New-onset IgG autoantibodies in hospitalized patients with COVID-19

COVID-19 is associated with a wide range of clinical manifestations, including autoimmune features and autoantibody production. Here we develop three protein arrays to measure IgG autoantibodies associated with connective tissue diseases, anti-cytokine antibodies, and antiviral antibody responses in serum from 147 hospitalized COVID-19 patients. Autoantibodies are identified in approximately 50% of patients but in less than 15% of healthy controls. When present, autoantibodies largely target autoantigens associated with rare disorders such as myositis, systemic sclerosis and overlap syndromes. A subset of autoantibodies targeting traditional autoantigens or cytokines develop de novo following SARS-CoV-2 infection. Autoantibodies track with longitudinal development of IgG antibodies recognizing SARS-CoV-2 structural proteins and a subset of non-structural proteins, but not proteins from influenza, seasonal coronaviruses or other pathogenic viruses. We conclude that SARS-CoV-2 causes development of new-onset IgG autoantibodies in a significant proportion of hospitalized COVID-19 patients and are positively correlated with immune responses to SARS-CoV-2 proteins.
Coronavirus Disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection, is associated with many different clinical features that are commonly found in autoimmune diseases, including arthralgias, myalgias, fatigue, sicca, and rashes. Less common manifestations of autoimmunity have also been observed in COVID-19 patients, including thrombosis, myositis, myocarditis, arthritis, encephalitis, and vasculitis. These clinical observations, and the increasing proportion of “recovered” patients with persistent post-COVID-19 symptoms (so-called “long haulers,” or “long COVID”) suggest that inflammation in response to SARS-CoV-2 infection promotes tissue damage in the acute phase and potentially some of the long-term sequelae.

Autoantibodies, a hallmark of most but not all autoimmune disorders, have been described in COVID-19 patients. In the earliest report, approximately half of hospitalized patients at an academic hospital in Greece had high levels of serum autoantibodies, often associated with clinical findings such as rashes, thrombosis, and vasculitis. Serum anti-nuclear antibodies (ANA) were detectable in approximately one-third of patients. Wodruff et al. reported that 23 of 48 (44%) critically-ill COVID-19 patients have positive ANA tests. Zuo described an even higher prevalence of thrombogenic autoantibodies, reporting that up to 52% of hospitalized COVID-19 patients have anti-phospholipid antibodies. They further showed that autoantibodies have the capacity to cause clots in mouse models. In a large autoantibody screen, Gruber et al. demonstrated that Multisystem Inflammatory Syndrome in Children (MIS-C) patients develop autoantibodies, including autoantibodies against the lupus antigen SSb/La. SSA/Ro autoantibodies have also been described. The apparent link between clinical manifestations resembling those seen in patients with classifiable autoimmune diseases, and those observed in COVID-19 patients, has prompted searches for candidate target autoantigens that may be useful for diagnosis and for improving understanding of COVID-19 pathogenesis. The molecular targets of autoantibodies in individual patients with COVID-19 are largely unknown, as are their associations with anti-viral immune responses, and the timing of their appearance in regard to infection with SARS-CoV-2.

We hypothesized that SARS-CoV-2 induces the production of antibodies against traditional autoantigens and cytokines/chemokines de novo, and these correlate with anti-viral responses. We assembled three different custom bead-based protein arrays to measure IgG antibodies found in CTDs, ACA, and anti-viral responses in 197 COVID-19 samples. Samples were obtained from 147 hospitalized patients infected by SARS-CoV-2, some of which were collected longitudinally, in three geographically distinct locations. Our results demonstrate that a large cadre of autoantigens are targeted by circulating antibodies in a substantial proportion of hospitalized patients with COVID-19, but less commonly in uninfected healthy controls (HC). Our studies confirm emerging reports of IgG autoantibodies in hospitalized COVID-19 patients and demonstrate that a significant subset of patients develops new-onset autoantibodies that could place them at risk for progression to symptomatic, classifiable autoimmunity in the future.

**Results**

**Anti-nuclear antibodies (ANA) are produced by one in four hospitalized COVID-19 patients.** To determine if hospitalized patients with COVID-19 produce autoantibodies against prototypical autoantigens associated with systemic autoimmunity, we measured ANA using an indirect immunofluorescence assay in one of our cohorts (University of Pennsylvania). We found that ten out of 73 patients (14%) were positive at a dilution of 1:160, six were positive at 1:320, three were positive at 1:640, and one was positive with greater than 1:1280 (Supplementary Fig. 1a). ANA positive samples were further diluted at 1:320, 1:640, and 1:1280 to determine titers. A variety of ANA patterns were observed including diffuse, speckled, and nucleolar (Supplementary Fig. 1b, c and Supplementary Table 1). Three patients exhibited cytoplasmic staining but were negative for nuclear staining at 1:160. Given the finding of positive and weakly positive ANAs, we measured dsDNA antibodies. Only one individual out of 73 tested was positive for dsDNA antibodies at a dilution of 1:270, and this individual also was ANA positive with a speckled pattern (Supplementary Fig. 2). Since several patients who were severely or critically ill had thrombocytopenic and vascular events, we also analyzed the same 73 patients for Myeloperoxidase (MPO) and Proteinase 3 (PR3) antibodies, as these antibodies are associated with autoimmune vasculitis. Only one individual tested positive for PR3 antibodies (Supplementary Fig. 2). The levels of positivity in these clinical-grade assays are in line with those of one of the authors (J.J.) who reported that 17 of 113 (15.8%) hospital patients with positive SARS-CoV-2 serology had serum autoantibodies and/or antiphospholipid antibodies. These findings prompted us to “cast a wider net” for autoantibodies using additional patients and assays that detected larger numbers of not only common, but also unusual autoantigens.

**Protein microarrays identify autoantibody targets in hospitalized COVID-19 patients.** To systematically and simultaneously measure a large number of different autoantibodies in serum or plasma derived from patients acutely infected with SARS-CoV-2, we constructed a 53-plex COVID-19 Autoantigen Array (Fig. 1, left half of the panel). The array comprised well-characterized autoantigens (Supplementary Table 2) across multiple rheumatologic diseases (Supplementary Fig. 3). Included are prominent antigens targeted in systemic sclerosis (scleroderma, SSc, left panel; myositis and overlap syndromes, second panel); systemic lupus erythematosus (SLE), Sjögren’s syndrome, and mixed connective tissue disease (MCTD, third panel); gastrointestinal and endocrine autoimmune disorders (fourth panel); chromatin-associated antigens (fifth panel); and miscellaneous antigens, including proteins targeted in vasculitis or in which autoantibodies are postulated to be directly pathogenic (sixth panel). Most antigens have been validated in previous publications and were also validated using commercially available autoimmune disease prototype sera (Fig. 1, bottom panel), or using previously characterized serum from Stanford’s biobank and the Oklahoma Immune Cohort in the Oklahoma Medical Research Foundation Arthritis & Clinical Immunology Biorepository (SLE, SSc, MCTD, primary biliary cirrhosis, and other disorders).

We characterized 51 cross-sectional COVID-19 serum or plasma samples from patients who provided samples within seven days of hospitalization (Fig. 1). As expected, prototype reference samples from patients with classifiable autoimmune diseases were strongly positive for autoantibodies, recognizing 25 of the 53 arrayed proteins (Fig. 1, bottom left panel, and Supplementary Fig. 3). Serum from only four HC recognized a single autoantigen each (signal recognition particle 54, SRP 54; Smith/ribonuclear protein, Sm/RNP; guanosine nucleotide-binding protein alpha subunit, GNAL, a candidate autoantigen in autoimmune hypophysitis; and Ku 70/80, respectively, Fig. 1, middle panel). HC06 and HC30 each had high MFI anti-thyroidperoxidase (TPO) that exceeded the 5 SD cutoff if excluded from calculating the average TPO MFI using the other 29 HC samples. Both samples were therefore considered “positive” in our analysis, but we included them in calculating the 5 SD cutoff on the COVID samples. In striking contrast, 25 of 51 (49%) hospitalized patients...
with COVID-19 had autoantibodies recognizing at least one traditional CTD autoantigen (Fig. 1, top panel). Using a stringent 5 SD cut-off, serum antibodies from eleven COVID-19 patients identified a single antigen, thirteen recognized 2-3 antigens, and one subject (Subject UP40) recognized nine different autoantigens. Ribosomal P proteins (P0, P1, and P2) were most prominently targeted (10 of 50 patients, 20%), but were not found in any of the HC. Similar results were observed in 48 Kaiser subjects analyzed using an earlier-generation 26-plex autoantigen microarray, identifying overlapping RNA-containing autoantigen complexes including RPP14 Th/To, the Ro/La particle, the U1-small nuclear ribonucleoprotein particle (U1-snRNP), thyroid antigens, and chromatin proteins as targets in hospitalized COVID-19 patients, but in none of the HC (Supplementary Fig. 4).

Rare antigens are seen in patients with autoimmune myositis (MDA5, Mi-2, and tRNA synthetases such as PL-7 and Jo-1), and candidate autoantigens in autoimmune myocarditis (troponin...
and MYH6, Fig. 2a), were observed in individual patients, as were rare SSc autoantigens (Th/To (RPP25), fibrillarin, and the U11/U12 snRNP, Fig. 2b). A subset of autoantibodies (e.g., antibodies that bind the complement inhibitor C1q, thrombosis-associated antibodies that target beta 2 glycoprotein 1 (β2GP1), and vasculitis-associated antigens such as bactericidal permeability inducing protein (BPI)) that have been implicated in pathogenic inflammation in target organs, were also found in individual patients (Fig. 2c4–6,14–16). Relatively common autoantigens such as Scl-70, CENP A/B, and Sm/RNP were infrequent. Thyroid autoantibodies were also commonly observed (12/147 subjects across our entire study, 8.2%, using cutoffs of 3000 MFI and 5 SD above HC). Thyroid dysfunction, which is relatively common in the general population, has been reported in COVID-19 patients4,5. In all cases where samples from more than one time point were available, anti-TPO and anti-thyroglobulin (TG) were already present at high MFI levels in the baseline sample. Taken together, these findings reveal that hospitalized patients with COVID-19 produce an increased frequency of autoantibodies, but that there is a substantial inter-individual variation in which autoantigens are targeted.

Secreted proteins are common autoantigens in hospitalized COVID-19 patients. In a pair of elegant studies, Bastard5 and Wang17 independently identified anti-cytokine antibodies (ACA) in patients with severe COVID-19. Both groups showed that a subset of ACA prevents binding of soluble factors to their cognate
cell surface receptors and have been postulated to play a patho-
genic role by thwarting protective immune responses to COVID-
19. We created a 41-plex array comprising secreted proteins and
cell surface receptors, modeled on arrays we and others have used
previously to characterize “secretome” antibodies in immunode-
ficiency disorders18,19, SLE18, and systemic sclerosis patients20
(Supplementary Table 3). We observed even more striking results
with the secretome array, which revealed that serum antibodies in
41/51 (80%) of hospitalized COVID-19 patients recognized at
least one secreted or cell surface autoantigen (Fig. 1, the upper
right half of panel), while only 2/31 (6%) HC subjects recognized
a single antigen (interferon-gamma, IFN-γ in one and CD74 in
the other, Fig. 1, middle right half of the panel). Interestingly, the
IFN-γ+HC subject (HC27) also had serum antibodies specific for
Sm (a subunit of the U1-snRNP, using 5 SD cutoff) and for both
Ro60 and La (using a 3 SD cutoff), suggesting this “healthy”
subject is in preclinical evolution toward developing SLE, a
disease in which we have previously described multiple different
ACA including anti-IFN-α and anti-B cell-activating factor
(BAFF)18.

Interferons, particularly the Type 1 interferon IFN-α2, were
targeted in multiple COVID-19 patients at frequencies (n = 23
across all interferons, 45%) higher than recently published
findings from other groups17,21. Serum from five subjects
(UP11, UP38, UP41, UP42, and UP46) recognized two or more
interferons. In some COVID-19 patients, MFI values were
comparable to or even exceeded those observed in previously
characterized prototype patients with autoimmunity polyendocrine
syndrome type 1 (APS-1), pulmonary alveolar proteinosis (PAP),
and atypical mycobacterial infections (AMI) (see Fig. 3d).

Many interleukins were also prominently identified as auto-
antibody targets in this screen (e.g., interleukins −1, −6, −10,
−15, −17A, −22, and −31), as were cytokines with well-
characterized functions such as leukemia inhibitory factor (LIF),
the chemotactic chemokine macrophage inflammatory protein-1
alpha (MIP-1α), and vascular endothelial growth factor-B
(VEGF-B). Several striking reactivities were observed in individu-
al COVID-19 patients, including IL-12p70 (Subject UP47); the
SARS-CoV-2 receptor angiotensin-converting enzyme-2 (ACE-2, Subject
UMR19); granulocyte-macrophage colony-stimulating
factor which is the causative autoantibody target in PAP (GM-
CSF, Subject UP25); oncostatin-M (OSM, Subject UP40); and
soluble receptor activator of nuclear factor kappa B (sRANK-
ligand, Subject UP19). Subject UP17 was being treated with a
tumor necrosis factor-alpha (TNF-α) inhibitor at the time of
SARS-CoV-2 infection, explaining the high MFI reactivity to TNF
(Fig. 1). MFI for all antigens except IL-12p70 were very high
(>10,000) in individual patients. Autoantibodies against all
interleukins, cytokines, and ACE-2 identified in the initial screen
were also observed using a 5 SD cutoff in a second COVID-19
cohort (n = 98 longitudinal samples from 48 different patients,
see Fig. 3, Supplementary Figs. 5 and 6), with few exceptions (e.g.,
IL-1α, although IL-1β was targeted using a 3 SD cutoff; IL-31,
which met a 3 SD cutoff; and GM-CSF).

A subset of autoantibodies is triggered by SARS-CoV-2 infec-
tion. To determine if autoantibodies targeting traditional auto-
antigens or cytokines were generated de novo (versus existed
prior to infection), we analyzed 48 hospitalized COVID-19
patients (Stanford University, University of Pennsylvania, and
Marburg University) in whom samples were available at two or
more different time points. Twenty-four patients had an avail-
able sample from the day of hospitalization or (day 0). The
interval between the collection of the second sample ranged from
2–58 days (mean interval = 15.8 days). Two subjects (UP70 and
UP71) also had a third sample drawn 14–21 days post ICU
admission. To reduce batch effects, all samples at all time points
were analyzed on the 53-plex COVID-19 Autoantigen Array in
the same instrument run (Supplementary Fig. 5 top panel)
together with HC (Supplementary Fig. 5, middle panel, n = 16)
and serum samples from prototype autoimmune diseases served
as positive controls (Supplementary Fig. 5, bottom panel, n = 8).

As with the unpaired samples described in Fig. 1, autoanti-
bodies from patients with paired samples had high MFI in
individual patients. Some patients have again identified whose
serum recognized a large number of autoantigens (Supplementary
Figs. 5 and 6). Twenty-five (52%) of hospitalized COVID-19
patients had autoantibodies against at least one autoantigen.
Serum autoantibodies recognized two or more antigens (range
2–7 antigens) in seven patients (15%) (Fig. 3a, and Supplemen-
tary Fig. 6). The longitudinal analysis identified prominent
increases in autoantibodies at the second available time point
(Fig. 3b). In 9 individual patients (19%), autoantibody measure-
ments were above the average for HC at the earliest available time
point and MFI increased by at least 50%, exceeding the 2500 and
3000 MFI cutoff at the later time point (Fig. 3b, e.g., MDA5,
subject UP50; BPI, subject UP52; Supplementary Fig. 6). Some
autoantibodies were at or below the average for HC at the first
time point and increased over time (e.g., histones and histone H3,
subject UP65; and β2GPI, subjects UP65 and UP52), suggesting
these autoantibodies were directly triggered by SARS-CoV-2
infection. Others were already elevated at the first time point
and did not have large increases in MFI over time (n = 22, 45%)
(Fig. 3c and Supplementary Fig. 6). In a small number of cases,
autoantibody MFI levels decreased below the SD and MFI cutoffs
over time (n = 5, 10%), suggesting that their development might
be transient (e.g., PL-7, subject UP70, Fig. 3b). Anti-TPO and
anti-Scl-70 (Fig. 3c) remained elevated at high levels in all
seropositive subjects regardless of the time of measurement,
suggesting that these autoantibodies were already present
at hospitalization and likely represent preclinical (asymptomatic),
unreported, or undiagnosed autoimmunity.

To further evaluate the potential evolution of autoantibodies,
we performed ANA testing on 21 individuals with paired
samples. Eight individuals (38%) had positive or weak positive
ANA reactivity. Among these 8 individuals, ANAs were present
at both time points in three, changed in the intensity of staining
in two, and were positive at only one of the two time points
in the final three (Supplementary Fig. 1b and Supplementary Table 1).
Taken together, these data indicate that autoantibody levels
change over time in individual COVID-19 patients, consistent
with their production and, in some cases, transience during acute
illness.

We next examined whether IgG ACA is triggered by SARS-
CoV-2 infection. Paired samples from the same 48 subjects
described above were used to probe the 41-plex cytokine array,
again in a single, batched run. As observed with unpaired samples
(Fig. 1), 28 of 48 (58%) of COVID-19 patients had at least one
ACA (Supplementary Fig. 6). Of these twenty-eight, sera from
fifteen patients recognized one cytokine, five recognized two
cytokines, and eight recognized three or more cytokines (range
3–12 antigens). Interferons, IL-17, and RANK-L were the most
common targets, and interferons, IL-17, and IL-22 were new
targets in some patients (Fig. 3d). In addition to Subject UMR19
(Fig. 1), a second patient with high MFI ACE-2 autoantibodies
was also identified (Subject UMR12, Fig. 3d). Increased MFI was
observed for one or more autoantibodies at later time points in 12
patients (24%). Several were present at MFI levels near or below
the average for HC at baseline and were induced to high MFI
levels at the second time point (e.g., anti-IFN-α, subject UMR07;
anti-IFN-ε, subjects UP63 and UP65; and anti-IL-22, subjects

UP54, UP63, UP65, and UMR10, Fig. 3d). In many patients, ACA MFI levels were significantly elevated at the first time point and decreased at the later time point, suggesting that some ACA were pre-existing and/or developed transiently following SARS-CoV-2 infection. Subject SU09 had very high MFI levels of anti-TNF-α at both time points, attributed to anti-TNF therapy. We conclude that antibodies against cytokines, chemokines, growth factors, and receptors are common in hospitalized COVID-19 patients. Many are triggered in response to SARS-CoV-2 infection, even at later time points distant from the time of infection (e.g., anti-IL-22, subject UMR10, day 29, Fig. 3d).

To further evaluate the change in autoantibodies targeting traditional autoantigens or cytokines over time, we performed a targeted analysis of 21 of the 48 patients who had paired autoantibody data specifically at D0 and D7 of hospitalization (Supplementary Fig. 7). Almost all patients (18/21, 86%) had demonstrable changes in the number of antibodies, defined at varying thresholds of sensitivity (>3 SD vs. 3–5 SD vs. >5 SD) between D0 and D7 (Supplementary Fig. 7a). When combining the number of autoantibodies targeting traditional autoantigens or cytokines (Supplementary Fig. 7b), there is a trend towards increased numbers both of autoantibodies and ACAs per subject.
over time. Higher numbers of individuals with more autoantibodies and ACAs at D7 compared to D0 at the 3–5 SD threshold were observed (Supplementary Fig. 7b and 7c), but the difference in medians between D0 and D7 was not statistically significant. Nevertheless, these data clearly show that there is an ongoing evolution in both the numbers and levels of autoantibodies and ACAs with time in hospitalized COVID-19 patients.

We next sought to quantify how much more frequently autoantibodies targeting traditional autoantigens and cytokines are observed in hospitalized COVID-19 patients than in HC. We conducted 2-sample tests for equality of proportions comparing the number of ANA or ACA+ COVID-19 patient subjects and HC. Any subject with an MFI value that was >5 SD above the mean MFI of the HC and >3000 for at least one of the 94 antigens (combining traditional autoantigens and cytokines) was considered ANA or ACA+. Autoantibodies were found to be significantly more common in the unpaired patient subjects than in HC (p = 4 × 10^−11 using a one-tailed, 2-sample test for equality of proportions). Forty-five of 51 (88%) of the paired hospitalized patients with COVID-19 were ANA or ACA+ compared to 5 of 31 (16%) HC. Autoantibodies were also found to be significantly more common in the paired patient subjects than in HC (p = 2 × 10^−5 using a one-tailed, 2-sample test for equality of proportions). Twenty-eight of 48 (58%) of the paired hospitalized patients with COVID-19 were ANA or ACA+ compared to 0 of 16 HC.

Broad anti-viral immune responses target internal viral proteins in hospitalized COVID-19 patients. We have used protein arrays for epitope mapping and to measure antibody responses in influenza vaccines and in a nonhuman primate human immunodeficiency virus (HIV) vaccine study. We used a similar approach here to characterize anti-viral responses following SARS-CoV-2 infection. We created a 28-plex COVID-19 viral array that included structural and surface proteins from SARS-CoV-2 as well as eight commercially available recombinant non-structural proteins localized to the interior of the virus (Supplementary Table 4). As an initial validation, we compared array-based detection and measurement using a clinical-grade ELISA (R = 0.81, Spearman’s, p < 0.0001 for anti-SARS-CoV-2 nucleocapsid; R = 0.60, Spearman’s, p < 0.0001 for anti-SARS-CoV-2 RBD, Supplementary Fig. 8a and 8b, respectively). By studying the anti-viral antibody (AVA) response, we hoped to understand if certain viral antigens might correlate with the development of autoreactive immune responses. We hypothesized that poorly controlled SARS-CoV-2 infection leads to the development of serum antibodies that recognize not just structural proteins such as the SARS-CoV-2 spike protein, but also nonstructural proteins, and that a subset of these viral proteins might correlate with the development of autoimmunity. Proteins from related coronaviruses were also included to explore whether pre-existing antibodies to seasonal coronaviruses might correlate negatively or positively with disease severity, and with autoimmunity.

Figure 4 depicts a heatmap representation of IgG reactivity based on MFI (Fig. 4a, left panel) and calculation of SD above average MFI for HC (Fig. 4b, right panel). As expected, nearly all patients had broad immune responses to viral structural proteins (first seven antigens on left, Fig. 4a, b). Twelve patients had low MFI levels at the earliest time point (almost all were day 0, defined as a collection within the first 24–72 h of hospitalization but developed high MFI IgG SARS-CoV-2 antibodies when tested at later time points, consistent with previously published findings in the setting of acute illness. Other subjects (e.g., subject UP50) already had broad AVA responses at day 0, suggesting the subject had been infected for a significant period of time prior to hospital admission.

IgG antibody levels against non-structural SARS-CoV-2 proteins were significantly elevated in a majority of patients (n = 35, 73%), particularly papain-like protease (PLPro, n = 13 patients), open reading frame proteins (Orf 8, n = 14 patients and Orf 3a, n = 18 patients); and nonstructural proteins (NSP 1, n = 20 patients, and NSP 9 n = 31 patients, but not NSP 7 (n = 0), NSP8 (n = 1) or 3C-like protease (n = 3)). The number of targeted non-structural proteins increased over time in 20 of 49 (40%) patients when compared with the earliest available time point, and were absent (n = 14), did not change (n = 7), or decreased (n = 8) in the remaining patients. Of the eight patients who showed a decrease in the number of targeted non-structural SARS-CoV-2 antigens over time, all but one decreased by a single antigen, and three were patients who had samples collected at an interval of 37 days, making it likely that the immune responses were transient. A majority of patients had linked antibody responses in which multiple non-structural antigens were targeted in the same subject. In rare patients (e.g., subject UP65, see SARS-CoV-2 protein PLpro, Figs. 4a and 4b), the initial immune response was focused on an internal protein (or was pre-existing) and later evolved to target spike and other SARS-CoV-2 surface or structural proteins. We conclude that antibody responses in hospitalized COVID-19 patients are not limited to structural proteins, that linked responses to multiple non-structural proteins are observed over time, and that NSP9 is the most commonly recognized internal SARS-CoV-2 protein of those tested on the array.

New-onset IgG autoantibodies are temporally associated with anti-SARS-CoV-2 IgG responses. We next identified a subgroup of patients (n = 12) whose anti-SARS-CoV-2 antibody responses suggested that they had been infected at a time point that was proximate to hospitalization and capture of the first sample. Our analysis identified patients without an anti-viral response at the first time point suggesting the sample was collected close to the time of infection. Selection criteria for patients who were early in their anti-viral responses included (i) the first available sample was within three days of hospitalization; (ii) anti-spike S1 IgG levels were <5000 MFI at baseline; (iii) anti-RBD IgG levels were <20,000 MFI at baseline; and (iv) at least a 2-fold increase in MFI for IgG against both S1 and RBD was observed at the next available time point. We then studied these patients to further determine if new IgG autoantibodies appeared at the second time point, providing evidence that SARS-CoV-2 directly triggers the development of autoantibodies.

We compared IgG reactivities at both time points for all twelve subjects who met the above criteria on COVID-19 autoantigen arrays (Fig. 5a, left panel) and cytokine arrays (Fig. 5b, middle panel) with anti-viral responses using the virus array (Fig. 5c, right panel). Four of twelve patients were found to have at least one newly induced autoantibody at the later time point (white boxes). Two of these four patients had two or more new autoantibodies (Subjects UP52, n = 5 antigens; and subject UP65, n = 10 antigens). β2GPI, histones, and the 54 kD component of the myositis autoantigen signal recognition particle (SRP 54) were the most common antigens identified (n = 2 subjects each). Given the small sample size, no clear correlations were identified between individual autoantibodies and IgG response to a specific viral protein (Fig. 5d and Supplementary Fig. 9).

Although 4 patients developed one or more new-onset autoantibodies using these criteria, the number of patients with longitudinal samples meeting these criteria was small (12 patients total), preventing us from drawing conclusions about the prevalence of this phenomenon. To further explore this finding, we performed a secondary analysis of the paired samples’ data to
Fig. 4 Measurement of anti-viral IgG responses using a COVID-19 viral array. a Heatmap depicting IgG antibodies using a 28-plex bead-based protein array. Viral protein antigens are grouped based on sixteen proteins from SARS-CoV-2 (left panel), other coronaviruses (middle panel), and other viruses (right panel), labeled on the x-axis. Most recombinant viral proteins were engineered to include a 6X-His-tag, which was used to validate conjugation to beads using an anti-epitope monoclonal antibody (bottom of the panel). The same COVID-19 patients from Fig. 3 (see Supplementary Figures 9 and 10) were analyzed (top panel, $n=94$ longitudinal COVID-19 samples, including paired samples from 44 subjects and 2 subjects who had 3 available time points each, subjects UP70 and UP71). HC ($n=16$, middle panel). Two patient sample pairs (UP63 and UMR20) were excluded from analysis due to technical failure on the viral array assay. Colors correspond to the MFI values shown at right. b Heatmap depicting statistically significant anti-viral IgG responses. Colors indicate IgG antibodies whose MFI measurements are $>5$ SD (red) or $<5$ SD (black) above the average MFI for HC samples collected prior to the COVID-19 pandemic. Source data are provided as a Source Data file.
identify those autoantibodies against traditional autoantigen or autoantibodies against cytokines that met all four of the following criteria: (1) MFI was at or below the mean for HC at first time point; (2) MFI increased by at least 2-fold at second time point; (3) MFI increased by at least 3 SD above the mean for HC at second time point; and (4) MFI was at least 3000 at the second time point. Although similar to the analysis we performed to obtain the data shown in Fig. 5, this new analysis separated out those patients whose earliest time point also may have included strong anti-viral responses. This new analysis shows that even after a strong anti-viral response has occurred, 23% of patients developed at least one new autoantibody later in the hospitalization.

Finally, we correlated autoantibodies targeting traditional autoantigens or cytokines with anti-viral IgG responses using array data from the cohort described in Fig. 3, focusing on Penn and Marburg samples which had been collected at time points as proximate to the day of hospitalization as possible. We compared patients who had one or more autoantibodies \(( n = 15 \text{ first time point}, \ n = 13 \text{ second time point})\) with patients who had no autoantibodies \(( n = 21 \text{ first time point}, \ n = 23 \text{ second time point})\). Anti-SARS-CoV-1 RBD correlated positively with the autoantibody-positive group \(( p = 0.002 \text{ at the second time point using one-tailed Wilcoxon rank-sum test, } p = 0.044 \text{ using Bonferroni correction for each time point})\). NSP1 \(( p = 0.03 \text{ at second time point, } p = 0.08 \text{ at first time point})\) and ME \(( p = 0.04 \text{ at first time point, } p = 0.06 \text{ at the second time point})\) trended positively when correlating with autoantibodies but were not statistically significant after Bonferroni correction. An identical analysis was performed on ACA+ vs. ACA− patients, showing no correlations with IgG responses to any viral proteins, including influenza, SARS-CoV-1, and seasonal coronaviruses (OC43 RBD, 229E-FL-GCN4, NL63 RBD, and HKU1 RBD).

**Discussion**

We have used a multiplexed, bead-based platform to identify circulating antibodies in hospitalized patients with COVID-19 and have generated integrated results from three different protein microarrays to discover COVID-19 associated autoantigens and link them to anti-viral responses. Our studies have led to several important findings that provide further insights into COVID-19 pathogenesis. First, we found that approximately half of hospitalized COVID-19 patients develop serum autoantibodies against one or more antigens on our array even though only a quarter of all patients are ANA+. Increased levels of autoantibodies are not simply a reflection of hypergammaglobulinemia because they are produced out of proportion to total IgG serum concentration. In...
most individuals, only a small number of autoantigens are targeted, which is more consistent with a sporadic loss of self-tolerance than a global increase in autoantibody production. Second, the autoantibodies we discovered are found in relatively rare connective tissue diseases that are not typically measured in clinical labs, and some are predicted to be pathogenic. Third, a surprisingly large number of ACA were identified, far more than just the interferon autoantibodies described recently. Fourth, antibodies recognizing nonstructural SARS-CoV-2 proteins were identified that correlate positively with autoantibodies. Finally, and perhaps most importantly, some autoantibodies are newly triggered by SARS-CoV-2 infection, suggesting that severe COVID-19 can break tolerance to self.

Approximately 60–80% of all hospitalized COVID-19 patients in our study had at least one ACA, with a greater number of different ACA specificities generated in individual patients than observed for traditional autoantigens. Two recent studies demonstrated that IFN-α and IFN-α-blocking ACA are found in patients with severe COVID-19. Anti-IFN antibodies with blocking activity were absent in all 663 tested patients with mild COVID-19, strongly linking the presence of anti-IFN to disease pathogenesis and severity. Another study reported that type I interferon (IFN) deficiency could be a hallmark of severe COVID-19, while other investigators pointed towards an untuned anti-viral immune response due to delayed-type I/III interferon signaling pathways, and expression of ACE-2 in vascular endothelia. Although not yet explored for COVID-19-associated inflammation, cytokine storm in lung and skin from COVID-19 patients correlate with fibrin deposition and thrombus formation. SARS-CoV-2 membrane proteins including the spike protein (but not SARS-CoV-2 RNA) colocalize with activated complement in ACE-2+ microvascular endothelia of COVID-19 lung tissue and normal-appearing skin. It has been suggested that these responses resemble classical IgG antibody responses and perhaps most importantly, some autoantibodies are newly triggered by SARS-CoV-2 infection, suggesting that severe COVID-19 can break tolerance to self.

We postulate that a subset of the autoantibodies we have identified contribute to the formation of inflammatory immune complexes in situ, particularly at endothelial surfaces. For example, neutrophil extracellular traps (NETs) have been implicated in COVID-19 patients with vasculitis. Antineutrophil Cytoplasmic Antibody (ANCA) associated vasculitis (AAV) has been strongly associated with neutrophil activation and generation of pro-inflammatory NETs containing necleic acids, histones, and inflammatory peptides. While we did not observe elevated levels of MPO or PR3, we identified high MFI anti-BPI antibodies in 6% of COVID-19 patients. The detection of autoantibodies to BPI and core as well as linker histones raises the possibility that NETs contribute to the generation of autoantibodies in severe COVID-19, a possibility that is in line with the neutrophilia that accompanies severe acute disease. Disseminated microvascular coagulopathy and microvascular injury in lung and skin from COVID-19 patients correlate with fibrin deposition and thrombus formation. SARS-CoV-2 membrane proteins including the spike protein (but not SARS-CoV-2 RNA) colocalize with activated complement in ACE-2+ microvascular endothelia of COVID-19 lung tissue and normal-appearing skin. Macrophages and dendritic cells in COVID-19 lung tissue and normal-appearing skin.

Severe infection may also result in an “all-hands-on-deck” immune response that results in loss of tolerance due to the presence of pro-inflammatory mediators that may lessen the requirement for T cell help. Some patients with severe acute COVID-19 appear to mount extrafollicular B cell responses that are characterized by expanded B cells and plasmablasts, loss of germinal centers, and loss of expression of Bcl-6. Antibody repertoires analyzed from hospitalized COVID-19 patients during acute disease include massive clones with low levels of somatic mutation (SHM) and elongated CDR3 sequences which can be associated with polyreactivity and are reminiscent of immune responses seen in acute Ebola and salmonella infection. It has been suggested that these responses resemble SLE flares in which autoreactive B cells are also activated via an extrafollicular, TLR7-dependent pathway. Although SARS-CoV-2 genomic RNA could itself serve as a costimulatory TLR7 ligand, many of the autoantigens we have identified also bind to structural RNAs such as the U1-snRNA (found in Sm/RNP complexes), 7S RNA (a component of SRP), and tRNAs (e.g., Jo-1, PL-7, and PL-12) which might activate dendritic cells in a TLR7-dependent manner.

One of the most important unanswered questions raised by our studies is why specific molecules are targeted in hospitalized COVID-19 patients. For newly triggered ACA, the most likely explanation is that they arise as a consequence of severe disease along with high levels of viremia, tissue injury, and elevated local levels of pro-inflammatory cytokines and chemokines. However, it is also possible that the presence of ACAs could affect the regulation of self-reactive lymphocytes by altering the half-lives of the receptor interactions of the target molecules. For traditional autoantigens, one possibility is that viral proteins or the SARS-
CoV-2 RNA genome and self-molecules physically interact, and that the initial immune response to the viral protein in a highly inflammatory microenvironment expands to include self-proteins through linked recognition and intermolecular epitope spreading. Another possibility is molecular mimicry in which one or more viral proteins or epitopes cross-react with self-proteins leading to loss of tolerance and development of autoimmunity. Experiments to explore these mechanisms are ongoing.

The vast majority of studies on SARS-CoV-2 proteins have focused on viral structural proteins to develop efficient and accurate diagnostic assays, and to identify specific epitopes on surface proteins for the development of vaccines and therapeutic monoclonal antibodies. These proteins are also the major focus of the immune response in most infected individuals. Here, we developed a multiplexed viral protein array that enables simultaneous measurement of antibody responses against 28 different proteins from 13 different viruses. We determined that non-structural proteins are recognized by antibodies in a large proportion of hospitalized COVID-19 patients, suggesting that B cell responses expand over time to involve additional viral molecules. IgG antibody levels against NSP1 and ME correlated positively with the presence of at least one autoantibody. Our viral array results (Figs. 4 and 5) confirm that the adaptive immune response to target non-structural viral proteins, some of which might physically interact or cross-react with autoantigens in the context of an intense local or systemic inflammatory environment, exceeding a threshold for breaking tolerance to self. In contrast, patients who rapidly mount neutralizing antibody responses to the viral spike protein abort “intraviral epitope spreading” and may be less likely to develop autoantibodies. Why anti-SARS-CoV-1 RBD IgG responses associate with autoantibody-positive patients is unclear. Future longitudinal studies are needed to determine whether broad B cell responses play any direct pathogenic role in patients with prolonged hospital courses or in patients with long-term sequelae of COVID-19 infection; to correlate anti-viral responses with autoantibodies targeting traditional autoantigens or cytokines over time using much larger COVID-19 cohorts including patients who are asymptomatic or have a mild disease; and to explore whether specific SARS-CoV-2 proteins might cross-react with autoantigens discovered in our screens.

Many studies of hospitalized COVID-19 patients, including our study, suffer from important limitations. First, confounding variables exist including heterogeneous demographics, medications at hospitalization, individualized treatment approaches, and, in some cases, unknown history of pre-existing medical or autoimmune conditions. Second, “Day 0” is not day 0 of infection but instead refers to a time point most proximate to hospitalization. Our viral array results (Figs. 4 and 5) confirm that the time between initial infection and sample acquisition was heterogeneous, potentially confounding interpretation of autoantibody results. Third, not all antigens (e.g., lipids, hydrophobic proteins, and carbohydrates) are compatible with our screening methodology, and as a result we have certainly missed some reactivities. Fourth, we did not include patients who were asymptomatic (e.g., patients with mild COVID-19, were vaccinated for SARS-CoV-2, had other severe viral illnesses, or were children). Finally, our analysis was limited to hospitalized patients during acute illness, with follow-up times of days rather than months or years.

Although beyond the scope of these studies, our data generate many more questions that need to be addressed in the coming years – questions that can only be answered by generating large cohorts of prospectively enrolled subjects with new-onset viral syndromes, including patients with COVID-19, respiratory illnesses which resemble COVID-19, and subjects enrolled in COVID-19 vaccine trials. Are autoantibodies targeting traditional autoantigens or cytokines specific to COVID-19, or is their presence shared more broadly in patients with influenza and other severe acute illnesses? Are autoantibodies found in convalescent serum used to treat patients with severe COVID-19? Do any of these autoantibodies underlie some of the signs and symptoms observed in “long COVID”, do they lead to classifiable autoimmune disease, and can they be used as predictive markers or identify subsets of patients who would benefit from targeted immunotherapies?

Our studies have begun to quantify the impact of SARS-CoV-2 on autoimmunity, identifying which antigens and specific autoimmune diseases to surveil in patients who have been infected, and contributing to our mechanistic understanding of COVID-19 pathogenesis. These studies provide a starting point for large-scale epidemiology studies to determine the extent of autoimmunity that results from SARS-CoV-2 infection, and its long-term impacts on the health care system and the economy. While the COVID-19 pandemic is leaving a wake of destruction as it progresses, it also provides an unprecedented opportunity to understand how exposure to a new virus could potentially break tolerance to self, potentially giving rise to autoimmunity and other chronic, immune-mediated, diseases.

Methods

Serum and plasma samples. Hospitalized COVID-19 patients. Serum or plasma samples were obtained following protocols approved by local institutional review boards (IRB) from 147 unique hospitalized subjects (n = 99 unpaired; n = 98 paired longitudinal samples from 48 distinct subjects). Samples were obtained from four centers in three distinct geographic areas: Northern California (Kaiser Permanente Health Care System, n = 48 unpaired samples from hospitalized subjects, IRB# 53718) collected in March and April 2020; and Stanford Occupational Health Clinic, 20 paired samples from 10 unique hospitalized subjects IRB# 55689) collected between April and June 2020; Philadelphia, Pennsylvania (University of Pennsylvania, n = 50 unpaired; and 44 paired samples from 21 unique hospitalized subjects, IRB # 808542) obtained between April and June 2020; and Marburg, Germany (Philips University Marburg, 1 unpaired; and 34 paired samples from 17 unique hospitalized subjects collected between April and June 2020, IRB# 57/20). Clinical characteristics of the cohorts can be found in Supplementary Tables 5–7 and in Supplementary Figs. 10 and 11.

Healthy Controls. Serum and plasma samples from anonymous healthy controls (HC, n = 41) were obtained prior to the COVID-19 pandemic from Stanford Blood Bank and Stanford Hospital and Clinics.

Bead-based antigen array content. We created three different custom, bead-based antigen arrays modelled on similar arrays that we previously used to study autoimmune and immunodeficiency disorders, and for characterizing vaccine responses. Antigens were selected based on our published datasets; literature searches that have implicated specific antigens in COVID-19; potential for a meaningful contribution to COVID-19 pathogenesis; and compatibility with bead-based platforms. A complete list of all antigens, vendors, and catalog numbers can be found in Supplementary Tables 2-4. The “COVID-19 Autoantigen Array” included 53 commercial protein antigens associated with CTDs (Supplementary Table 2). The “COVID-19 Cytokine Array” comprised 41 proteins including cytokines, chemokines, growth factors, acute phase proteins, and cell surface proteins (Supplementary Table 3). Specific “secretome” proteins included a subset of molecules identified in previous large screens in patients with systemic lupus erythematosus (SLE) (59-plex screen), systemic sclerosis (scleroderma or SSC, 221-plex screen, manuscript in preparation), Autoimmune Polyendocrinopathy Syndrome Type 1 (APS-1), Atypical Mycobacterial Infections (AMI), Immunosuppression Polycydomonocytopathy Enteropathy X-linked (IPEX), and more recently in COVID-19. The “COVID-19 Array” included 54 recombinant, purified SARS-CoV-2 proteins from commercial sources, or recombinant proteins produced in the labs of several of the authors (Kim and Wang, Supplementary Table 4). We also included proteins or protein fragments from SARS-CoV-1, Middle East Respiratory Syndrome (MERS), nonpathogenic coronaviruses (OC43, 229E, NL63, and HKU1), Hepatitis B, Mumps, Rubella, Rubeola, Ebola, and Influenza (hemagglutinin (HA) from A/California/07/2009 H1N1).

Array construction. Antigens were coupled to carboxylated magnetic beads (MagPlex-C, Lumineex Corp.) such that each antigen was linked to beads with a unique barcode. In brief, in order to avoid non-specific signal, control antibody was diluted in phosphate-buffered saline (PBS) and transferred to 96-well plates. Diluted antigens and control antibodies were conjugated to 1 x 10^6
carboxylated magnetic beads per ID. Beads were distributed into 96-well plates (Greiner BioOne), washed, and re-suspended in phosphate buffer (0.1 M NaH2PO4, pH 6.2) using a well plate washer (Biomek). The bead surface was activated by adding 100 μl of phosphate buffer containing 0.5 mg 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (Pierce) and 0.5 mg N-hydroxysuccinimide (Pierce). After 20 min incubation on a shaker, beads were washed and resuspended in activation buffer (0.05 M 2-N-Morpholino Ethanesulfonic acid, MES, pH 5.0). Diluted antigens and control antibodies were incubated with beads for 2 h at room temperature. Beads were washed three times in 100 μl PBS-Tween, re-suspended in 60 μl storage buffer (Blocking reagent for Enzyme-Linked Immunosorbent Assay, ELISA, Roche) and stored at plates in 4°C. Immobilization of some antigens and controls together with the correct bead IDs was confirmed by analysis using commercially available mouse monoclonal antibodies, or antibodies specific for epitope tags such as 6X-histidine. In addition, prototype human plasma samples derived from participants with autoimmune diseases with known reactivity patterns (e.g. ds-DNA, Sc-l, centromere, SSA, SSb, cardiolipin, whole histones, and RNP, all purchased from ImmunoVision; also from Stanford Autoimmune Diseases Biobank and OMRF); APS-1, IPEX, PAP, or AMI associated with anti-γ2 blocking antibodies; as well as normal human sera (ImmunoVision, Product # HNP-0300, certified to be nonreactive to Hep-2 cell lysates at a titer of 1:100), were used for validation.

**Array probing.** Serum or plasma samples were first heat-inactivated at 56°C for 1 h, then tested at 1:100 dilution in 0.05% PBS-Tween supplemented with 1% (w/v) bovine serum albumin (BSA) and transferred into 96-well plates in a randomized layout. The bead array was distributed into a 384-well plate (Greiner BioOne) by transfer of 5 μl bead array per well. 45 μl of the 1:100 diluted sera were transferred into the 384-well plate containing the bead array. Samples were incubated for 60 min on a shaker at room temperature. Beads were washed with 3 x 60 μl PBS-Tween on a plate washer (EL406, Biotek) and 50 μl of 1:50 diluted R-phycocerythrin (R-PE) conjugated Fcy-specific goat anti-human IgG Fab fragment (Jackson ImmunoResearch, Cat # 109-116-098) was added to the 384-well plate for detection of bound human IgG. After incubation with the secondary antibody for 50 min, the plate was washed on 3 x 60 μl PBS-Tween and re-suspended in 60 μl PBS-Tween prior to analysis using a FlexMap3D™ instrument (Luminex Corp.) and Luminex xPONENT® version 4.2 software. A minimum of 100 events per bead ID were counted. Binding events were displayed as Mean Fluorescence Intensity (MFI). To ensure reproducibility and rigor, all samples were run in duplicate in each experiment. Most samples were analyzed twice using the same array run on different days, showing that the mean Pearson correlation coefficient (mean SD) of 0.09 for one representative dataset). Prototype autoimmune sera were also heat-inactivated and compared with untreated prototype autoimmune serum on the same arrays, with similar results.

**Anti-dsDNA ELISA.** Anti-DNA ELISA was performed on calf thymus DNA (Sigma, Cat # D3664)-coated Immobilon (Immuno-1B) 96-well plates (Thermo Scientific, Cat # 3355) that were then blocked with 1% BSA (Sigma, Cat # A7906) in PBS (Corning, Cat # 21-031-CV). Dilution of patient and control plasma in blocking buffer were incubated in the DNA-coated 96-well plates, unbound Ig was washed off, and bound IgG was measured with goat anti-human IgG alkaline phosphatase conjugates (Southern Biotech, Cat # 2040-04) at a dilution of 1:2000. Samples were analyzed at dilutions of 1:30, 1:90, 1:270, and 1:810. Of these dilutions, 1:270 was chosen as optimal for distinguishing background binding from positive staining. Samples from five healthy donors were tested under the same conditions and an arbitrary cut-off of 2× the highest measured value at 1:270 (which was 0.390 arbitrary units) was used to distinguish positive from negative levels of binding.

**Anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3) ELISAs.** MPO (Cat # 708705) or purified proteinase 3 PR3 (Cat # 708700) pre-bound to the wells of microtiter plates were purchased from Inova Diagnostics (San Diego, CA). Assays were performed as recommended by the manufacturer using a dilution of 1:100 plasma in diluent (provided in the assay kit). Assay controls included strong positive (S), weak positive (W), and negative samples provided by the vendor. Additional controls included samples from five healthy donors and three de-identified patients known to have clinically elevated PR3 and MPO antibody levels. Results were scored as positive or negative based upon the kit instructions.

**ANA and imaging.** ANAs were performed by indirect immunofluorescence using fixed and permeabilized Hep-2 cells affixed to glass slides (Inova Diagnostics, Cat # 708800) as recommended using a FITC-conjugated goat anti-human IgG antibody following vendor instructions. Samples were screened in a blinded fashion at a dilution of 1:80 with ultraviolet (UV) microscopy by clinical laboratory staff (A.G. and J.G.) who have extensive experience in the interpretation of ANA patterns. Positive and weak positive samples (with evidence of either nuclear or cytoplasmic or mixed staining patterns) were tested further at 1:160 (the dilution at which ANAs are considered to be positive in the clinical lab assay, which uses the same assay kit) and if still positive at 1:160, further titrations were performed at 1:320, 1:640, and 1:1280. In addition to the kit positive and negative controls (which were included on every slide), de-identified clinical samples from patients with known clinically detectable ANAs were used.

For ANA image analysis, all images were collected with a Nikon Eclipse Ti with widefield illumination equipped with a Nikon Plan Apo VC 60x 1.4 oil objective. FITC and Evans Blue fluorescence images were collected with a Chroma dichroic/ beamsplitter (part no 98402) and a Chroma quadset CoolLED300 light source, using FITC 480/30x excitation and 519/26 emission filters, as well as Cy5 640/30x excitation and 697/60 emission filters. Images were acquired with a Hamamatsu ORCA-ER B&W CCD Digital Camera controlled with Metamorph V7.10.3.390 software and 1 x 1 camera binning. Multiple stage positions were collected using a Ludl XY linear encoded stage and Z motor. Minimum and maximum pixel values all set to the same level on a 12-bit camera (4096 gray levels–FITC 500 min, 2500 max, and Cy5 300 min, 1300 max), gamma set to 1, with acquisition times and light source intensities consistent for all images for comparison purposes.

**Anti-SARS-CoV-2 ELISAs.** RBD ELISAs were performed as described in a previous study with several modifications. SARS-CoV-2 RBD proteins (gift of Scott Hengley) were coated on the ELISA plates (Cat # 1193A15, Thomas Scientific, Swedesboro, NJ) at a final concentration of 2 μg/ml in 50 μl of 1x PBS. Serum and plasma samples were diluted at 1:100 in sample dilution buffer (PBS-Tween with 1% non-fat milk powder by weight) and incubated for 1 h at room temperature. Goat anti-human IgG-HRP (Cat # 109-035-008, Jackson ImmunoResearch Laboratories West Grove, PA) was diluted 1:10,000 with sample dilution buffer and 50 μl of secondary antibody was added to each well. After washing 3x (PBS-Tween), plates were incubated for 0.5 h at room temperature and washed again 3x (PBS-Tween), 50 μl of TMB substrate (Cat # 555214, Fisher Scientific, Waltham, MA) was applied for the color development at room temperature for 10 mins and stopped with 50 μl of 250 μM hydrochloric acid. All samples were run in duplicate. Anti-SARS-CoV-2 Nucleocapsid Assays were run with a commercially produced kit (Cat # CV3002, LifeSensors, Malvern, PA) and performed according to the manufacturer’s instructions. Serum and plasma samples were diluted at 1:100 and plates were read at OD450 nm. The RBD and nucleocapsid ELISAs were repeated to confirm the results. ELISA plates were read at OD450 nm (CLARIOstar plate reader, BMG LABTECH Inc., Cary, NC).

**Statistical analyses.** All data analysis and statistics were performed using R and various R packages. For normalization, average MFI values for “bare” bead IDs were subtracted from average MFI values for antigen conjugated bead IDs. The average MFI for each antigen was calculated using samples from healthy subjects known to be uninfected with SARS-CoV-2 (all obtained before December 2019). Antibodies were considered “positive” if MFI was > 3 SD above the average MFI for HC for that antigen, and MFI was > 3000 units, a threshold which is more stringent than commonly published in related literature. A less stringent 3 SD cutoff used in a Luminex assay to measure SARS-CoV-2 immunoglobulins in blood and saliva was also employed for comparison in some experiments. An example can be found in Supplementary Figs. 3 and 4. ELISA and antibody number data were visualized in GraphPad Prism v9.0.0 (86). Complexheatmap v2.7.7 was used for all heatmaps. Detailed availability data are published on the Gene Expression Omnibus (GEO) database. The accession code is provided in our data availability statement.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** All raw and normalized Luminex protein array data have been made publicly available in Gene Expression Omnibus (GEO) database with the SuperSeries accession code GSE180743. All other data provided to researchers are available from the corresponding author upon request. Clinical data other than data already shown in Supplementary Figures 10 and 11 are not available, to remain compliant with HIPAA requirements. Source data are provided with this paper.

**Code availability.** Code used for data analysis and figure generation is available from the corresponding authors upon reasonable request.

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Author contributions
S.E.C., A.F., P.J.U., C.S. and E.T.L.P. designed experiments and array panels. S.E.C. and S.D. performed antigen array, ACA, and viral array production, quality control, sample runs, and data acquisition. W.M., J.G., A.F., K.B. and E.T.L.P. performed and/or interpreted ANA assays. W.M., I.N., M.R. and E.T.L.P. performed and/or interpreted ELISAs. T.W., S. Chakraborty, P.A.W., A.E.P. and P.S.K. generated key antigens, provided pre-characterized sera, and assisted with data analysis and interpretation of viral array assays. W.H.R. generated key reagents for the autoantigen arrays and supervised A.H. M.G., E.D., I.C., S.G. collected and processed patient blood samples. M.G., S.G., E.M., S.A., N.J.M., H.M.G., M.M. and O.O. collected, extracted, and analyzed clinical data. E.M., A.N., H.R., G.M., M.M., R.P., R.W. and S. Chinhtahaj assisted in designing, recruiting, and/or following inpatient and HC cohorts. J.S. provided pre-characterized COVID-19 samples Kaiser Permanente that were used to establish the array platforms and to select exoantigens for the final array. N.J.M., E.J.W. designed the inpatient and HC Penn cohorts and obtained funding. H.M.G., O.O., N.J.M. S.A. screened and enrolled human subjects at Penn and collected biosamples. M.A., N.A., M.M. and K.N. supervised clinical data management in the CROWN clinic and performed chart reviews that enabled the correlation of array results with clinical parameters. L.B., M.M., E.D., I.C. and M.A. from the Stanford CROWN Research Team contributed to the collection and storage of patient samples, collection of patient laboratory and clinical data, and distribution of blood samples. P.J. and U.S. provided and characterized blood samples from an interferon lambda trial in patients with mild COVID-19 that were used to establish assays and compare with results from hospitalized patients. S.E.C., A.F., S.D., S.A., W.M., H.R.C., A.K., P.C., A.H., W.H.R., C.S., E.T.L.P. and P.J.U. analyzed and interpreted array data. S.E.C., A.F., S.D., S.A., W.M., C.S., E.T.L.P. and P.J.U. created figures and tables. S.E.C., A.F., R.M., C.S., E.T.L.P. and P.J.U. wrote the paper. J.I. provided unpublished preliminary data and assisted with data interpretation and manuscript editing. P.J.U., C.S., E.T.L.P., E.J.W. and K.N. supervised the research.

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