

Platelet Dysregulation in the Pathobiology of COVID-19

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Abstract

Coronavirus disease 2019 (COVID-19) encompasses a broad spectrum of clinical manifestations caused by infection with severe acute respiratory syndrome coronavirus 2. Patients with severe disease present with hyperinflammation which can affect multiple organs which often include observations of microvascular and macrovascular thrombi. COVID-19 is increasingly recognized as a thromboinflammatory disease where alterations of both coagulation and platelets are closely linked to mortality and clinical outcomes. Although platelets are most well known as central mediators of hemostasis, they possess chemotactic molecules, cytokines, and adhesion molecules that are now appreciated as playing an important role in the regulation of immune response. This review summarizes the current knowledge of platelet alterations observed in the context of COVID-19 and their impact upon disease pathobiology.

Keywords

- ▶ platelets
- ▶ COVID-19
- ▶ SARS-CoV-2
- ▶ immunothrombosis

Introduction

The global pandemic of coronavirus disease 2019 (COVID-19) is characterized by an increased susceptibility to thrombosis due to a combination of excessive inflammation, endothelial dysfunction, and platelet activation.

In some COVID-19 patients, systemic inflammation and microvascular thrombosis of the lungs, heart, and liver correspond with progressive thrombocytopenia. Autopsies from COVID-19 patients revealed the presence of platelet-rich microvascular thrombi in the lungs, heart, kidneys, and skin.¹ While the breadth of mechanisms underlying the coagulopathy observed in COVID-19 is not fully known, recent studies provide evidence that hyperreactive platelets and abnormally elevated numbers of megakaryocytes in the lungs contribute to dysregulated hemostasis. In addition to their essential role in maintenance of vascular integrity, platelets participate in immunity during infection via multiple mechanisms.² Platelets have essential roles in the activation of innate defense mechanisms by regulating recruitment, migration, activation of immune cells including

adaptive immunity, and modulating B- and T-cell activity by secretion of granule content. Platelet-mediated immune functions in response to viral infections have emerged as key participants in host defense which when dysregulated can contribute to disease.³ In the context of viral infection, platelets are capable of internalizing viruses such as influenza and human immunodeficiency virus (HIV), which can promote heterotypic aggregate formation with circulating immune cells. The thrombotic state in COVID-19 arises from alterations in coagulation and endothelial dysfunction, which has been the subject of considerable attention.^{4–7} In this article, we review the clinical and laboratory findings of platelet dysregulation caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the consequences in COVID-19.

Thrombocytopenia Associated with COVID-19

Many viral and bacterial infections are commonly associated with thrombocytopenia.⁸ Independent studies from across the globe have produced abundant reports that COVID-19

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follows this trend as well,^{4–9} routinely observing thrombocytopenia in patients with SARS-1 and Middle East respiratory syndrome.^{9–20} Initial descriptions of COVID-19 patients ($n = 1,099$) reported thrombocytopenia in 31.6% of non-severe patients and 57.7% of patients with severe disease. While the majority of COVID-19 patients exhibit only mild thrombocytopenia at hospital admission, significantly decreased platelet counts ($<31 \times 10^9/L$) are associated with increased mortality in critically ill patients.^{20–23} In a study of 61 critically ill patients admitted into intensive care unit (ICU), 41% of ICU patients had severe thrombocytopenia with platelet counts $<50 \times 10^9/L$, and all except one were non-survivors.²⁴ Mild thrombocytopenia appears to be a common feature of SARS-CoV-2 infection, while significantly reduced platelet counts are rare and likely represent developing coagulopathy. Thrombocytopenia at admission is associated with mortality of COVID-19, and reduction in circulating platelets postadmission associates with the deterioration of lung function leading to respiratory failure, vascular leakage, and septic shock.²⁵

A number of potential mechanisms underlying COVID-19-associated thrombocytopenia have been suggested, and thrombotic coagulopathy is a critical factor. The lungs of COVID-19 patients are characterized by diffuse damage to the alveoli, pulmonary congestion, fluid retention, fibrosis, and expansion of hyaline membrane.²⁶ The damaged lung tissue and pulmonary microvascular endothelium likely result in platelet activation, aggregation, microvascular thrombi formation, and platelet sequestration by the lungs. Mounting evidence has shown that two discreet populations of megakaryocytes exist within the lung: those in circulation of extrapulmonary origin likely released from the sinusoids of the spleen or bone marrow, and those resident in the lung interstitium.²⁷ Murine studies have demonstrated that megakaryocytes within the lung are capable of releasing platelets into pulmonary circulation,²⁸ and several lines of indirect evidence point to an important role of the human lung in thrombopoiesis.²⁷

Megakaryocytes are present at increased levels in lung autopsies from COVID-19 patients,^{29,30} where lung inflammation, ventilation, and oxygen therapy could affect lung thrombopoiesis by resident megakaryocytes and simultaneously result in increased consumption of platelets due to thrombotic microangiopathy. While the definitive mechanisms underlying thrombocytopenia in COVID-19 may partially depend in part on disease stage and severity, coronaviruses have been demonstrated to directly infect bone marrow cells and cause abnormal hematopoiesis.³¹ Like SARS-1, SARS-CoV-2 may directly infect hematopoietic cells, as ACE2 is expressed in up to 65% of cord-blood-derived hematopoietic stem cells. Further, the precursors to megakaryocytes, multipotential progenitor cells, expand less effectively and exhibit dysfunctional colony-forming capacity in the presence of SARS-CoV-2 spike protein.³² This observation has implications not only for the immune response, but for plausible mechanisms that could lead to direct viral alterations of platelets and megakaryocytes.

While direct viral infection could explain COVID-19-associated thrombocytopenia via growth inhibition or apoptosis, the observation that thrombocytopenia associates with more severe clinical outcomes suggests that alternative mechanisms, including antiplatelet autoantibodies and immune complexes, are also major contributors to low platelet counts. Elevated antiphospholipid antibody (aPL) titers have been reported in COVID-19,³³ and can react with the endothelium, leukocytes, and platelets where they can induce expression of thrombotic factors and adhesion molecules.³⁴ In the context of platelets, aPLs can promote platelet activation, aggregation,³⁵ increased expression of integrin $\alpha\text{IIb}\beta\text{3}$,³⁶ and synthesis of thromboxane A₂,³⁷ which result in sensitization of platelets to aggregating stimuli similar to observations of hyperreactive platelets from COVID-19 patients. It is important to note that the presence of transient aPL positivity has been reported in up to 58% of patients tested for aPL,³⁸ and follow-up studies suggest that 67% of patients continue to test positive for aPL at 1 month postinfection.³⁹ Given that patients with aPLs often do not present with thrombocytopenia except in extremely severe cases, it remains unclear whether there exist unique targets generated by patients with COVID-19. These studies are suggestive of some clinical similarities between severe COVID-19 and antiphospholipid syndrome, and are hypothesis-generating and require future study.

Given the widespread use of heparin as a prophylactic anticoagulant in COVID-19 patients, sparse observations of heparin-induced thrombocytopenia (HIT) began to emerge in the early stages of the pandemic.^{40–42} HIT involves the formation of an immune complex consisting of heparin: platelet factor 4 (PF4) antibodies, which interact with the Fc γ R2a receptor on the platelet surface leading to platelet activation and clearance in a neutrophil extracellular trap (NET)-mediated mechanism.⁴³ Subsequent studies have demonstrated the presence of anti-heparin:PF4 antibodies in COVID-19 patients, but results demonstrating whether these immune complexes are capable of activating platelets are conflicting.^{42,44} Recently, several cases of cerebral venous thrombosis and moderate-to-severe thrombocytopenia were observed in healthy individuals following vaccination with AstraZeneca's COVID-19 vaccine (AZD1222). These cases resemble HIT with a different serological profile.^{45,46} Initial study suggests that these individuals produce immunoglobulin class G platelet-activating antibodies which recognize PF4 independent of heparin, unlike classic HIT. Further analysis of immunogenic anti-PF4 and anti-heparin:PF4 antibodies purified from patients receiving AZD1222 shows that affinity-purified antibodies do not bind to full-length spike protein, the S1 domain, or the receptor-binding domain.⁴⁶ Intriguingly, there are reports of high levels of anti-PF4 antibodies present in severe COVID-19.^{24,40} Structural features between the SARS-CoV-2 spike protein sequences share potential immunological epitopes with PF4, and antibodies purified from 4 of 222 patients with COVID-19 were capable of inducing PF4-dependent platelet activation.^{45,46} Together, these studies suggest that an underlying mechanism could exist by which antigen

presentation of the SARS-CoV-2 spike protein may very rarely lead to development of immune thrombotic thrombocytopenia by platelet-activating anti-PF4 antibodies, which presents in clinically similar fashion to HIT. However, it is important to note that 5 to 7% of healthy individuals have detectable PF4-heparin antibodies, and nearly all healthy adults have a reservoir of B-cells specific for PF4-heparin complexes,^{47,48} and alternate mechanisms such as breakdown of immune tolerance could also explain the presence of anti-PF4 antibodies in the context of COVID-19.

The Platelet Hyperactivated State in COVID-19

The prothrombotic state in COVID-19 likely arises as a consequence of increased coagulation, decreased fibrinolysis, immune activation, and increased platelet activation and hyperactivity. One of the first functional studies of platelets isolated from COVID-19 patients demonstrated that platelets are hyperreactive to traditional agonists such as adenosine diphosphate (ADP), collagen, and thrombin as compared with responses in healthy donor platelets, indicating a virus-induced sensitization of platelets.⁴⁹ The platelet activation state results from integration of both stimulatory and inhibitory signaling pathways, and enhanced platelet activation in COVID-19 appears to be partially due to increased downstream factors such as protein kinase C delta, extracellular signal-related kinase, and p38 signaling.⁴⁹ Other investigators have similarly demonstrated increased platelet activation and aggregation in response to low-dose agonist stimulation, as well as enhanced ADP release by platelets isolated from COVID-19 patients.⁵⁰ Both ADP and thromboxane A2 act as local, autocrine agonists which amplify platelet activation, thereby triggering further release of platelet granule contents. This hyperactivity in COVID-19 platelets likely explains increased release of extracellular vesicles, α granules, and dense granules into circulation. Indeed, several molecules released from activated platelets including serotonin, soluble P-selectin, soluble CD40L, platelet-derived growth factors, and PF4 are increased in COVID-19 patients, and COVID-19 platelets are more prone to release some cytokines such as sCD40L and interleukin (IL)-1 β upon stimulation.⁵¹ High levels of PF4 are present in both severe and nonsevere COVID-19 cases, while sP-selectin is consistently elevated in severe COVID-19 cases, including those complicated by respiratory distress.⁵⁰ Importantly, while activation leads to increased P-selectin at the platelet surface,⁵² P-selectin can also be shed from the platelet membrane *in vivo* after activation. Pathological levels of P-selectin, either soluble or on platelets, can trigger activation of leukocytes, induce tissue factor (TF) expression by monocytes, and stimulate neutrophils to form extracellular traps, all of which contribute to a hypercoagulable state.^{53,54} Enhancement of platelet degranulation and cytokine release by platelets may have a major contribution to the cytokine storm observed in COVID-19. Several cytokines and growth factors relevant to viral and inflammatory responses (sCD40L, eotaxin, interferon- α and - γ , IL-1 β , tumor necrosis factor- α and - β), and other platelet α - and dense-granule

content (PF4 and serotonin, respectively) are reported to be decreased in platelets, but are present at elevated levels in severe and mild COVID-19 patient plasma samples as compared with healthy donors.⁵¹

In other viral infections, including HIV and dengue, and in critical illnesses such as sepsis, a subpopulation of strongly activated procoagulant platelets has been reported to contribute to increased risk of thrombosis.⁵⁵⁻⁵⁷ These platelets are distinct in that while they support thrombin generation and increased interactions with leukocytes, procoagulant platelets also demonstrate reduced aggregation. Formation of procoagulant platelets relies upon a mitochondrial permeability transition pore, resulting in decreased mitochondrial membrane potential, release of mitochondrial Ca²⁺ which initiates phosphatidylserine (PS) exposure, and the assembly of prothrombinase complexes to generate thrombin.^{58,59} In contrast to HIV and dengue infections, platelets isolated from COVID-19 patients have reduced procoagulant formation in response to dual agonist stimulation with thrombin and convulxin as measured by PS exposure in both non-ICU and ICU-admitted patients due to altered mitochondrial function and reduced mitochondrial membrane potential when compared with healthy donors.⁶⁰ Constitutive exposure to platelet agonists could explain these observations, as prolonged stimulation is known to significantly affect mitochondrial membrane potential and subsequent exposure of PS.⁵⁹ However, another observational study demonstrated increased PS externalization, depolarization of mitochondrial transmembrane potential, and cleavage of caspase-9 in unstimulated platelets isolated from ICU-admitted COVID-19 patients as compared with platelets isolated from either nonsevere COVID-19 or healthy donors.

Further, incubation of immunoglobulin G (IgG) fractions from severe COVID-19 patients with platelets isolated from healthy donors led to similar levels of PS exposure and increased cytosolic calcium, indicating that IgG antibodies present in severe COVID-19 patients may induce procoagulant responses *in vivo* via Fc γ RIIIa, which upon binding the constant fragment of IgG triggers platelet activation and aggregation.⁶¹ To date, the contribution of procoagulant platelets to thrombosis risk in COVID-19 has not been well defined, and may not be the most detrimental aspect of platelet activation. Based on the evidence available to date, coagulopathy in COVID-19 is most likely a result of thromboinflammatory disease which promotes increased P-selectin, α IIb β 3 activation, and pathological interactions between platelets, leukocytes, and endothelial cells.

Together, these studies demonstrate that platelets in COVID-19 patients are more responsive to agonists at a lower threshold for activation, similar to previous reports of platelet hyperreactivity in patients with dengue and influenza.^{57,62} While the roles of platelets as mediators of inflammation are well established, the mechanisms by which platelets become primed to respond to stimuli are not clear in the context of COVID-19. COVID-19 induces significant changes to the platelet transcriptome,⁴⁹ and platelets may become primed due to alterations in gene expression or in response to increased procoagulant molecules in circulation. Viral infection can

indirectly activate platelets during disease by promoting an inflammatory microenvironment and vascular endothelial dysfunction, which can include release of endogenous danger signals and factors from inflamed endothelial cells. Platelets may also degrade matrix components and releasing fragments of glycosaminoglycans, such as hyaluronan and heparan sulfate, which can promote inflammation in immune cells and drive endothelial barrier dysfunction.^{63–65} Circulating levels of von Willebrand factor and fibrinogen are markedly increased in COVID-19 and are generally associated with vascular inflammation, disease severity, and hypercoagulability.^{66,67} The autocrine signaling roles of ADP, ATP, thromboxane A₂, and β -thromboglobulin released by platelets^{68,69} could further amplify platelet activation and granule release, thereby fueling a condition where platelet reactivity is increased in COVID-19. Another possible mechanism involves direct viral infection of platelets or megakaryocytes; although viremia is considered rare, mRNA corresponding to SARS-CoV-2 has been reported in platelets from COVID-19 patients. Multiple reports confirmed that the SARS-CoV-2 virus can be found within platelets,^{49,51} and at least one study indicates that platelets not only express ACE2 and TMPRSS2, but that SARS-CoV-2 and spike protein directly bind ACE2 and enhance platelet activation *in vitro*.⁷⁰ Direct interactions between viruses and platelets are not uncommon. Platelets bind to HIV via lectins CLEC-2 and DC-SIGN, to adenovirus via α IIb β 3, and to influenza/IgG immune complexes by Fc γ RIIA.³ Human platelets respond to single-stranded RNA predominantly by toll-like receptor 7 (TLR7), located within endolysosomes.

Activation of TLR7 leads to α -granule release in an AKT- and p38-dependent manner and promotes platelet–neutrophil interactions via P-selectin and CD40L,⁷¹ supporting the notion that SARS-CoV-2 could directly lead to platelet activation via multiple pathways.

Platelet Interactions with Immune Cells in COVID-19

Platelets play multiple roles in COVID pathogenesis, including participation via interactions with immune cells. Platelet recruitment of leukocytes to sites of inflammation is crucial, and corresponds with increased abundance of platelet–leukocyte aggregates in COVID-19 patients.^{49,72} When stimulated by proinflammatory cytokines, monocytes are a source of procoagulation TF that initiates coagulation pathways mediated by thrombin.^{73,74} Expression of TF on the monocyte surface promotes the generation of thrombin, and monocyte-derived TF expression increases in COVID-19 patients (positively correlating with disease mortality), and is highly expressed on platelet–monocyte complexes within patient plasma.⁷² Interestingly, neutralization of P-selectin, a key receptor in the formation of platelet–monocyte aggregates, resulted in loss of COVID-19-induced TF monocyte expression as well as platelet–monocyte complexes.⁷² Thus inflammatory environment present in COVID-19 can trigger activation of TF macrophages and other cell types including neutrophils and endothelial cells within the lung, amplifying the coagulation cascade and leading to microvascular dysfunction.

Given the clinical importance of thrombosis in COVID-19, several studies have investigated the role of neutrophils and subsequent NET formation. High circulating neutrophil levels are indicative of COVID-19 infection and positively correlate with patient symptom severity.⁷⁵ Platelets and neutrophils modulate one another during inflammation, with neutrophils releasing proteases that promote coagulation through degradation of anticoagulant molecules,^{76–78} while activated platelets have been shown to bind to circulating neutrophils, presumably leading to neutrophil activation,^{49,50,74,75,79} initiate NETs through platelet TLR4 signaling,⁸⁰ and secretion of granule contents⁸¹ in COVID-19, leading to immunothrombosis. This pattern of high neutrophil count and disease severity correlates with increase of released NET markers in COVID-19 patients,^{75,79,82,83} and while NETs can be protective against bacterial infections, dysregulated NET formation can be cytotoxic to the host and damage surrounding tissues, and is found embedded in fibrin clots in close association with platelets in the lungs, heart, and kidneys of patients with COVID-19.^{79,81} Not only can the expelled chromatin scaffold that comprises NETs allow for platelet aggregation and thrombi formation,⁸⁴ but the exposed histone proteins can also activate the platelets as well; leading to thrombin generation through activation of TLR4 and TLR2 in platelets.⁸⁵ Compared with healthy controls, patients with COVID-19 have increased levels of circulating myeloperoxidase–DNA (MPO–DNA) complexes, which are used as biomarkers of circulating NETs.⁸¹ MPO–DNA and thrombin–antithrombin complexes positively correlate in patients with COVID-19 and return to normal levels in convalescent patients, suggesting an important role for NETs in immunothrombosis.^{74,81} The neutrophil serine proteases released during NET formation also target and degrade TF pathway inhibitor, the inhibitor of thrombin development,⁷⁷ allowing for coagulation to proceed. In the context of COVID-19, the interplay between hyperreactive platelets and neutrophils leads to immunothrombosis and an accumulation of NETs within the lung of critically ill patients.⁸¹

A connection between platelets and adaptive immunity has not been thoroughly investigated in COVID-19. However, many of the soluble factors contained within α granules of platelets and increased in COVID-19 including CD40L, PF4, and tumor growth factor β (TGF- β) have immunomodulatory activities. Both forms of CD40L, soluble and membrane-associated, can modulate B cell isotype switching and enhance CD8+ T cell responses.^{86,87} Platelets are significant sources of circulating TGF- β , and platelet-derived TGF- β is known to regulate differentiation of CD4+ T cells into immunosuppressive regulatory T-cells, which can prevent cytokine storm and accelerate resolution of lung injury.^{88,89} Platelets are also capable of processing antigens through major histocompatibility complex (MHC) class I to activate the adaptive immune system. Resting platelets express a partially denatured MHC I molecule adsorbed from plasma and are generally thought to be unable to activate CD8+ T cells. However, activated platelets express functional MHC I molecules released from α granules and are capable of

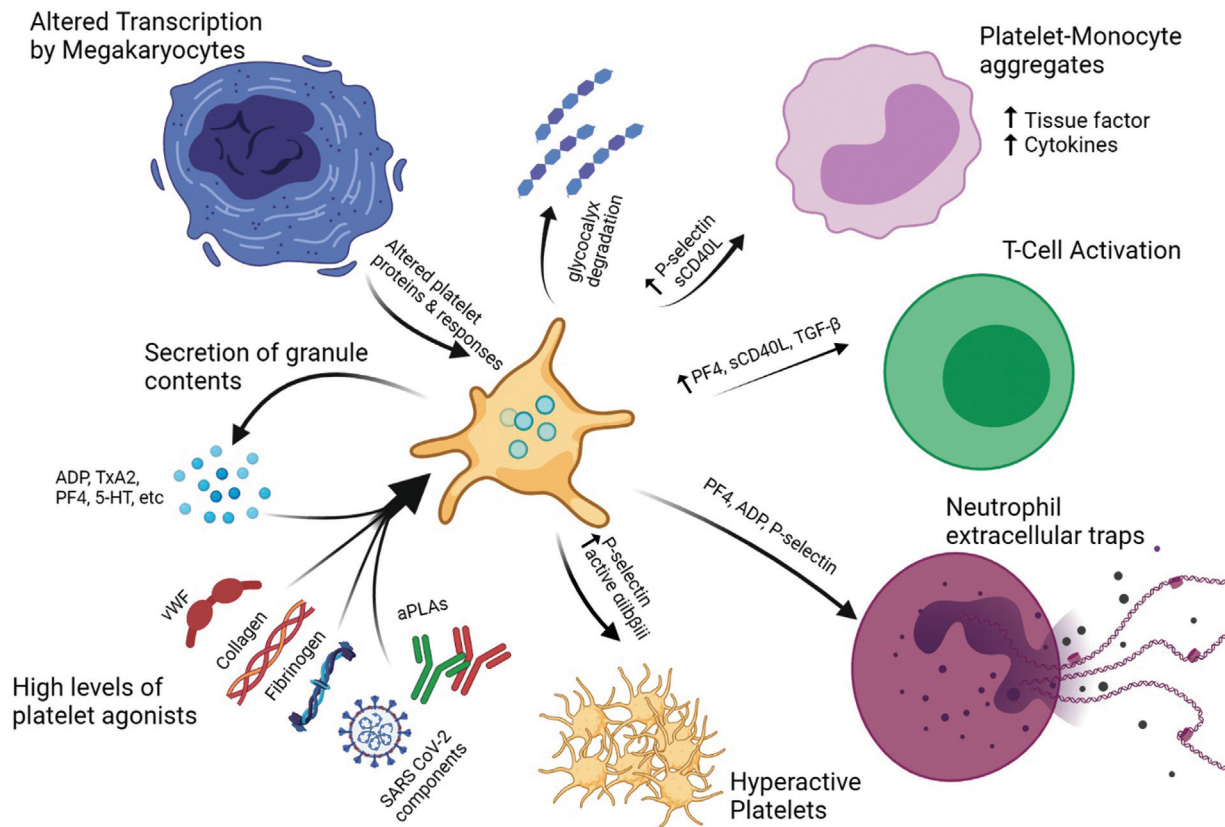


Fig. 1 COVID-19 drives platelet activation and pathological interactions with immune cells. Several pathways likely work in parallel and lead to hyperactive platelets in COVID-19. These include classical platelet agonists which are produced in patients during disease pathogenesis (von Willebrand factor, collagen, fibrinogen, ADP, antiphospholipid antibodies, etc.). Direct viral interactions are also speculated to activate platelets and megakaryocytes. Platelets from COVID-19 patients also exhibit increased activation potential, which platelets may inherit due to disease-driven alterations in megakaryocytes. Signals coming from platelets themselves amplify the inflammatory response by secretion of granule contents, increased proadhesive receptors (P-selectin, GPIIb/IIIa), and binding to endothelial and immune cells. Hyperactivated platelets can promote degradation of the endothelial glycocalyx and extracellular matrix, fueling barrier dysfunction. Platelet-leukocyte aggregates participate in amplification of cytokine release, activation of T-cells, and neutrophil extracellular trap formation. This figure was generated using BioRender. ADP, adenosine diphosphate; aPLAs, antiphospholipid antibodies; PF4, platelet factor 4; sCD40L, soluble CD40 ligand; TGF- β , transforming growth factor β ; TxA2, thromboxane A2.

presenting endogenous antigens as well as exogenous antigens by cross-presentation.⁹⁰ In response to dengue infection, a proteomic study revealed that proteasome subunits and the class I antigen presentation pathway are the most significantly increased proteins in platelets during infection.⁹¹ Given the reports of SARS-CoV-2 association with platelets, it seems possible that similar to dengue, platelets could be capable of degrading viral components and presentation of antigen peptides via MHC I. Together with costimulatory molecules, platelets have the potential to either inhibit or activate CD8⁺ T cells in the context of COVID-19 depending on their activation state and MHC expression.

Conclusions

As our knowledge of COVID-19 increases, the complexity of the disease mechanisms continues to increase. There remains a pressing need to delineate the immunological processes which determine whether infected individuals experience mild symptoms or progress into critical illness. Dysregulation of platelets and coagulation clearly plays a

part in multiple aspects of disease triggered by SARS-CoV-2 infection (**~Fig. 1**) and leads to life-threatening thrombosis. The spectrum of dysregulated platelet responses correlates with clinical outcomes and several lines of evidence point to platelets as important regulators of disease in COVID-19. Future studies of platelets as players in innate immunity and in COVID-19 will better determine how platelet function impacts disease progression.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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