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REVIEW

Identification of key signaling pathways induced by SARS-CoV2 that underlie thrombosis and vascular injury in COVID-19 patients

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Abstract

The SARS-CoV-2 pandemic has led to hundreds of thousands of deaths and billions of dollars in economic damage. The immune response elicited from this virus is poorly understood. An alarming number of cases have arisen where COVID-19 patients develop complications on top of the symptoms already associated with SARS, such as thrombosis, injuries of vascular system, kidney, and liver, as well as Kawasaki disease. In this review, a bioinformatics approach was used to elucidate the immune response triggered by SARS-CoV-2 infection in primary human lung epithelial and transformed human lung alveolar. Additionally, examined the potential mechanism behind several complications that have been associated with COVID-19 and determined that a specific cytokine storm is leading to excessive neutrophil recruitment. These neutrophils are directly leading to thrombosis, organ damage, and complement activation via neutrophil extracellular trap release.

KEYWORDS

chemokines, cytokines, Kawasaki disease, mast cells, preeclampsia

1 | INTRODUCTION

The immune system responds to each pathogen in a different and distinct way in order to efficiently resolve an infection. This involves several converging pathways necessary for the recognition of the pathogen and recruitment and activation of the immune response necessary for controlling and eliminating the invading pathogen.[1] Epithelial cells are usually the first line of host defense against pathogens and express an expansive complement of pattern-recognition receptors that identify specific microorganisms and respond to them by releasing cytokines/chemokines that are able to initiate and modulate the immune response against the pathogen.[1] Although the immune system is essential for the protection of the body from invading microorganisms, complications can arise where the immune system causes damage to the body due to over response or lack of response.[2] Consequently, the regulation of the immunologic response is critical in order to maintain the adequate balance between protection and maintaining tissue homeostasis.[3]

The novel coronavirus, SARS-CoV-2, has evolved into a global pandemic that has infected globally more than 3 million people and killed over 200,000.[4] Although several clinical trials are taking place, there are no approved therapeutic options for patients infected with SARS-CoV-2. Fever, dry cough, and shortness are currently thought to be the first and most common symptoms seen in COVID-19 cases.[5] Less common symptoms include loss of taste/smell, diarrhea, and vomiting.[5] Moreover, this initial symptomatology associated with COVID-19 was thought to be associated with an inflammatory storm that would damage the epithelium of the lungs leading to respiratory complications described in multiple patients.[6] However, as the number of patients in the United States and other parts in the world has increased, the list of complications associated with this virus is rapidly evolving. Secondary complications such as thrombosis, liver and kidney injuries, preeclampsia, and Kawasaki disease (KD) are starting to be seen in an alarming number of COVID-19 patients.[7–10] With a vaccine expected to take up to a year to make, and still no approved

Abbreviations: CRS, Cytokine release syndrome; KD, Kawasaki disease; LCN2, Lipocalin 2; NETs, Neutrophil extracellular traps; TF, Tissue factor.

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drugs available, and continuously increasing case numbers showing additional complications, it is of utmost importance to understand the immune response to SARS-CoV-19 and the mechanism underlying the cause(s) of these secondary complications.

The objective of this study was to review and evaluate the potential biologic pathways by which SARS-CoV-2 infection could trigger thrombosis and then investigate therapeutic approaches that could prevent it. Because SARS-CoV-2 infects type I and II alveolar epithelial cells, which are among the first cell type to produce proinflammatory cytokines and recruit immune cells in order to mount an adequate antiviral response, we took a bioinformatics approach and used RNAseq data from human lung and transformed lung alveolar cells infected with SARS-CoV-2. We examined the specific biologic pathways and genes involved in the immune response elicited from SARS-CoV-2-infected lung alveolar cells, which could explain the prothrombotic events that have being reported in COVID-19 patients. We discuss the involvement of this specific cytokine profile on the formation of neutrophil extracellular traps (NETs) and its association with the prothrombotic process and organ damage described in SARS-CoV-2-infected patients.

2 | CYTOKINE RESPONSE ASSOCIATED WITH NEUTROPHIL RECRUITMENT

COVID-19 patients often develop a cytokine release syndrome (CRS) that is responsible for the respiratory distress syndrome and the multi-organs injuries as result of secondary haemophagocytic-lymphohoistocytosis. The CRS observed in COVID-19 patients resembles the immune dysregulation caused by other highly pathogenic respiratory viruses such as SARS-CoV and MERS-CoV.[11] In order to understand the potential triggers of CRS, we evaluated the general response of lung epithelial cells as a result of SAR-CoV-2 infection. We analyzed RNAseq data from primary human lung epithelial that were mock treated or infected with SARS-CoV-2 (USA-WA1/2020) at an multiplicity of infection (MOI) of 2. This data set is available in GEO as the GSE147507 data set. Using iPathwayGuide (www.advaitabio.com) we determined what cytokines and chemokines were differentially expressed in these groups. All statistical applications were performed by the iPathwayGuide software, which includes GO analysis, network analysis, pathway analysis, and differentially expressed genes.[12–15]

In the cytokine-cytokine receptor and chemokine signaling pathways, we found several proinflammatory cytokines/chemokines that were differentially expressed (Fig. 1A,B and Supporting Information Fig. S1). Two chemokines with the highest fold change were GM-CSF2 (logFC = 2.981 and P = 1.000e-6) and GM-CSF3 (logFC = 4.852 and P = 1.000e-6) (Fig. 1A, B and Supporting Information Fig. S1).

CSF3 is mainly an inducer of neutrophil colony formation and it is associated with the regulation of neutrophil proliferation and maturation.[16–19] CSF2 is involved in the regulation of multiple immune cell types, especially macrophages but, in addition to stimulation of macrophage colonization, CSF2 also promotes the generation of neutrophils.[18–21] Furthermore, CSF2 plays a role in the generation of immature dendritic cells.[22] All of these processes of differentiation are mediated through the direct action of CSF2 and CSF3 on the bone marrow where it promotes the generation and release of mature colonies of myeloid cells.[20] Additionally, both colony-stimulating chemokines are capable of inducing the expression of proinflammatory cytokines, which in turn enhance the inflammatory response.[23] CSF3 expression has been linked to pulmonary neutrophilia that is seen in acute respiratory distress syndrome.[23] Furthermore, both of these factors are able to act locally on these cell subtypes to induce various aspects involved in an immune response, such as enhanced immune cell survival and increased production of inflammatory cytokines.[24,25] The up-regulation of CSF2 and CSF3 found in this analysis suggests that these SARS-CoV-2-infected epithelial cells are sending signals to the bone marrow to produce more macrophages, eosinophils, and neutrophils to eliminate the threat.[23]

A second set of related cytokines that were up-regulated during SARS-CoV-2 infection were CXCL1, CXCL2, CXCL3, CXCL6, CXCL5, and CXCL8 (or IL-8) (Fig. 1 and Supporting Information Fig. S1). All six of these cytokines are part of the CXC chemokine family. This specific set of CXC chemokines bind to the CXCR2 receptor.[26,27] One of the primary roles of this set of cytokines is to act as a chemoattractant for neutrophils as well as to promote adherence to endothelial cells.[28–34] CXCL1 (logFC = 1.419 and R = 1.000e-6) and CXCL2 (logFC = 1.393 and R = 1.000e-6) have been shown to be critical for neutrophil recruitment to the lungs.[35,36] CXC chemokines are produced by different cell types in response to proinflammatory signals such as IL-1β (logFC = 1.065 and R = 1.000e-6), IL-1α(logFC = 1.074 and R = 1.000e-6), and TNF-α (logFC = 1.809 and R = 2.865e-4), all of which were up-regulated in SARS-CoV-2-infected epithelial cells.[37] In addition to their roles in neutrophil recruitment to sites of infection, this set of CXC chemokines are also involved in the recruitment of various other cell types. CXCL8 (logFC = 2.335 and R = 1.000e-6) is a chemoattractant for basophils, eosinophils, and peripheral blood T lymphocytes.[38–40] More importantly, CXCL8 is also the primary neutrophil chemoattractant in the lungs.[41] CXCL1-3 are closely related and are important for the recruitment and activation of basophils, eosinophils, monocytes, and lymphocytes, in addition to neutrophil recruitment.[42] CXCL5 (logFC = 3.487 and R = 1.000e-6) has been found to be involved in different types of inflammatory diseases and has been shown to be expressed by alveolar type II epithelial cells in response to LPS.[43–46]

Interestingly, we observed that CXCL14 (logFC = −1.446 and R = 7.498e-6) was down-regulated in these data sets (Fig. 1 and Supporting Information Fig. S1). CXCL14 is a potent inhibitor of epithelial cell chemotaxis and function as a negative feedback to decrease immune cells recruitment.[47] Inhibition of CXCL14 allows a sustained and enhanced immune cell recruitment. Therefore, the finding of down-regulation of CXCL14 in SARS-CoV-2-infected epithelial cells suggest a removal of the regulator of immune cell recruitment leading to a persistent and enhanced enrollment of immune cells to the lungs, the site of the infection. This enhanced recruitment might lead to a disproportionate inflammatory

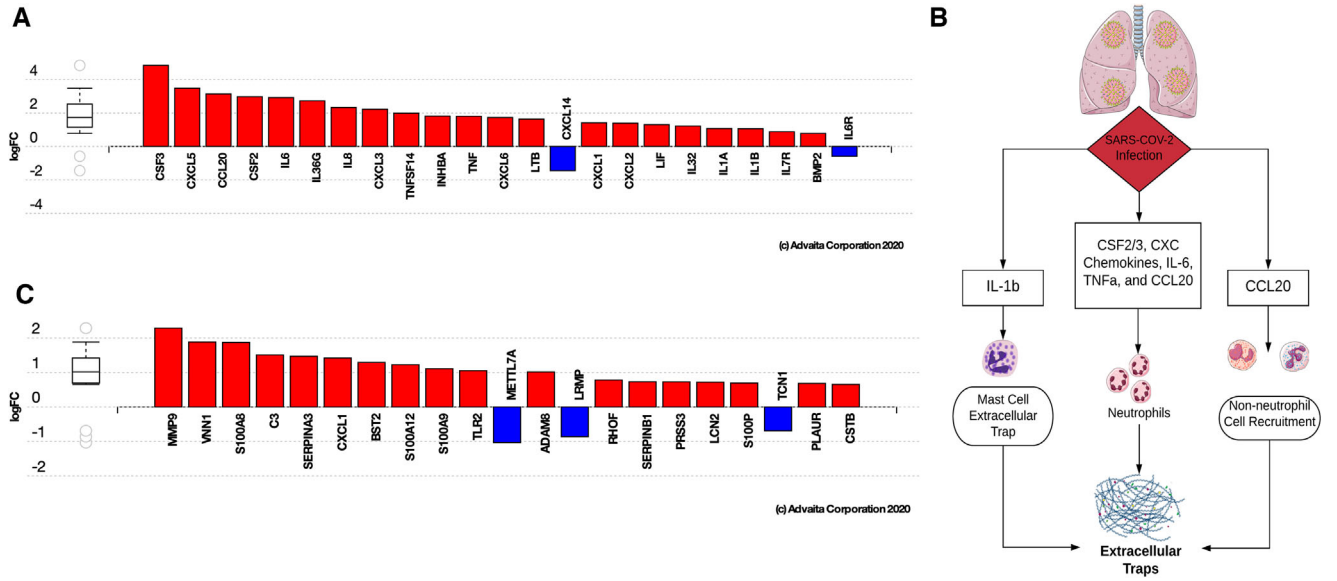

FIGURE 1 **Differentially expressed genes associated with chemokine signaling and neutrophil function in COVID-19.** (**A**) The panel shows the logFC of the main cytokines significantly expressed in primary human lung epithelium infected with SARS-COV-2 (USA-WA1/2020) (multiplicity of infection [MOI] = 2) that are induced in the receptor-cytokine interaction pathways showed in (**A**). (**B**) Model of the cellular pathways associated with neutrophil extracellular trap (NET) formation during SARS-COV-2 infection based on the pathway analysis. (**C**) Differentially expressed genes from primary human lung epithelium infected with SARS-COV-2 (USA-WA1/2020) (MOI = 2) that are associated with neutrophil function and activation

response, a characteristic of CRS observed in patients infected with SARS-CoV-2.

When we analyzed the potential cell type targeted by these chemokines, we observed that the up-regulated CXCL1, CXCL2, CXLC3, CXCL5, and CXCL8 have a predominant function related to the recruitment of neutrophils to the lungs. Additionally, the down-regulation of CXCL14 fosters the capacity of epithelial cells to release chemokines, which will lead to further neutrophil recruitment. These data suggest that lung epithelial cells respond to SARS-CoV-2 infection by secreting a group of proinflammatory cytokines and chemokines that promote neutrophil recruitment into the infected tissues.

3 | REGULATORY FACTORS ASSOCIATED WITH NEUTROPHIL ACTIVATION AND FUNCTION

This first analysis demonstrates a major chemokine profile leading to neutrophil recruitment. We next analyzed whether in the infected lung tissue we can observe immunologic signals that could support the functional activity of recruited neutrophils. Indeed, we observed that CCL20 (logFC = 3.146 and R = 1.000e-6) was up-regulated in the lung samples infected with SARS-CoV-2 (Fig. 1A, B and Supporting Information Fig. S1). CCL20 is produced by neutrophils and plays a role in recruiting additional immune cell types to sites of inflammation.[48–50] CCL20 is the ligand for the CCR6 receptor and is known to play a role in mucosal immunity by recruiting immature dendritic cells, memory T cells, memory B cells, as well as macrophages to sites of infection.[48–55] Therefore, up-regulation of CCL20 in SARS-CoV-2-infected tissues suggest the presence of activated neutrophils at the SARS-CoV-2-infected cells and their potential role in further enhancing the inflammatory syndrome through the recruitment of additional adaptive and innate immune cells (Fig. 1B).

4 | TYPE I IFN RESPONSES DURING SARS-COV-2 INFECTION

Type I IFN responses are critical for an efficient and regulated response to viral infections.[56] The main role of type I IFNs is to restrict viral replication and spread through the induction of a family of genes known as IFN-stimulated genes (ISGs).[56,57] In addition to controlling the antiviral response, an important role of these ISGs is to modulate the immune response in order to prevent an exacerbated inflammatory process.[57] Because of its critical role in controlling viral responses, the components of the type I IFN pathway are viral targets as a mechanism to escape immune surveillance. Contrary to patients infected with pathogenic influenza virus, minimal amount of IFNα or β have being detected in the blood of COVID-19-infected patients suggestive of a blunted type I IFN.[58] The dysregulated IFN response implies an effective immunoregulatory strategy developed by SARS-CoV2 that allows a successful infection and replication, as well as excessive inflammation.[59]

We looked at the type I IFN response in lung epithelial cells infected with SARS-CoV2 and found that the only ISG that was up-regulated was IL-6 (logFC = 2.929 and R = 1.000e-6) (Fig. 1). Elevated IL-6 serum levels have been closely linked to severe COVID-19 cases.[60] As with the other cytokines that were previously discussed, IL-6 is also

important for neutrophil recruitment, function, and survival.[61–64] Resolution of the infection accelerates neutrophil apoptosis; however, continued expression of IL-6 has been shown to delay neutrophil apoptosis in addition to increasing their superoxide production capacity.[61,65] Up-regulation of IL-6 may lead to a larger population of neutrophils that produce superoxide at a greater rate than normal neutrophils, causing tissue damage. These profiles confirm the observed blunting of type I IFNs and the consequent IL-6 related inflammatory syndrome (Fig. 1C).

5 | NEUTROPHILS RECRUITMENT AND ACTIVATION

Altogether, the data described earlier suggest that neutrophils are the primary cell type recruited to the lungs during SARS-CoV-2 infection. This is further supported by a meta-analysis done by Langunas-Rangel that examined severe COVID-19 cases, which showed that an increased neutrophil to lymphocyte ratio (i.e., more neutrophils) was associated to an enhanced inflammatory process and poor survival rate.[66] The specific cytokine profile seen in the analysis of these data links neutrophilia to the cause of several complications in COVID-19 cases. This is highly relevant because we observed the differential expression of several different genes that are associated with neutrophil activation, degranulation, and neutrophil-mediated immunity (Fig. 1C), which implies that not only are neutrophils heavily recruited to sites of inflammation, but they are highly active as well. As stated earlier, epithelial cells are heavily involved in the immune response, which suggests that these factors are being secreted by them and are acting on neutrophils to induce these specific responses. Based on the gene analysis and published clinical data, we propose that excessive neutrophil recruitment may be involved in the severity of COVID-19 cases and lead to the complications seen in some patients as well.[66,67] The data discussed here provide the potential molecular and cellular pathways responsible for the clinical outcomes described for COVID-19 patients.

6 | MECHANISMS UNDERLYING THROMBOSIS AND VASCULITIS: NEUTROPHILIC CONNECTION

It has become evident that COVID-19 patients are experiencing abnormal vascular issues, such as abnormal clotting, thrombosis, microvascular injuries, and preeclampsia.[4,8,68–72] Therefore, we sought to find a link to the cytokine profile shown in the analysis of these data and these abnormal vascular issues. First, it has been well established that neutrophils have been associated with thrombosis and microvascular injury.[73–77] In addition to the direct damage caused by neutrophil ROS production, we suspect that the main culprits behind these vascular issues in COVID-19 patients are NETs. NETs are structures released from neutrophils that contain nuclear chromatin bound by histones and a number of granular antimicrobial proteins.[78] Furthermore, an expanding amount of evidence suggests that the release of NETs (NETosis) is a new form of cell death that is different from apoptosis.[78,79] NETotic neutrophils do not release apoptotic signals, do not show membrane blebbing, or go through nuclear chromatin condensation.[78,79] In the context of COVID-19, NETs have been suggested to be linked to organ damage and mortality seen in severe COVID-19 cases.[80,81] Similarly, NET formation has also been confirmed to be important for pulmonary lung disease pathology, such as COPD, acute lung injury, and lung transplant rejection.[82–84] In the analysis of these data, we observed the up-regulation of several key cytokines that are highly relevant for NET regulation and production, including CCL20, IL-6, TNFα, CXCL8, and IL-1β. Their involvement will be outlined in the following text.

As discussed earlier, we see up-regulation of CCL20 (logFC = 3.146 and R = 1.000e-6) (Fig. 1A), which in addition to promote the recruitment of several cell types to sites of infection and inflammation, CCL20 is a major inducer of the formation of extracellular traps in neutrophils as well as other cell types such as mast cells, monocytes/macrophages, and eosinophils.[85] The presence of CCL20 up-regulation in COVID-19 patients suggests that extracellular traps maybe formed within the lung tissue or any tissue that is infected with SARS-CoV-2.

Other cytokines involved in NET release are IL-6, CXCL8, TNFα, and IL-1β, which are also up-regulated in SARS-CoV-2-infected samples (Fig. 1A). Both IL-6 and TNFα have been shown to be a potent inducer of NETosis.[86] Additionally, NETosis is at least partially dependent on CXCL8, whereas IL-1β is important for the induction of mast cell extracellular traps.[87,88] Last, activated endothelial cells under inflammatory conditions are thought to be involved in inducing NETosis, which may lead to their own demise.[87]

NETs have been described to be induced during viral infections, such as influenza A and respiratory syncytial virus and are an important component to control viral spread.[89] Two specific studies also support our hypothesis that neutrophils and extracellular traps are important for COVID-19 pathology.[90,91] A study by Radermecker et al. shows that NETs are important for the immunothrombotic events seen in COVID-19 cases.[91] Veras et al. also show that NETs are induced by SARS-CoV-2, in addition to seeing an augmented concentration of NETs in the plasma of COVID-19 patients.[90] This not only suggests that neutrophils are acting locally in the lungs but also that the effects from the neutrophils are reaching the vasculature that is away from the lungs. Therefore, it is feasible that neutrophil recruitment and NET release is an important component of the innate immune response to SARS-CoV-2 infection.

Altogether, the analysis of the inflammatory response found in the lungs and epithelial cells infected with SARS-CoV-2 suggests that the antiviral response to SARS-CoV-2 is leading to excessive neutrophil recruitment and NETosis. As mentioned earlier, histones are a component of NETs and have been shown to be cytotoxic to epithelial and endothelial cells, especially in the lungs.[92] This may explain why COVID-19 patients are experiencing microvascular injuries.[93]

Although neutrophils are the well characterized source of extracellular traps we cannot disregard the potential contribution of other granulocyte extracellular traps and their effect to the tissue damage seen in COVID-19 patients.

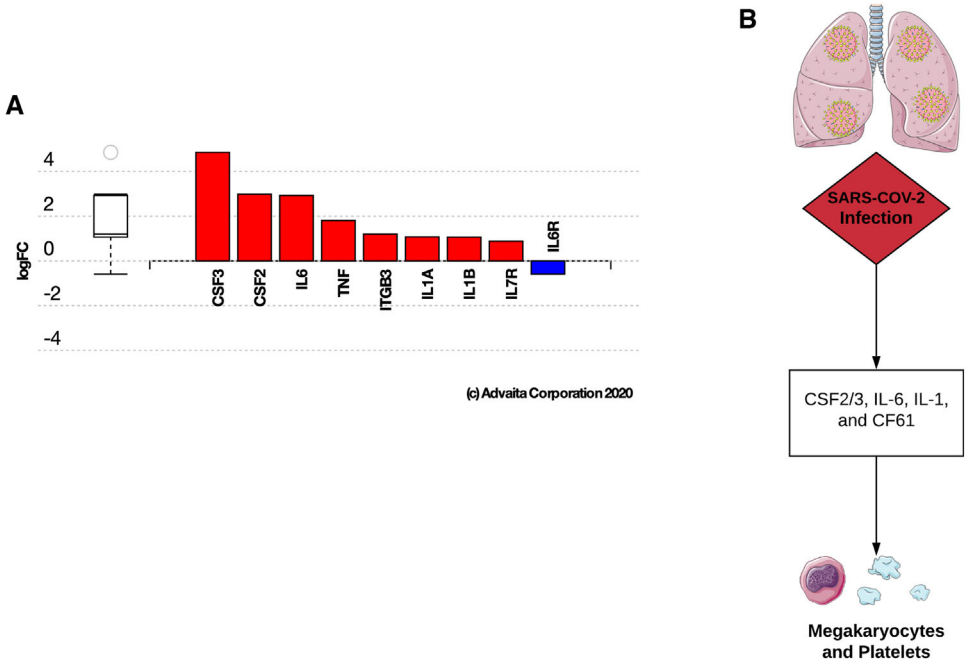

FIGURE 2 **Genes associated with hematopoietic stem cell lineage pathway in COVID-19.** (**A**) The logFC of all the main differentially expressed cytokines seen in primary human lung epithelium that are infected with SARS-COV-2 (USA-WA1/2020) (multiplicity of infection [MOI] = 2) that are associated with the hematopoietic stem cell lineage pathway. (**B**) Model linking the involvement of SARS-COV-2 infection in megakaryocyte activation and platelet production

7 | NETS AND THROMBOSIS

Several physicians taking care of patients with SARS-CoV-2 have reported an increased incidence of thromboembolic events such as catheter thrombosis, deep venous thrombosis, and pulmonary embolism. Recent studies in China and Europe have also suggested that patients with SARS-CoV-2 might have a hypercoagulable state that could predispose them to the occurrence of thromboembolic events.[94] The results of that case series suggest that pulmonary microvascular thrombosis might be responsible for the high mortality rate in patients with SARS-CoV-2 and acute respiratory distress syndrome.[95,96] The theory regarding pulmonary microvascular thrombosis has been further corroborated by a Chinese study, which performed postmortem examinations of patients who had died of SARS-CoV-2. They reported that thrombosis was commonly found in the small vessels, lungs, and, even, some extrapulmonary organs.[11,94,96]

One of the main components of thrombosis is platelets. Interestingly we observed significant up-regulation of CSF2, CSF3, IL-6, and IL-1β in samples infected with SARS-CoV-19 (Figs. 1 and 2 and Supporting Information Figs. S1 and S2). These cytokines are known to be involved in megakaryocytic function and platelet production[97–101] (Fig. 2A and Supporting Information Fig. S2). This suggests that these factors are being secreted from the epithelium that is infected with SARS-COV-2 and acting to remotely induce these effects. Thus, these differentially expressed genes suggest a potential response to the infection that could have an effect on platelet production (Fig. 2A, B).

To better understand the potential immunologic response associated with these thrombotic events, we performed a network analysis to determine the differentially expressed genes associated with various aspects of coagulation (Fig. 3). Interestingly we did not identify differentially expressed genes that are associated with the activation of the extrinsic, intrinsic, and common coagulation pathways (Fig. 3). This suggests that there is a noncanonical mechanism that is leading to coagulation in COVID-19 patients, which is capable of circumventing the normal coagulation pathways.

Between the main pathways affected by SARS-CoV-19 and associated with thrombotic events, we observed up-regulation of the IL-17 pathway (Fig. 4A and Supporting Information Fig. S3). Mast cell and NETs have been shown to release IL-17.[88] A study by Boer et al. showed an increase in IL-17 in the thrombi of acute myocardial infarctions, which suggests IL-17 may play a role in thrombosis.[93] Thus, IL-17 found in extracellular traps (NETs) may also be taking part in the prothrombotic environment seen in COVID-19 patients (Fig. 4B).

Several reports suggest that COVID-19 is associated with complement activation and complement mediated microvascular damage and thrombosis.[70,102–104] In this analysis, we see that another pathway that was affected by SARS-CoV-19 infection was the complement pathway. We observed up-regulation of C3 (logFC = 1.511 and R = 1.000e-6) and complement factor B (CFB; logFC = 1.857 and R = 1.000e-6) (Fig. 4C, D and Supporting Information Fig. S4). Additionally, we find up-regulation of several other complement pathway associated genes, including C1R (logFC = 0.832 and R = 0.045), C1S (logFC = 0.8 and R = 0.15), and C1Q (logFC = 1.874 and R = 1.000e-6) (Fig. 4D).

The classical and alternative complement pathways can be activated near platelets or on their own cell membranes.[105–107] This process ties the complement pathway into thrombosis by allowing

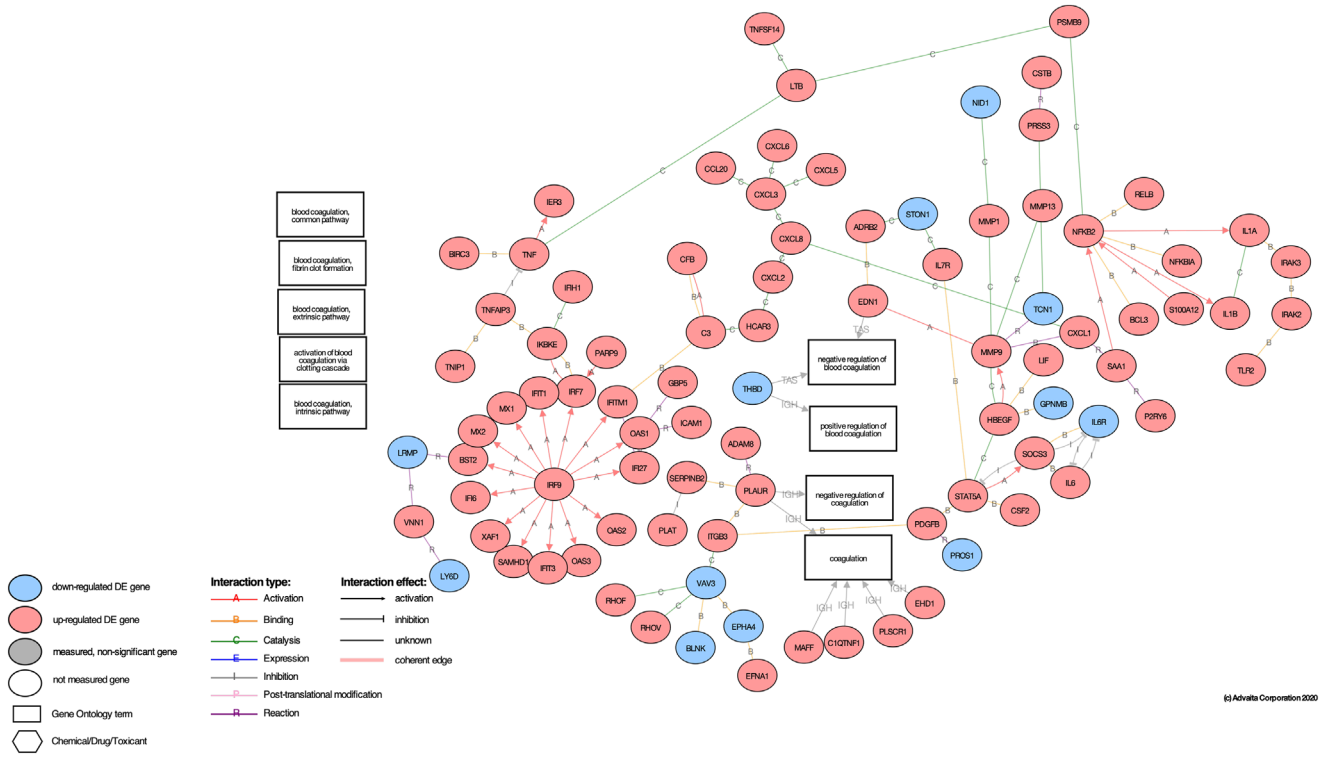

FIGURE 3 **Network analysis of COVID-19 and coagulation.** Network analysis of differentially expressed genes that are associated with various aspects of the coagulation process in response to SARS-COV-2 infection in primary human lung epithelium (USA-WA1/2020) (multiplicity of infection [MOI] = 2). Interestingly, none of the identified genes responsible for coagulation during SARS-COV-2 infection are associated with activation of the classical extrinsic and intrinsic pathways. Red circles and arrows indicate activation; blue circles indicate inhibition

complement to prime cell membranes to be more receptive to tissue factor (TF).[108] Additionally, complement directly activates platelets and also increases the expression of TF from various cell types.[108] Complement also switches mast cells away from a profibrinolytic phenotype to a prothrombotic phenotype.[104] Furthermore, the c1q subunit from the complement pathway has been shown to induce IL-6 and IL-8 expression; all of which are up-regulated in lung samples infected with SARS-CoV-2.[109] Neutrophils and NETs carry various key components that are involved in the complement pathway, such as complement factor P, CFB, and C3.[110] This may suggest a positive feedback loop where complement is inducing IL-6 and IL-8 expression, which in turn lead to further complement activation via NETosis (Fig. 4E). To summarize all this information, we propose that in COVID-19 patients, NETs lead to complement activation, which compounds the prothrombotic nature of NETs (Fig. 4E).

8 | COVID-19 AND KIDNEY INJURY

Kidney disease and acute kidney injury have been shown to be highly prevalent in COVID-19 patients.[9] Additionally, kidney injuries in COVID-19 patients have been associated with an increased in-hospital mortality rate.[9] This led us to hypothesize that neutrophils and NETs may be the culprit behind the onset of these kidney disorders in COVID-19 patients. One of the key biomarkers thought to be important for early detection of kidney injury is neutrophil gelatinase-associated lipocalin or lipocalin 2 (LCN2).[111] LCN2 is produced by neutrophils, and its gene expression is up-regulated (logFC = 0.719 and $R = 1.446e\text{-}5$) in these data, which indicates the potential role of neutrophils in kidney injuries seen in COVID-19 patients.[112] Furthermore, NETs and their histones are linked to kidney injury.[113] Similar to histone induced epithelial cell death, histones from NETs induce tubular epithelial cell death and lead to kidney injury.[113] Complement proteins and their activation have been confirmed to play roles in a diverse set of renal diseases and disorders, suggesting that the kidneys are particularly susceptible to complement mediated injury.[114–117] Last, individuals with kidney disease show a higher thrombotic risk.[118] This suggests that our proposed model of SARS-CoV-2 infection leading to neutrophilia, NETs, and complement activation may lead to kidney damage and thrombosis in the kidneys (Fig. 5). Additionally, there is a possibility that the kidney damage itself may lead to thrombosis.

9 | COVID-19 AND LIVER INJURY

As with kidney disease, there is an abnormally high number of COVID-19 patients that develop liver disease or injury. Thus, liver disease and injury have also been linked to SARS-CoV-2 infection.[10,119–122] As with kidney disease and injury, neutrophils, NETs, complement, and thrombosis all play roles in liver injury. Neutrophils have been extensively proven to be involved in a wide variety of different types of liver dysfunction and damage, including hepatic ischemia-reperfusion

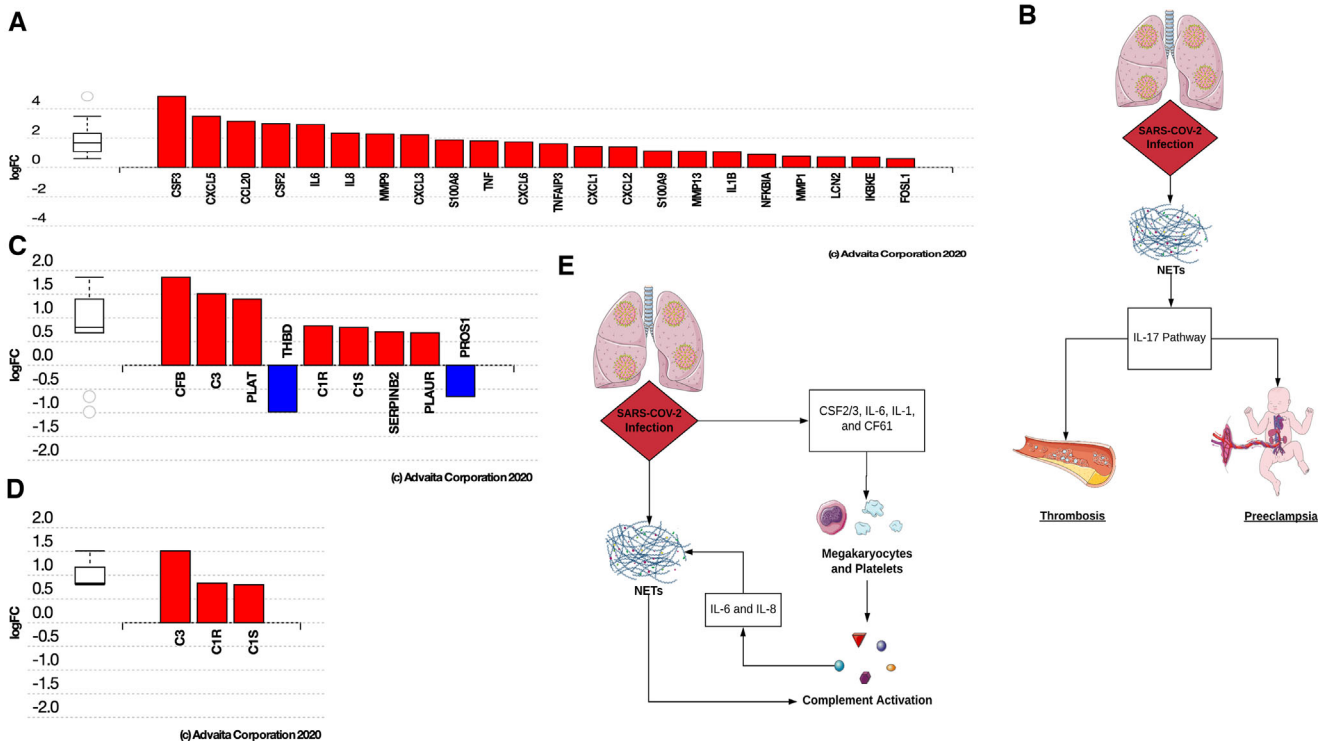

FIGURE 4 **IL-17 and complement pathways associated with COVID-19**. (**A**) The logFC of all the main cytokines that were differentially expressed in the IL-17 signaling pathway in cells infected with SARS-COV-2 (USA-WA1/2020) (multiplicity of infection [MOI] = 2). (**B**) Proposed model of the pathways behind IL-17 induced inflammation following SARS-COV-2 infection of lung epithelial cells which may be involved in thrombosis and pregnancy complications such as preeclampsia. (**C**) The logFC of the genes activated in the complement pathway following SARS-COV-2 infection (USA-WA1/2020) (MOI = 2). (**D**) The logFC of the genes that are most commonly associated with classical complement pathway and are differentially expressed in primary human lung epithelium infected with SARS-COV-2 (USA-WA1/2020) (MOI = 2). (**E**) Model linking the identified complement activation pathway in response to SARS-COV-2 infection

injury, endotoxin shock, alcoholic hepatitis, obstructive cholestasis, and concanavalin A or alpha-naphthyl isothiocyanate-induced liver injury.[123–131] NETs have been shown to accelerate liver damage during hepatic ischemia-reperfusion injury.[132] Moreover, extracellular histones released from NETs induced fatal liver injury in mice via TLR-2 and TLR-4.[133] Furthermore, the complement system has also been implicated to be involved in liver dysfunction and damage.[134] Particularly, the C3 complement component is seen to be up-regulated during early stages of liver damage.[135] Last, certain types of liver diseases, such as cirrhosis and chronic liver disease, show an increased risk for thrombosis.[136] Nearly identical to the mechanism for COVID-19 induced kidney damage, these data suggest that liver damage caused by COVID-19 may be associated with neutrophilia, NETs, and complement (Fig. 5).

10 | COVID-19 AND KAWASAKI DISEASE

KD is a rare hyperinflammatory shock syndrome that has been identified in asymptomatic SARS-CoV-2-infected children.[7,137] To date, eight children that tested positive for SARS-CoV-2 were affected by this disease. Interestingly, these children were all asymptomatic for the common COVID-19 symptoms.[7] KD is an acute systemic vascular disease usually seen in children under the age of five. The main complication of KD is the destruction of the epithelial lining in small and medium sized vessels that can lead to a coronary aneurysm.[138] Although the pathophysiology of this disease is poorly understood, it is currently thought that neutrophils, NETs, and the complement system may all be involved, all of which are included in our model. Elevated neutrophil levels have been reported in KD.[139,140] Additionally, neutrophils in this disease show an increased ROS production and neutrophil elastase expression during the early stages of KD, further suggesting that neutrophils play a role in epithelial cell destruction in KD.[139,141] It has also recently been reported that NETosis is enhanced in acute KD and takes part in KD vascular injury.[140] Last, the classical complement pathway is enhanced in KD. The inflammatory response in this disease also results in the production and activation of complement components.[142] Altogether, these findings suggest that the elevated neutrophils in SARS-CoV-2 infections may contribute to KD.

11 | COVID-19 AND PREECLAMPSIA

Finally, we want to discuss the potential involvement of SARS-COV-2 in preeclampsia. There have been several reports showing that pregnant mothers infected with SARS-CoV-2 develop symptoms

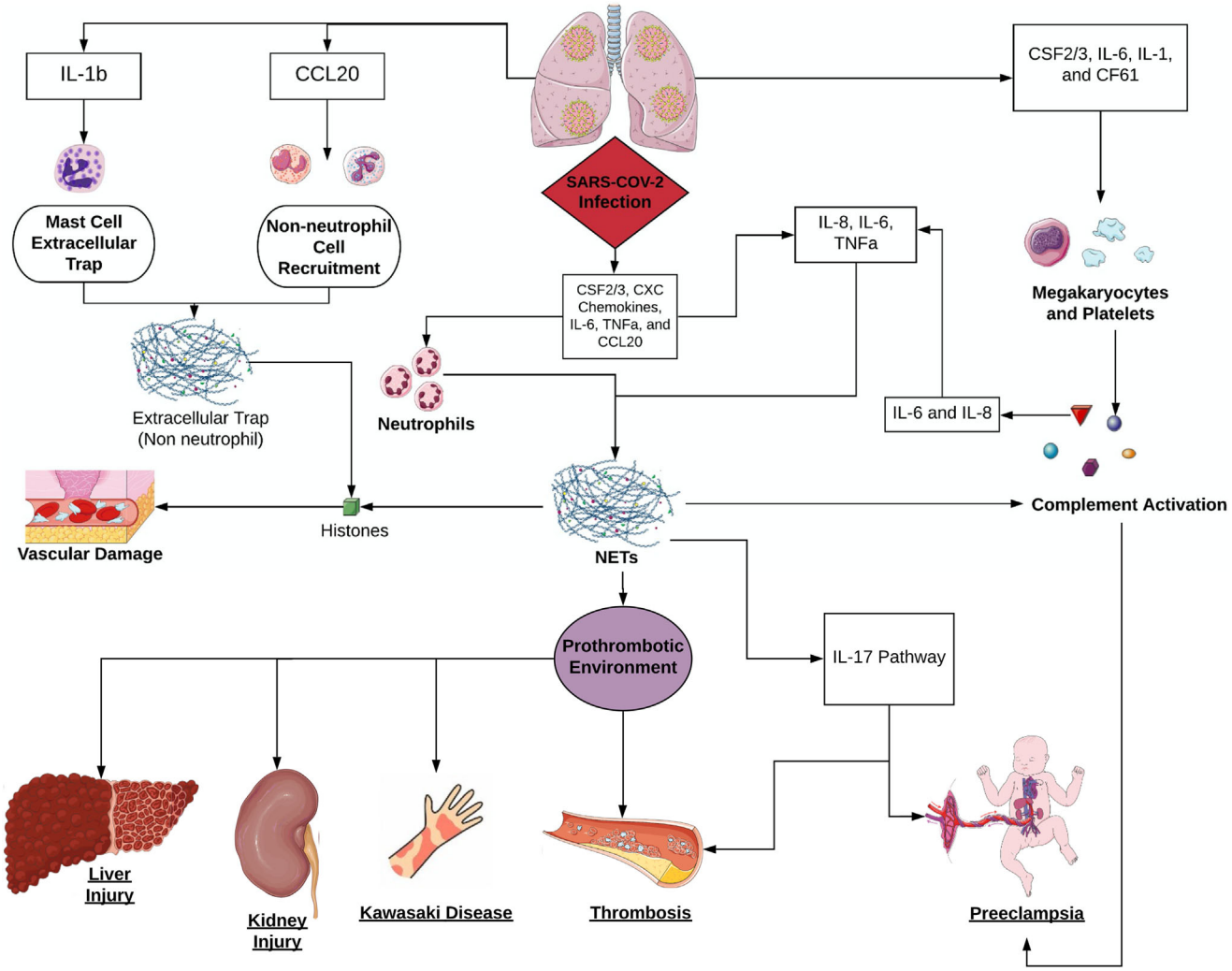

FIGURE 5 **Immunologic response to COVID-19 infection has an impact on the process of thrombosis and induce vascular injury**. Model of SARS-COV-2 induced activation of thrombosis and consequent tissue injuries. SARS-COV-2 infection of the epithelium of the lung triggers an inflammatory response, which promotes neutrophil activation and neutrophil extracellular trap (NET) formation. This response is responsible for the initiation of a thrombotic event and vascular injury leading multiple organ damages. If the process occurs in pregnant women, it can lead to symptoms associated with preeclampsia

that resemble patients with preeclampsia.[68,69,143] Similar to the nonpregnant patients, the role of COVID-19 in preeclampsia may be linked to the specific cytokine profile described earlier. First, NETs have been shown to be present in the intervillous spaces of preeclamptic placentae suggesting their involvement in the pathophysiology of preeclampsia.[144] Second, elevated IL-17 serum levels have been associated with preeclampsia.[145] As mentioned previously, NETs contain IL-17. This elevated IL-17 in preeclamptic mothers may be due to neutrophils producing NETs. Third, the complement pathway has been linked to the onset of preeclampsia.[146] Complement deposits have been seen on the trophoblasts of preeclamptic placentae.[147,148] All three of these factors are proposed by our model and are also linked to thrombosis and a procogaulatory environment. Moreover, high levels of TF mRNA have been seen in the vasculature of preeclamptic placentae.[149] Thus, preeclampsia appears to be associated with procoagulation and microvascular injuries, both of which are indicated in these data. Last, a recent study by Shanes et al. showed that mothers infected with SARS-CoV-2 displayed placentae in a hypercoagulative environment that were at risk for developing thrombi in addition to a hyperinflammatory environment.[150] This study further solidifies our model and is important for the fields of reproductive immunology and fetal development. Upward transmission from the mother to the fetus has yet to be confirmed, but maternal infection with SARS-CoV-2 could lead to severe developmental issues in the fetus similar to what was seen during the 2016 Zika virus epidemic.[151]

12 | CONCLUSION

In this review, we used a bioinformatics approach to help elucidate the immune response elicited during a SARS-CoV-2 infection by examining the cytokine profile of human lung epithelium cells infected with

SARS-CoV-2. Additionally, we propose the potential mechanisms underlying the increased thrombotic events and vascular injuries occurring in COVID-19 patients. We propose that SARS-CoV-2 infection causes neutrophilia via a specific cytokine storm. The elevated neutrophil levels in the body in turn generate a large number of NETs, which consequently lead to a variety of complications, such as vascular injury, thrombosis, and organ damage. NETs seem to be the most important factor for several reasons. First, NETs are procoagulant and create a prothrombotic environment. Thrombosis has been linked to nearly all of the other complications associated with SARS-CoV-2 infections, including liver damage, kidney damage, and preeclampsia. Thus, it can explain the mechanism responsible for the increased occurrence of thrombosis and other related complications. Second, NETs promote the activation of the complement pathway. Again, the complement pathway has been linked to the severity of COVID-19 cases, that is, thrombosis, injuries of liver, kidney, and vascular system, as well as KD. Finally, IL-17 contained in NETs is associated with thrombosis and preeclampsia. For the first time to our knowledge, our proposal suggests a system that well explains why SARS-CoV-2 infection causes pathologic thrombosis, vascular and organ injuries, preeclampsia, and KD. This pathway provides a useful guide for researchers to fully elucidate the mechanisms underlying the complications associated with COVID-19 and to develop novel therapeutic strategies to combat this disease (Fig. 5). Most importantly, this pathway extends beyond just being useful for the SARS-CoV-2 research and allows us to have a better understanding of the activation of innate immune system in response to the complications associated with COVID-19.

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AUTHORSHIP

A.J.M., J.D., and Y.Y. performed experiments, interpreted data, and assisted with preparation of the manuscript. Z.D., J.H.C., A.A., performed statistical analyses. Y.M., assisted with clinical data, S.D. provided analytic advice and reviewed the manuscript. G.M. conceived and designed the study, interpreted data, and wrote the manuscript.

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SUPPORTING INFORMATION

Additional information may be found online in the Supporting Information section at the end of the article.

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