Arthritis & Rheumatology
Vol. 74, No. 7, July 2022, pp 1132–1138
DOI 10.1002/art.42094
© 2022 The Authors. *Arthritis & Rheumatology* published by Wiley Periodicals LLC on behalf of American College of Rheumatology.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

COVID-19

Endothelial Cell–Activating Antibodies in COVID-19

Hui Shi,[1] Yu Zuo,[2] Sherwin Navaz,[2] Alyssa Harbaugh,[2] Claire K. Hoy,[2] Alex A. Gandhi,[2] Gautam Sule,[2] Srilakshmi Yalavarthi,[2] Kelsey Gockman,[2] Jacqueline A. Madison,[2] Jintao Wang,[3] Melanie Zuo,[2] Yue Shi,[4] Michael D. Maile,[2] Jason S. Knight,[2] and Yogendra Kanthi[5]

Objective. While endothelial dysfunction has been implicated in the widespread thromboinflammatory complications of COVID-19, the upstream mediators of endotheliopathy remain, for the most part, unknown. This study was undertaken to identify circulating factors contributing to endothelial cell activation and dysfunction in COVID-19.

Methods. Human endothelial cells were cultured in the presence of serum or plasma from 244 patients hospitalized with COVID-19 and plasma from 100 patients with non–COVID-19–related sepsis. Cell adhesion molecules (E-selectin, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1 [ICAM-1]) were quantified using in-cell enzyme-linked immunosorbent assay.

Results. Serum and plasma from COVID-19 patients increased surface expression of cell adhesion molecules. Furthermore, levels of soluble ICAM-1 and E-selectin were elevated in patient serum and correlated with disease severity. The presence of circulating antiphospholipid antibodies was a strong marker of the ability of COVID-19 serum to activate endothelium. Depletion of total IgG from antiphospholipid antibody–positive serum markedly reduced the upregulation of cell adhesion molecules. Conversely, supplementation of control serum with patient IgG was sufficient to trigger endothelial activation.

Conclusion. These data are the first to indicate that some COVID-19 patients have potentially diverse antibodies that drive endotheliopathy, providing important context regarding thromboinflammatory effects of autoantibodies in severe COVID-19.

INTRODUCTION

There are several likely synergistic mechanisms by which SARS–CoV-2 infection may result in COVID-19–associated coagulopathy, including cytokine release that activates leukocytes, endothelium, and platelets; the direct activation of various cells by viral infection; and high levels of intravascular neutrophil extracellular traps (NETs) (1). The latter are inflammatory cell remnants that contribute to thrombosis (2). COVID-19–associated coagulopathy may manifest with thrombosis in venous, arterial, and microvascular circuits. The incidence of venous thromboembolism is particularly notable in severe COVID-19 (10–35%), with autopsy specimen findings suggesting that it affects as many as 60% of patients in association with COVID-19–related mortality (3).

Recently, there have been several descriptions of what appears to be de novo autoantibody formation in individuals with severe COVID-19. One example replicated in multiple studies is the detection of antibodies similar to the antiphospholipid

Dr. Y. Zuo's work was supported by the Rheumatology Research Foundation and the Arthritis National Research Foundation. Dr. Madison's work was supported by the VA Healthcare System. Dr. Knight's work was supported by the Rheumatology Research Foundation, the Michigan Medicine Frankel Cardiovascular Center, the A. Alfred Taubman Medical Research Institute, the NIH (grant R01-HL-115138), Burroughs Wellcome Fund, and the Lupus Research Alliance. Dr. Kanthi's work was supported by the Intramural Research Program of the National Heart, Lung, and Blood Institute, NIH, the Lasker Foundation, the University of Michigan Frankel Cardiovascular Center, the A. Alfred Taubman Medical Research Institute, and the Falk Medical Research Trust Catalyst Award.

[1]Hui Shi, MD, PhD: University of Michigan, Ann Arbor, and Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2]Yu Zuo, MD, Sherwin Navaz, BS, Alyssa Harbaugh, BS, Claire K. Hoy, BS, Alex A. Gandhi, BS, Gautam Sule, PhD, Srilakshmi Yalavarthi, MS, Kelsey Gockman, BS, Jacqueline A. Madison, MD, Melanie Zuo, MD, Michael D. Maile, MD, Jason S. Knight, MD, PhD: University of Michigan, Ann Arbor; [3]Jintao Wang, PhD: National Heart, Lung and Blood Institute, Bethesda, Maryland; [4]Yue Shi, PhD: Shanghai University of Sport, Shanghai, China; [5]Yogendra Kanthi, MD: National Heart, Lung and Blood Institute, Bethesda, Maryland, and University of Michigan, Ann Arbor.

Author disclosures are available at https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42094&file=art42094-sup-0001-Disclosureform.pdf.

Address correspondence to Jason S. Knight, MD, PhD, 1150 West Medical Center Drive, Ann Arbor, Michigan (email: jsknight@umich.edu); or to Yogendra Kanthi, MD, Clinical Center, 10 Center Drive, Bethesda, Maryland (yogen.kanthi@nih.gov).

Submitted for publication August 24, 2021; accepted in revised form February 13, 2022.

antibodies (aPLs) that mediate antiphospholipid syndrome (APS) in the general population. In APS, persistent autoantibodies are formed to phospholipids and phospholipid-binding proteins, such as prothrombin and β_2-glycoprotein I (β_2GPI). These autoantibodies then engage cell surfaces, where they activate endothelial cells, platelets, and neutrophils and thereby trigger thrombosis in the blood vessel wall interface. While viral infections have long been known to trigger transient aPLs (4), the mechanisms by which these potentially short-lived antibodies may be pathogenic have not been thoroughly characterized. To this end, our group recently found that IgG fractions from COVID-19 patients were enriched for aPLs and amplified thrombosis when injected into mice (5). Intriguingly, the circulating B cell compartment in COVID-19 appears similar to lupus, an autoimmune disease whereby naive B cells rapidly become antibody-producing cells using an extrafollicular pathway (6), and, in doing so, bypass the normal germinal center tolerance checkpoints against autoimmunity.

Here, we were initially interested in the extent to which circulating NET remnants might be an important activator of endothelial cells. We then also turned our attention to aPLs as potential markers of COVID-19 serum and polyclonal IgG fractions with strong endothelial cell–activating potential.

MATERIALS AND METHODS

For detailed methods see Supplementary Materials and Methods (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42094). This study complied with all relevant ethics regulations and was approved by the Institutional Review Board of the University of Michigan (approval nos. HUM00179409 and HUM00131596).

Serum and plasma samples from COVID-19 patients and cell culture conditions. All 118 patients in the primary cohort and 126 patients in the second cohort had a confirmed COVID-19 diagnosis based on US Food and Drug Administration–approved RNA testing. Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial cell growth basal medium 2 (CC-3156; Lonza) supplemented with EGM-2 MV SingleQuot kit (CC-4147; Lonza).

Protocols for purification of human IgG, in-cell enzyme-linked immunosorbent assay (ELISA), and characterization of neutrophil adhesion. IgG was purified as described previously (5). In-cell ELISA and neutrophil adhesion were also conducted according to protocols described previously (7).

Quantification of antibodies and biomarkers. We quantified aPLs using Quanta Lite kits (Inova Diagnostics). Soluble E-selectin and intercellular adhesion molecule 1 (ICAM-1) levels were quantified by ELISA (DY724 and DY720; R&D Systems). Myeloperoxidase (MPO)–DNA complexes were quantified as previously described (8). Cell-free DNA was quantified using a Quant-iT PicoGreen double-stranded DNA Assay kit (P11496; Invitrogen). Citrullinated histone H3 was quantified by ELISA (501620; Cayman Chemical), and calprotectin was quantified by ELISA (DY8226; R&D Systems).

RESULTS

Activation of HUVECs by COVID-19 serum. Serum samples were collected from 118 patients hospitalized with COVID-19 at an academic hospital. The mean age of patients in this cohort was 62 years, 47% of patients were women, and 42% were Black African Americans (Supplementary Table 1, http://onlinelibrary.wiley.com/doi/10.1002/art.42094); 35% were receiving mechanical ventilation. Serum from COVID-19 patients was added to early-passage HUVECs, and the expression of cell adhesion molecules was determined after 6 hours using a custom in-cell ELISA compatible with a biosafety level 3 facility per institutional guidelines (Figure 1A). As compared to serum samples from 38 healthy controls, the samples from COVID-19 patients triggered an activated endothelial cell phenotype, as evidenced by increased surface expression of the cell adhesion molecules E-selectin (Figure 1B and Supplementary Figure 1A, http://onlinelibrary.wiley.com/doi/10.1002/art.42094), vascular cell adhesion molecule 1 (VCAM-1) (Figure 1C and Supplementary Figure 1B), and ICAM-1 (Figure 1D and Supplementary Figure 1C). Relative activation (as compared to unstimulated cells) was similar to activation observed with 20 ng/ml tumor necrosis factor stimulation, which was included in all experiments as a positive control (Supplementary Figure 2, http://onlinelibrary.wiley.com/doi/10.1002/art.42094).

We obtained sufficient serum from an additional 126 hospitalized patients with COVID-19 to expand ICAM-1 testing. These patients had similar clinical severity profiles compared to the original 118 patients (see Supplementary Table 1 http://onlinelibrary.wiley.com/doi/10.1002/art.42094) and had similarly up-regulated expression of surface ICAM-1 (Supplementary Figure 3). Considering all 244 patients together, serum from patients requiring mechanical ventilation up-regulated ICAM-1 more strongly than serum from patients who were hospitalized but did not require mechanical ventilation ($P < 0.01$) (Figure 1E). We also measured levels of soluble ICAM-1 and E-selectin in patient serum. We found significantly higher levels of both soluble ICAM-1 and E-selectin in COVID-19 serum as compared to healthy control serum (Figure 1F and Supplementary Figure 4, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). Soluble ICAM-1 levels positively correlated with HUVEC ICAM-1 expression (Figure 1G), as well as with clinical parameters that correlated with severity, including C-reactive protein level ($r = 0.27$, $P = 0.0002$), D-dimer ($r = 0.29$, $P = 0.0001$), and oxygenation efficiency ($r = -0.37$, $P < 0.0001$) (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). Soluble

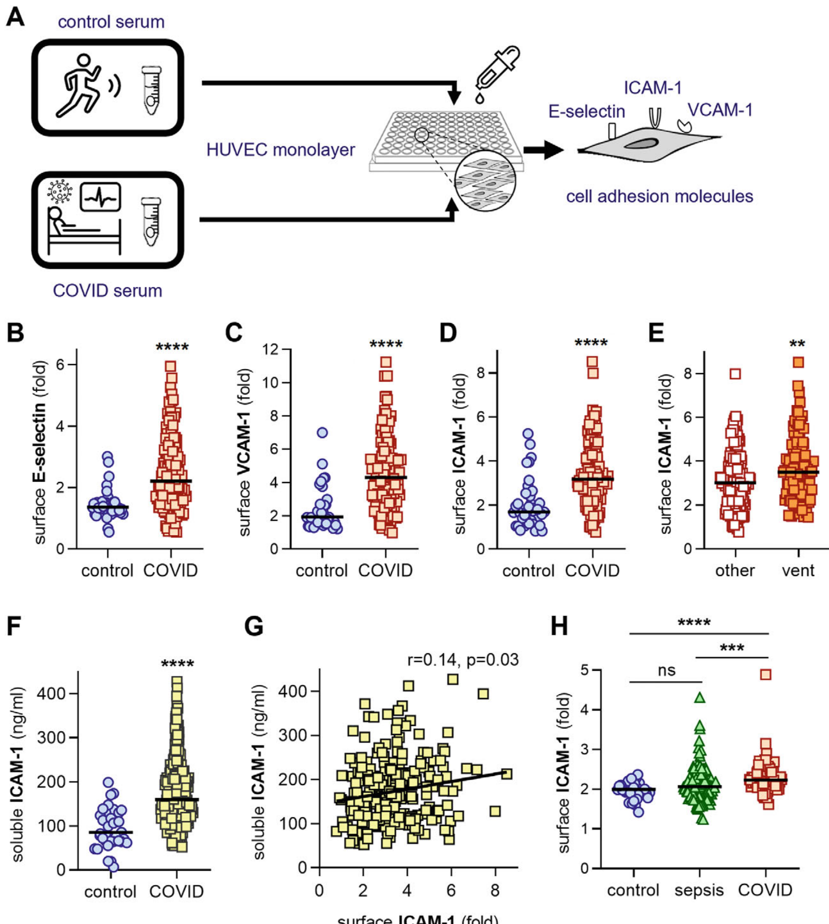


Figure 1. Activation of human umbilical vein endothelial cells (HUVECs) by control or COVID-19 serum. **A**, Schematic workflow of in-cell enzyme-linked immunosorbent assay is shown. HUVECs were cultured for 6 hours with serum from either healthy controls (collected prepandemic) (n = 38) or patients hospitalized with COVID-19 (n = 118). **B–D**, Cells were fixed, and surface expression of E-selectin (**B**), vascular cell adhesion molecule 1 (VCAM-1) (**C**), or intercellular adhesion molecule 1 (ICAM-1) (**D**) was quantified. **E**, Beyond the 118 COVID-19 patients tested in **D**, surface expression of ICAM-1 was tested in an additional 126 unique patient samples. Patients requiring mechanical ventilation (vent) (n = 101) were compared to hospitalized patients who did not receive mechanical ventilation (n = 143). **F**, Serum from healthy controls (n = 37) and from COVID-19 patients (n = 232) was assessed for soluble ICAM-1. **G,** In samples from COVID-19 patients, the correlation between expression levels of soluble ICAM-1 and HUVEC expression of surface ICAM-1 was assessed by Spearman's test. **H**, HUVECs were cultured for 6 hours with plasma from healthy controls (n = 36), patients with non–COVID-19–related sepsis admitted to the intensive care unit (n = 100), or patients hospitalized with COVID-19 (n = 72). Cells were then fixed, and surface expression of ICAM-1 was quantified. In **B–F** and **H**, symbols represent individual subjects; bars show the median. In **B–F**, ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$, by Mann-Whitney test in **B–F** and by Kruskal-Wallis test with Dunn's correction for multiple comparisons in **H**. NS = not significant.

E-selectin had similar correlations to all of the above (Supplementary Figure 4).

To determine the extent to which this phenomenon might extend to critically ill patients without COVID-19, 100 age- and comorbidity-matched patients with non–COVID-19–related sepsis (Supplementary Table 2, http://onlinelibrary.wiley.com/doi/10.1002/art.42094) admitted to the intensive care unit (plasma obtained in the pre–COVID-19 era) were matched with patients in our COVID-19 cohort (n = 72 available plasma samples). More patients in the non–COVID-19–related sepsis cohort required mechanical ventilation (65% versus 46%). We then stimulated HUVECs with plasma and determined levels of surface expression of ICAM-1. While a subset of the non–COVID-19–related sepsis plasma elicited high levels of expression of surface ICAM-1, that group as a whole was not significantly different from the control group (Figure 1H). Additionally, COVID-19 plasma demonstrated greater activation than either control or non–COVID-19–related sepsis plasma (Figure 1H). Taken together, these data indicate that COVID-19 serum and plasma contain factors capable of activating endothelial cells.

Association of NET remnants and other biomarkers with the endothelial cell–activating potential of COVID-19 serum. Given that NETs are known activators of endothelial cells,

Table 1. Correlation of HUVEC cell adhesion molecule expression with aPL expression in COVID-19 patients*

	E-selectin		VCAM-1		ICAM-1	
	r	*P*	r	*P*	r	*P*
aPLs						
IgG aCL	0.446	<0.0001	0.421	<0.0001	0.346	<0.001
IgM aCL	0.369	<0.0001	0.252	<0.01	0.357	<0.0001
IgG anti-β_2GPI	0.156	NS	0.213	<0.05	0.076	NS
IgM anti-β_2GPI	0.009	NS	0.047	NS	0.150	NS
IgG aPS/PT	0.432	<0.0001	0.252	<0.01	0.299	<0.001
IgM aPS/PT	0.254	<0.01	0.115	NS	0.276	<0.01
NET remnants						
Cell-free DNA	0.073	NS	0.237	<0.05	0.135	NS
MPO–DNA complexes	0.156	NS	0.256	<0.01	0.277	<0.01
Cit-H3	0.079	NS	0.224	<0.05	0.076	NS
Other biomarkers						
C-reactive protein	−0.008	NS	0.173	NS	0.003	NS
D-dimer	0.175	NS	0.133	NS	0.258	<0.05
Calprotectin	0.206	<0.05	0.203	<0.05	0.197	<0.05

* Correlations were determined by Spearman's test. HUVEC = human umbilical vein endothelial cell; aPL = antiphospholipid antibody; VCAM-1 = vascular cell adhesion molecule 1; ICAM-1 = intercellular adhesion molecule 1; aCL = anticardiolipin; NS = not significant; β_2GPI = β_2-glycoprotein I; aPS/PT = antiphosphatidylserine/prothrombin; NET = neutrophil extracellular trap; MPO-DNA = myeloperoxidase-DNA; Cit-H3 = citrullinated histone H3.

we investigated whether NET remnants might predict the ability of a particular COVID-19 serum sample to up-regulate levels of surface E-selectin, VCAM-1, and ICAM-1. Specifically, we measured NETs in COVID-19 serum (n = 118) via quantification of cell-free DNA, myeloperoxidase (MPO)–DNA complexes, and citrullinated histone H3 (Cit-H3) (Table 1). Serum MPO–DNA complexes moderately correlated with surface expression of both VCAM-1 (r = 0.26, *P* < 0.01) and ICAM-1 (r = 0.28, *P* < 0.01). Cell-free DNA and Cit-H3 both correlated only with VCAM-1 (r = 0.24, *P* < 0.05 and r = 0.22, *P* < 0.05, respectively) (Table 1). Beyond these relatively specific markers of NETs, we also investigated correlations with more general markers of thromboinflammation, including C-reactive protein, D-dimer, and calprotectin (the latter previously shown to be an early predictor of respiratory failure in COVID-19). Of the 3, only calprotectin positively correlated with expression of all 3 endothelial cell surface markers (r = 0.19–0.20, *P* < 0.05), while D-dimer correlated with ICAM-1 only (r = 0.26, *P* < 0.05) (Table 1). Taken together, these data demonstrate modest correlations between NETs/thromboinflammation and the ability of COVID-19 serum to activate endothelial cells.

Association of aPLs with the endothelial cell–activating potential of COVID-19 serum. We reasoned that COVID-19–associated autoreactive antibodies might activate endothelial cells. In pursuit of such antibody fractions, we focused on the IgG and IgM isotypes of 3 types of aPLs: anticardiolipin antibodies (aCLs), anti-β_2GPI, and antiphosphatidylserine/prothrombin (aPS/PT). As shown in Supplementary Table 3 (http://onlinelibrary.wiley.com/doi/10.1002/art.42094), 45% of subjects were positive for at least 1 antibody based on the manufacturer's cutoff, and 25% were positive using a more stringent cutoff of ≥40. The vast majority of positive findings were either aCL positivity (for IgG and IgM, values were 3% and 25% of the cohort, respectively) or aPS/PT positivity (for IgG and IgM, values were 24% and 15% of the cohort, respectively). It should also be noted that there were strong correlations among many of the antibodies, for example IgG aCL and IgG aPS/PT (r = 0.51, *P* < 0.0001) and IgM aCL and IgM aPS/PT (r = 0.53, *P* < 0.0001) (all correlations are shown in Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). Furthermore, aPL levels correlated, to some extent, with clinical biomarkers, especially levels of circulating NETs (Supplementary Table 5, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). None of the aPLs were detected at significant levels in the healthy control serum (2 positive test results) and plasma (1 positive test result) in this study (Supplementary Tables 6 and 7, http://onlinelibrary.wiley.com/doi/10.1002/art.42094).

Interestingly, we detected strong correlations between aCL and aPS/PT antibodies and the 3 markers of endothelial cell activation (E-selectin, VCAM-1, and ICAM-1) (Table 1). The only correlation that was not significant was between IgM aPS/PT and VCAM-1 (Table 1). We additionally performed a logistic regression analysis after setting a positive/negative threshold for cell adhesion molecule up-regulation that was 2 standard deviations greater than the control mean (Supplementary Table 8, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). IgG aCL levels were significantly higher in E-selectin– and VCAM-1–positive patients (*P* = 0.001 and *P* = 0.003, respectively), while IgG aPS/PT levels were significantly higher in ICAM-1–positive patients (*P* = 0.018). We also measured the same aPL levels in the aforementioned non–COVID-19–related sepsis cohort (Supplementary Table 9, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). Of 100 patients, 29 were positive for any aPL, with 23 patients positive at the more

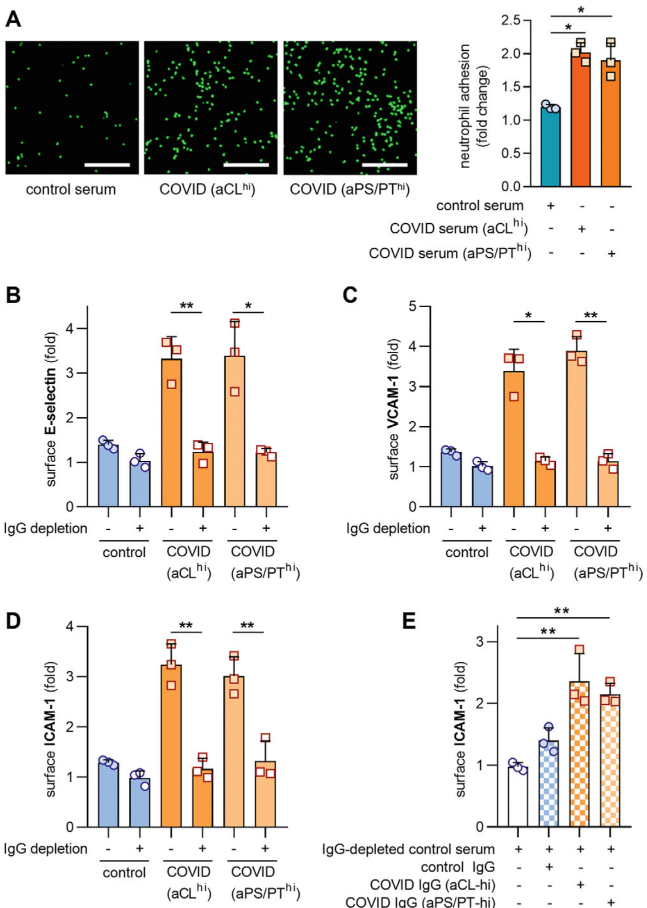


Figure 2. Depletion of IgG from sera with high levels of anticardiolipin (aCL) and antiphosphatidylserine/prothrombin (aPS/PT) antibodies alleviates HUVEC activation. **A,** Serum was pooled from 3 IgG aCL–positive patients, 5 IgG aPS/PT–positive patients, or 3 healthy donors. HUVEC monolayers were treated with 2.5% COVID-19 serum or control serum for 4 hours, and calcein AM–labeled neutrophils were added as described in Materials and Methods. Representative photomicrographs are shown (left) (bars = 200 μm) and results were quantified (right). **B–D,** IgG was depleted from each of the aforementioned pools. Activation of HUVECs, defined by surface expression of E-selectin (**B**), VCAM-1 (**C**), or ICAM-1 (**D**), was determined after culture for 6 hours. The experiment was repeated on 3 different days. **E,** IgG (100 μg/ml) was purified from the pooled samples in **A–C**, and then spiked into IgG-depleted control serum. HUVEC activation, defined by surface expression of ICAM-1, was determined after culture for 6 hours. Symbols represent individual samples; bars show the mean ± SD from 3 independent experiments. * = $P < 0.05$; ** = $P < 0.01$, by one-way analysis of variance with Dunn's correction, or Tukey's correction or by 2-sided paired *t*-test. See Figure 1 for other definitions.

stringent cutoff of ≥40. In contrast to the COVID-19 cohort, the vast majority of tests showed positivity for IgM aPS/PT, the levels of which correlated with the ability of plasma to up-regulate ICAM-1 ($r = 0.29$, $P < 0.01$) (Supplementary Table 10, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). To summarize, we detected correlations between various aPLs and endothelial cell activation in COVID-19, and to some extent in non–COVID-19–related sepsis.

Increased neutrophil adhesion upon serum-mediated HUVEC activation. To determine whether up-regulation of E-selectin, VCAM-1, and ICAM-1 is associated with increased adhesive functions of HUVECs, we performed a neutrophil adhesion assay. We pooled serum from 3 COVID-19 patients with positive IgG aCL activity (mean 29 IgG phospholipid units/ml) and from a separate 5 COVID-19 patients with positive IgG aPS/PT activity (mean 54). We then stimulated HUVECs with the pooled serum. Compared to control serum, both COVID-19 samples demonstrated significantly increased adhesion of neutrophils to endothelial cells (Figure 2A).

Depletion of IgG alleviates HUVEC activation. We next sought to determine the extent to which total IgG fractions from COVID-19 patients could directly activate HUVECs. To this end, we subjected the aforementioned pooled serum (IgG aCL– and IgG aPS/PT–positive) to either mock depletion or IgG depletion. IgG depletion did not result in a significant reduction of other serum proteins of note, such as calprotectin (Supplementary Figure 6, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). As compared to mock depletion, total IgG depletion completely abrogated the ability of both pooled sample groups to up-regulate E-selectin (Figure 2B), VCAM-1 (Figure 2C), and ICAM-1 in endothelial cells (Figure 2D). We next purified a small quantity of total IgG from each group of pooled serum and added it to control serum that had been depleted of its own IgG. The COVID-19 IgG-supplemented control serum demonstrated an ability to increase expression of surface levels of ICAM-1 (Figure 2E). In contrast, IgG purified from COVID-19 serum that tested negative for both IgG aCL and IgG aPS/PT did not have increased expression of ICAM-1 (Supplementary Figure 7, http://onlinelibrary.wiley.com/doi/10.1002/art.42094). Taken together, these data indicate that the presence of aPLs correlates with activation of endothelial cells in the presence of IgG in the serum of patients with COVID-19.

DISCUSSION

In the current study, we show that serum from COVID-19 patients activated cultured endothelial cells that express surface adhesion molecules integral to inflammation and thrombosis, namely ICAM-1, E-selectin, and VCAM-1. Furthermore, we found that, for at least a subset of serum samples from COVID-19 patients, activation could be mitigated by depleting total IgG. The role of aPLs in activating endothelial cells has been demonstrated both in vitro and in vivo (9). For example, IgG fractions from APS patients have long been known to activate HUVECs, as indicated by increased monocyte adherence and increased expression of adhesion molecules. It is intriguing that while most characterization of endothelium in APS has focused on activation

of the endothelium by anti-β_2GPI antibodies (10), these were only rarely detected in our cohort. An interesting recent study demonstrated activation of endothelium in APS by phospholipid-binding, cofactor-independent antibodies (11). Of course, it should be noted that all experiments performed in our study were with total IgG fractions and not affinity-purified aPLs. Therefore, aPLs may mark antibody profiles in severe illness (possibly polyclonal) that activate endothelium and influence inflammation and coagulation at the normally quiescent blood–vessel wall interface.

In addition to our findings (5), several other studies (12) have also provided evidence of the de novo formation of pathogenic autoantibodies in COVID-19. For example, in an interesting study, a high-throughput autoantibody discovery technique was used to screen a cohort of COVID-19 patients for autoantibodies against 2,770 extracellular and secreted proteins (13). That study found that there is a tendency for autoantibodies to be directed against immunomodulatory proteins, including cytokines, chemokines, complement components, and cell surface proteins. This is further supported by our data suggesting correlations among aPL species in serum from COVID-19 patients.

Beyond COVID-19, we were intrigued to find that ~25% of patients with non–COVID-19–related sepsis had at least 1 positive aPL test, mostly IgM aPS/PT. Given that IgM aPS/PT levels correlated with plasma activation of endothelial cells, we hypothesize a similar autoreactive antibody–mediated, endothelial cell–activating mechanism may occur in some patients with sepsis, a state in which interaction between the infection and the host immune response disrupts the vasculature (14). Of particular note, infections causing critical illness have long been known to be potential triggers of autoantibodies, and in particular aPLs (4,15). Although infection-associated aPLs have typically been described as transient (16), a recent systematic review showed that ~33% of individuals positive for aPLs in the setting of a virus-associated thrombotic event continue to have persistent aPL positivity for at least several months (15). Based on our data, studies with long-term clinical and serologic follow-up may be necessary to better define the natural history of COVID-19 and non–COVID-19–related sepsis.

There are several potential clinical implications of our findings. One consideration that warrants further investigation is whether patients with severe COVID-19 should be screened for aPLs to evaluate their risk of thrombosis and progression to respiratory failure, and whether patients with high aPL titers might benefit from treatments used in traditional cases of severe APS such as therapeutic anticoagulation, complement inhibition, and plasmapheresis. At the same time, determining the extent to which aPLs are direct mediators of the endothelial cell phenotypes observed here, and better understanding of which polyclonal antibody fractions are most likely to activate endothelial cells are important questions warranting future research. Our study has additional limitations, including lacking a direct readout of macrovascular thrombosis (available in other well-conducted studies [17]) given aggressive anticoagulation used at our center early in the pandemic and a currently incomplete understanding of the mechanisms by which aPL-associated IgG fractions activate endothelial cells. However, given the urgency of COVID-19 research, we believe these issues are counterbalanced by our relatively large sample size and the previously unknown discovery of endothelial cell–activating antibody profiles in some COVID-19 serum. Indeed, these data also provide context for diffuse organ involvement of COVID-19, where a nonspecific humoral immune response to illness may disrupt the normally quiescent endothelium and increase vascular inflammation. As we await definitive solutions to the pandemic, these findings provide important context to the complex interplay between SARS–CoV-2 infection, the human immune system, and vascular immunobiology.

ACKNOWLEDGMENTS

We would like to thank all the individuals with COVID-19 and those with non–COVID-19-related sepsis who participated in this study, as well as the frontline workers during the pandemic.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Knight and Kanthi had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Shi, Zuo, Maile, Knight, Kanthi.

Acquisition of data. Shi, Zuo, Navaz, Harbaugh, Hoy, Gandhi, Sule, Yalavarthi, Gockman, Madison, Wang, Zuo, Shi.

Analysis and interpretation of data. Shi, Zuo, Maile, Knight, Kanthi.

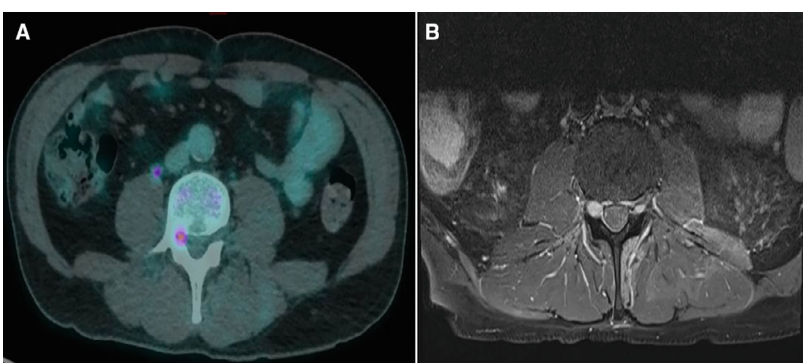
REFERENCES

1. Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, et al. Neutrophil extracellular traps in COVID-19. JCI Insight 2020;5: e138999.
2. Middleton EA, He XY, Denorme F, Campbell RA, Ng D, Salvatore SP, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 2020;136:1169–79.
3. Wichmann D, Sperhake JP, Lütgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Ann Intern Medicine 2020;173:268–77.
4. Abdel-Wahab N, Talathi S, Lopez-Olivo MA, Suarez-Almazor ME. Risk of developing antiphospholipid antibodies following viral infection: a systematic review and meta-analysis. Lupus 2018;27:572–83.
5. Zuo Y, Estes SK, Ali RA, Gandhi AA, Yalavarthi S, Shi H, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. Sci Transl Med 2020;12:eabd3876.
6. Woodruff MC, Ramonell RP, Nguyen DC, Cashman KS, Saini AS, Haddad NS, et al. Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. Nat Immunol 2020; 21:1506–16.
7. Shi H, Gandhi AA, Smith SA, Wang Q, Chiang D, Yalavarthi S, et al. Endothelium-protective, histone-neutralizing properties of the polyanionic agent defibrotide. JCI Insight 2021;6: e149149.
8. Kessenbrock K, Krumbholz M, Schonermarck U, Back W, Gross WL, Werb Z, et al. Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 2009;15:623–5.

9. Pierangeli SS, Colden-Stanfield M, Liu X, Barker JH, Anderson GL, Harris EN. Antiphospholipid antibodies from antiphospholipid syndrome patients activate endothelial cells in vitro and in vivo. Circulation 1999;99:1997–2002.
10. Allen KL, Fonseca FV, Betapudi V, Willard B, Zhang J, McCrae KR. A novel pathway for human endothelial cell activation by antiphospholipid/anti-β2 glycoprotein I antibodies. Blood 2012;119:884–93.
11. Muller-Calleja N, Hollerbach A, Royce J, Ritter S, Pedrosa D, Madhusudhan T, et al. Lipid presentation by the protein C receptor links coagulation with autoimmunity. Science 2021;371:eabc0956.
12. Chang SE, Feng A, Meng W, Apostolidis SA, Mack E, Artandi M, et al. New-Onset IgG autoantibodies in hospitalized patients with COVID-19. Nat Commun 2021;12:5417.
13. Wang EY, Mao T, Klein J, Dai Y, Huck JD, Jaycox JR, et al. Diverse functional autoantibodies in patients with COVID-19. Nature 2021;595:283–8.
14. Colling ME, Tourdot BE, Kanthi Y. Inflammation, infection and venous thromboembolism [review]. Circ Res 2021;128:2017–36.
15. Abdel-Wahab N, Lopez-Olivo MA, Pinto-Patarroyo GP, Suarez-Almazor ME. Systematic review of case reports of antiphospholipid syndrome following infection. Lupus 2016;25:1520–31.
16. Blank M, Asherson RA, Cervera R, Shoenfeld Y. Antiphospholipid syndrome infectious origin. J Clin Immunol 2004;24:12–23.
17. Borghi MO, Beltagy A, Garrafa E, Curreli D, Cecchini G, Bodio C, et al. Anti-phospholipid antibodies in COVID-19 are different from those detectable in the anti-phospholipid syndrome. Front Immunol 2020;11:584241.

DOI 10.1002/art.42108

Clinical Images: Motor deficiency and radicular pain secondary to sarcoidosis



The patient, a previously healthy 65-year-old man, presented with right L4 radiculopathy and motor deficit. Before presentation, he had asthenia for 1 month, which was associated with a weight loss of 5 kg. Physical examination revealed a complete leg extension deficit, absence of patellar reflex, and paresthesias of the anterolateral side of the right thigh. Spinal magnetic resonance imaging (MRI) showed no disc herniation. Laboratory investigations revealed a slightly elevated C-reactive protein level of 8.6 mg/liter and normal calcium and phosphate levels. His cerebrospinal fluid contained 1 white blood cell/mm^3 and showed a total protein level of 0.42 gm/liter. Further analyses of the cerebrospinal fluid included testing for viruses, bacteria, and other immunologic factors, all of which yielded negative results. The angiotensin-converting enzyme level was normal. An electromyogram showed signs of denervation in the right L3 and L4 regions. Positron emission tomography showed an intense focal hypermetabolism in the right L4 root (**A**) and multiple hypermetabolic mediastinal lymphadenopathies. Lymphoma was the suspected diagnosis. The patient underwent intravenous administration of gadolinium-based contrast agent followed by fat-suppressed T1-weighted MRI, which showed an enlargement of the right L4 dorsal root ganglion (**B**). Biopsy findings of mediastinal lymphadenopathy confirmed a diagnosis of sarcoidosis. The patient's symptoms quickly disappeared after initiation of glucocorticoid treatment. Sarcoidosis is an inflammatory disease that, like some lymphomas, can present as radicular pain and deficiency (1,2). The clinician should be alert to this possibility.

Author disclosures are available at https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42108&file=art42108-sup-0001-Disclosureform.pdf.

1. Shono T, Tamai M, Kobayashi M, Wakasaki H, Furuta H, Nakao T, et al. Neurosarcoidosis with spinal root pain as the first symptom. Intern Med 2004;43873–7.
2. Santos E, Scolding NJ. Neurolymphomatosis mimicking neurosarcoidosis: a case report. J Med Case Rep 2010;4:5.

Coralie Humann
Caroline Raymond, MD
CHRU de Besançon
Besançon, France
Daniel Wendling, MD, PhD
Frank Verhoeven, MD, PhD
CHRU de Besançon
and Franche-Comté University
Besançon, France