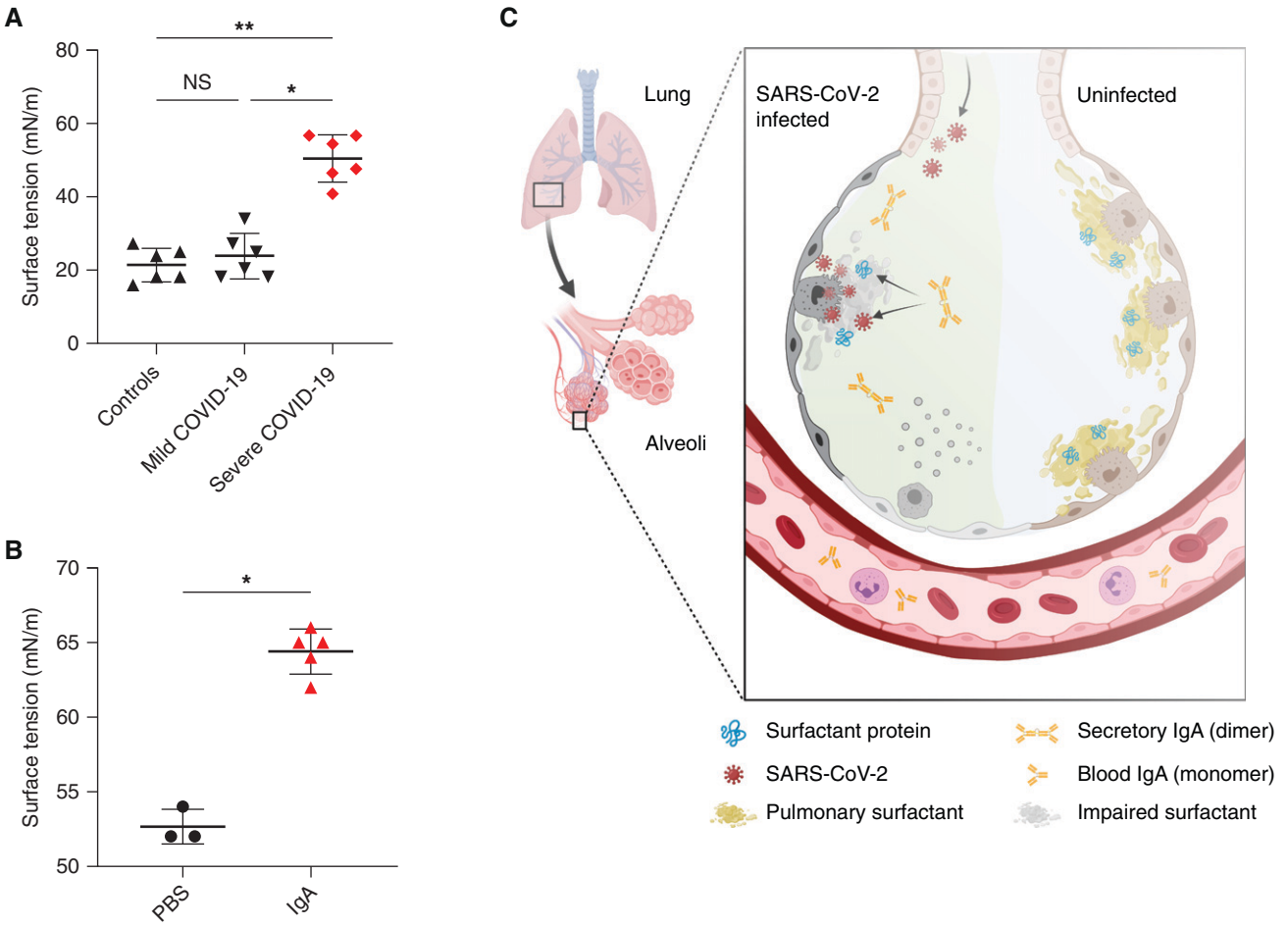



**Figure 5.** Plasma and isolated IgA from patients with severe coronavirus disease (COVID-19) inhibit the function of pulmonary surfactant. (*A*) Incubation of poractant alfa (40 mg/ml) with plasma from patients with severe COVID-19 resulted in a significant increase in surface tension, whereas plasma from patients with mild COVID-19 and patients in the noninfected healthy control did not experience notable changes. (*B*) Purified IgA (0.2 mg/ml) from plasma of patients with severe COVID-19 ($n = 5$) caused a significant increase of surface tension compared with PBS control subjects ($n = 3$). (*C*) Graphical summary of our results showing that auto-IgA occurs in the alveoli and blood of severely diseased patients with COVID-19, binds to surfactant proteins, and causes impairment of pulmonary surfactant function. All data are represented as mean ± SD. For single comparisons, a Mann-Whitney test was used, and for multiple comparisons, a Kruskal-Wallis test with Dunn's correction was applied. *$P < 0.05$ and **$P < 0.01$. NS = not significant; PBS = phosphate-buffered saline.

## Discussion

COVID-19 continues to burden healthcare systems, and the mechanisms that contribute to disease progression remain poorly understood. Here, we demonstrate that antisurfactant IgA in patients with severe COVID-19 impairs pulmonary surfactant proteins. Although the presence of autoantibodies in COVID-19 has been reported (17, 19, 21), our data reveal a potential mechanistic driver of disease, as we show that auto-IgA interferes with the ability of pulmonary surfactant to lower surface tension. This could lead to impaired stabilization of the pulmonary air sacs, thus contributing to alveolar collapse and insufficient oxygen exchange (34). In an ongoing clinical trial with bovine surfactant (Alveofact) for treatment of COVID-19, Postle and colleagues reported that pulmonary surfactant was compromised in patients with severe disease (35). Piva and colleagues showed that bronchoscopically administered poractant alfa as a surfactant replacement reduced the 28-day mortality of patients with severe COVID-19 (36). Avdeev and colleagues demonstrated that survivors of severe COVID-19 with surfactant substitution required less ventilation and were able to leave the hospital earlier than those without the replacement (37). These findings strongly suggest a crucial role of

**Figure 4.** (*Continued*). patients without pneumonia ($n = 10$). ELISAs consistently detected significantly higher concentrations of IgA against SP-B (left), SP-C (center), and poractant alfa (right) in patients with severe COVID-19 compared with patients without COVID-19. All data are represented as mean ± 95% confidence interval. For multiple comparisons, the Kruskal-Wallis test with Dunn's correction was used. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. NS = not significant; TMB = substrate for horseradish peroxidase.

pulmonary surfactant in COVID-19. Antibodies targeting surfactant proteins have been associated with respiratory insufficiency in rabbits (38), and the addition of SP-B antibodies to surfactant in newborn rabbits leads to widespread alveolar collapse (39). Similarly, Kobayashi and colleagues reported that surfactant replacement therapy was ineffective for treating surfactant-deficient immature newborn rabbits when SP-B antibodies were added. In contrast, the addition of SP-A antibodies did not impair the function of supplementary surfactant (40, 41). Importantly, Losada-Olivia and colleagues recently investigated the effect of SARS-CoV-2 infection on pulmonary surfactant by comparing its activity in patients with and without COVID-19 ARDS. In line with our data, changes in pulmonary surfactant activity were only found in patients with COVID-19 (42). Furthermore, ventilator-induced lung injury has been shown to impair pulmonary surfactant, possibly contributing to disease progression (43).

J-chain colocalization with IgA in lungs of patients with severe COVID-19 indicates that these are primarily sIgAs and, likely to a lesser extent, monomeric IgAs entering from damaged lung capillaries (44). These findings imply an expansion of local IgA-producing plasma cells, which is supported by a previous report showing compartment-specific IgA production (20). Furthermore, it is possible that IgAs bind to other COVID-19–relevant proteins, such as antimicrobial peptides, which are involved in defense against bacterial infection (45).

One limitation of our investigation is the number of enrolled patients. In addition, access to longitudinal samples would allow us to analyze antibody concentration changes over time, including the effects of immunosuppressive therapies such as corticosteroids. Furthermore, surfactant protein reduction because of the destruction of type 2 pneumocytes by SARS-CoV-2 cannot be ruled out. However, as incubation of pulmonary surfactant with plasma from patients with severe COVID-19 leads to an increase in surface tension, it is likely that auto-IgAs impact surfactant protein function. Given the region and time of our blood and tissue sample collection, we assume that most patients in our COVID-19 cohorts had been infected with either the SARS-CoV-2 wild type or α (B.1.1.7) strain. It is possible that this pathomechanism depends on virus variants and should be further explored in future studies.

## Conclusions

We identified SP-B– and SP-C–IgA in patients with severe COVID-19 and demonstrated that auto-IgAs impair the capability of pulmonary surfactant to lower surface tension. Our data further strengthen the notion that IgA antibodies against self-antigens in the lung corrupt crucial components of alveolar gas exchange, thereby providing novel mechanistic insights into the progression of COVID-19. ■

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