Platelet Function in Viral Immunity and SARS-CoV-2 Infection

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Abstract

Platelets, as nonnucleated blood components, are classically recognized for their pivotal role in hemostasis. In recent years, however, accumulating evidence points to a nonhemostatic role for platelets, as active participants in the inflammatory and immune responses to microbial organisms in infectious diseases. This stems from the ability of activated platelets to secrete a plethora of immunomodulatory cytokines and chemokines, as well as directly interplaying with viral receptors. While much attention has been given to the role of the cytokine storm in the severity of the coronavirus disease 2019 (COVID-19), less is known about the contribution of platelets to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Here, we give a brief overview on the platelet contribution to antiviral immunity and response during SARS-CoV-2 infection.

Keywords
- platelets
- hemostasis
- inflammation
- SARS-CoV-2
- COVID-19
- viral infections

Infectious diseases have significant impact globally, with high mortality and morbidity rates reported each year by the World Health Organization.[1] Over the past few decades, new challenges associated with infectious diseases have placed additional burdens on health care due to the emergence of antimicrobial resistance,[2] and viral pandemics including Ebola,[3] human immunodeficiency virus (HIV),[4] and most recently severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is causing the ongoing coronavirus disease 2019 (COVID-19) pandemic outbreak.[5]

Platelets are small anucleate cells derived from megakaryocytes, and are traditionally known for their role in prevention of bleeding and minimizing vascular injury.[6] While vital for hemostasis, there has been an increasing awareness that platelets also contribute to various human pathologies, including autoimmunity,[7] cancer,[8] and infectious diseases.[9] Thus, in addition to their key contribution to thrombus formation, there is increasing consensus that platelets would play important roles in modulating immune and autoimmune responses.[10,11]

The ability of platelets to participate in the immune response is in part due to their ability to release a myriad of inflammatory and bioactive molecules stored within their granules. These mediators are able to attract and modulate

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the activities of circulating leukocytes, important in orchestrating localized immune responses to pathogens.[11] Platelets have also been found to elicit direct effector functions, so as to be considered independent immune effector cells.[12] Indeed, megakaryocytes and platelets have been shown to express several immune-associated molecules and receptors, including Fc receptors,[13] complement receptors,[14] chemokine receptors,[15,16] and an array of toll-like receptors (TLRs).[17–25] The expression of these functional immune receptors raises the question whether platelets can also engage viruses and contribute to antiviral immunity. In this short narrative review, we explore how viruses engage circulating platelets and how they contribute to viral pathology.

The Role of Platelets in Viral Immunity

Viral immunity has traditionally focused on the roles of leukocytes, given their direct involvement in viral spread and antiviral responses. Clinically, platelet hyperactivity has been recognized as a hallmark of many viral infections, including dengue virus,[26] HIV,[27–29] influenza virus,[30] and SARS-CoV-2.[31] Given the prominent clinical presentations of platelet-driven events, along with their emerging immune role, having a better understanding of the role of platelets in viral infections may disclose and highlight novel therapeutic targets.

A key antiviral platelet response is to sequester viral particles, thus limiting viral spread within the host environment. Evidence of such activity has been seen in HIV, where platelets bind and endocytose HIV virions,[32–35] which is believed to help clearance of viral particles from circulation.[36] In addition to engaging viruses, platelets are also able to exert direct antiviral properties. During platelet activation, α-granules are trafficked to the cell surface and externalized, so releasing a wide spectrum of bioactive molecules, including platelet factor 4 (PF4; also referred to as the chemokine CXCL4). As well as being an important chemotactic agent for leukocytes, PF4 has direct antiviral activity, being found to suppress HIV infection of T cells.[37–39] Interestingly, platelets may also help control infection through the secretion of platelet antimicrobial peptides, such as PD1–PD4, which have been shown to have antiviral activity against the vaccinia virus.[40] A recent study has also presented data demonstrating that platelets contain virus-specific immunoglobulin G (IgG), which is able to potentially neutralize in vitro and in vivo viral infection against human cytomegalovirus (HCMV) and influenza A virus.[41] Platelet-derived IgG localizes to α-granules,[42] suggesting that megakaryocytes are able to take up IgG, where they are stored in α-granules for later secretion by mature platelets. Interestingly, IgG released from platelets was found to be more efficient at neutralizing virus compared with equal amounts of plasma IgG,[41] the biological significance of which is unclear.

Platelets are also able to orchestrate local immune responses to viral infection. HCMV can be recognized by platelet TLR-2. This engagement leads to platelet degranulation, leukocyte chemotaxis, and formation of platelet aggregates with neutrophils, monocytes, B cells, T cells, and dendritic cells.[43] Through these platelet–leukocyte interactions, platelets present viral antigens to leukocytes via major histocompatibility complex class I,[44] as well as providing costimulatory signals to antigen-presenting cells,[45] both of which can prime and mount an antiviral leukocyte response. Similar inflammatory activities have been observed in dengue virus infection, whereby dengue-infected platelets were able to induce monocyte activation.[46]

While platelets can exert a degree of antiviral immunity, viruses have evolved mechanisms of evading platelet recognition. Viruses are able to engage with receptors at platelet surfaces; for example, dengue virus and HIV both bind surface lectin receptors and dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) on platelets.[47] Such interactions lead to internalization of viral particles, where viruses such has HIV, HCMV, and hepatitis C virus (HCV) can continue to replicate and generate productive viruses within both megakaryocytes and platelets.[48–50] In addition to using platelets as a site of replication, some viruses use circulating platelets as cellular carriers to evade immune detection, such as the influenza virus[51] and HIV,[52,53] essentially forming latent viral reservoirs within the circulation. Interestingly, HCV is believed to utilize circulating platelets to transport itself to the liver, where enhanced platelet–hepatocyte interactions prolong the time for potential viral infection.[54]

Thrombocytopenia is a common feature among various viral infections, which is associated with more severe diseases.[55] Viruses have developed several mechanisms to target and reduce platelet production and/or integrity. A classic example can be seen with neuraminidase activity of influenza virus, which reduces platelet life span by targeting them for rapid clearance in the liver and spleen.[56] In addition to targeting platelets for destruction, neuraminidase activity also alters megakaryocyte ploidy, morphology, and subsequent platelet size.[57] Human herpes viruses have also adopted similar mechanisms, and can interfere with thrombopoietin activity, thus reducing megakaryocyte colony formation[58] and impairing megakaryocyte survival and differentiation.[59] Defective megakaryocyte differentiation can also be achieved by altering cytokine expression in the bone marrow, which has been found in dengue virus infection.[60] By targeting these megakaryocytic developmental checkpoints, abnormal platelet activation, mitochondrial dysfunction, reduced cellular integrity, and increased apoptosis are often seen in patients infected with dengue,[61–63] encephalomyocarditis virus,[23] and HIV.[64,65]

In addition to impacting platelet integrity, viral infection can also affect platelet function.[66] Coxsackievirus B virus (CVB) binds and enters platelets via the Coxsackie–Adeno receptor.[67] While unable to replicate inside the platelet, CVB modulates activity and enhances P-selectin release and phosphatidylserine exposure, which collectively promote platelet–leukocyte interactions and ultimately leads to platelet destruction and thrombocytopenia,[67] driving viral pathology. While vaccinia virus is known to bind and enter platelets,[68] the significance of this interaction to disease is

not completely understood. Early studies reported reduced in vitro platelet aggregation, but increased serotonin release in vaccinia-infected platelets,[68] which would suggest that platelet function may be suppressed. In contrast, in vivo models found vaccinia virus infection led to fatal intravascular coagulation,[69] implicating an augmented platelet response. This disparity may highlight that the vaccinia virus may impact endothelial function, which is known to be critical in regulating in vivo platelet responses.[70,71]

Given the complex nature of the immune network, the existence of indirect effects of viruses on platelets is unsurprising. Platelet hyperactivity in influenza infection can be partially attributed to influenza's impact on monocyte cytokine release, which then activates platelets.[72] Adaptive immune responses to HCV, HIV, HCMV, herpes viruses, and coronaviruses result in the production of antibodies targeting viral glycoproteins to help neutralize and suppress viral spread. These antiviral glycoprotein antibodies can, however, cross-react with platelet integrins and trigger platelet autoantibody-induced thrombocytopenia in several viral settings.[73] Viruses have also been found to infect the endothelium, with indirect effects on platelet function. For example, dengue virus and hantaviruses infect endothelial cells, promoting endothelial activation, endothelial–platelet interactions, and increasing vascular permeability.[74,75] This disruption of vascular integrity is thought to contribute to the increased platelet reactivity observed in virally infected patients and may represent one mechanism of enhanced platelet clearance.

An additional consideration of chronic viral infection, like HIV, is that patients require permanent therapeutic intervention to suppress viral replication. Some cohort studies have found that certain antiretroviral drugs are associated with increased risk of myocardial infarction,[76,77] which have subsequently been found to enhance platelet activation and aggregation.[78,79] These effects can be further enhanced by vascular endothelium, which is also impacted by antiretroviral drugs in ways that increase platelet reactivity.[80,81] Such data demonstrate that both viral infection and therapeutic measures to control infection can impact platelet reactivity.

The Platelet Response during SARS-COV-2 Infection

Dual activation of inflammation and coagulation pathways, combined with an excessive recruitment and activation of immune cells to sites of infection, is known as "immunothrombosis," a concept that was initially conceptualized by Engelmann and Massberg, in 2013, to accurately define the crosstalk between hemostasis and the innate immune system.[82] Given that aberrant platelet activation has been documented with other viral infections, researchers have begun to explore the potential contributory role of platelets to SARS-CoV-2 infection.

The core pathology of COVID-19 is pulmonary, with epithelial cell infection by SARS-CoV-2 ultimately resulting in pulmonary leukocyte infiltration and an excessive inflammatory response.[83] Clinical evidence supports this model, with several reports detailing signs of epithelial and endothelial inflammation, leukocyte recruitment, and platelet activation in the lung of COVID-19 patients.[84–86] Poorer prognoses in patients are shown to associate with abnormal coagulation parameters, primarily D-dimer, fibrinogen, fibrin degradation product levels, reduced mitochondrial depolarization, and phosphatidylserine exposure,[87–89] suggesting that thrombosis may be important to COVID-19 pathophysiology. Severe pulmonary inflammation and obstructive immunothrombosis in the lung microvascular network of COVID-19 patients, leading to pulmonary thrombosis/thromboembolism, underlie multiple organ failure and mortality in patients with advanced stages of illness.[90–92]

Elevated plasma levels of proinflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-6, IL-12, monocyte chemoattractant protein-1, interferon-γ, and tumor necrosis factor-α have been found in patients with COVID-19.[93–95] While there is evidence of elevated proinflammatory cytokines, it is important to note that there are also reports finding similar or lower levels of proinflammatory cytokines when compared with patients with COVID-19-unrelated acute respiratory distress syndrome or other cytokine release syndromes.[96–98] In addition, reports have also found elevated D-dimer concentrations in patients with COVID-19,[99] which is consistent with the observed systemic inflammation and macrovascular thrombotic complications seen in patients with SARS-CoV-2 infection,[100,101] and may therefore be linked to coagulation activation and diffuse macro- and microvascular thrombosis.[102,103]

While SARS-CoV-2 messenger RNA (mRNA) can be detected in platelets isolated from patients with COVID-19,[94,104–106] it is not clear whether SARS-CoV-2 is internalized by the platelets via receptor-mediated endocytosis. Although it is widely accepted that SARS-CoV-2 infects host cells via binding angiotensin-converting enzyme 2 (ACE2),[107] it is not known whether platelets express this protein. While some studies have shown that neither ACE2 mRNA nor protein could be detected in platelets,[94,104] others have reported robust ACE2 expression in platelets, associated to direct platelet activation by SARS-CoV-2 via spike/ACE2 interactions.[108,109] The reason for this disparity is unclear, but may stem from differences in washed platelet preparation given that one study used sodium citrate-evacuated blood tubes, while others used an acid/citrate/dextrose anticoagulant. It is also feasible that genetic differences between cohort populations may account for differences in ACE2 expression or protein polymorphisms. These discordant results were clearly highlighted in a recent review.[106] Interestingly, while Zaid et al demonstrated that platelets only associate with SARS-CoV-2 RNA, they reported substantial alterations in the platelet transcriptome and proteome profiles,[104] as well as platelet hyperreactivity.[94,104] The abilities of viruses including SARS-CoV-2 to associate and internalize with platelets are listed in ►**Table 1**.

These studies indicate that platelet activation contributes to COVID-19 pathophysiology. Autopsy studies found evidence of extensive thrombosis in multiple organs,[84]

Table 1 Associations and/or internalizations between platelets and different viruses

Virus		Platelet		References
Type	Nomenclature	Association	Internalization	
DNA	Herpes simplex virus type 1 (HSV-1)	Yes	?	108
	Human cytomegalovirus (HCMV)	Yes	?	118
	Vaccinia virus (VACV or VV)	Yes	?	43
RNA	Human immunodeficiency virus (HIV)	Yes	Yes	68
	Hepatitis C virus (HCV)	Yes	Yes	32
	Dengue virus (DENV)	Yes	Yes	119
	Influenza virus (flu virus)	Yes	Yes	120
	Coxsackievirus B (CVB)	Yes	Yes	121
	Encephalomyocarditis virus (EMCV)	Yes	Yes	67
	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Yes	?	94

suggesting enhanced platelet reactivity may be a driver of thrombosis in severe COVID-19. Abnormal platelet morphology has also been reported in COVID-19 patients, with evidence of large, hyperchromatic, and vacuolated platelets.[110] A recent study documented COVID-19 patients as having enhanced platelet hyperreactivity relative to non-COVID-19 patients and controls subjects.[111] Greater levels of platelet–monocyte and platelet–granulocyte aggregates can be seen in patients with COVID-19 pneumonia,[112] highlighting greater levels of systemic platelet activation. Further phenotypic analysis revealed that resting platelets in COVID-19 patients had similar levels of P-selectin expression as control platelets activated with collagen.[112] Taus et al also demonstrate that COVID-19 platelets contribute to the increased fibrinogen, von Willebrand factor, and factor XII reported in patients, while facilitating accelerated factor XII-dependent coagulation.[112] Moreover, platelets isolated from patients with severe COVID-19 were able to induce ex vivo tissue factor expression in monocytes isolated from health controls,[88] indicating platelet crosstalk into other circulating cells. Interestingly, normal platelet function is restored in patients who have recovered from SARS-CoV-2 infection, which suggests that platelet hyperreactivity may be a direct consequence of SARS-CoV-2 infection.[111] Together, these data suggest that in COVID-19, platelets are primed to spread proinflammatory and procoagulant activities within the systemic circulation.

►**Fig. 1** summarizes the role that the platelet could have following SARS-CoV-2 infection.

Conclusion

COVID-19 is a viral infection with variable clinical outcomes, determined by the amplitude of immunothrombosis response and extent of tissue injury. While hyperinflammation and the "cytokine storm" may be central to the most severe COVID-19 cases,[113] given the clinical spectrum of COVID-19, the absolute centrality of the "cytokine storm" may not be as straightforward. It could be argued that the extent and importance of increased cytokine release on pathology draws upon multiple factors including genetic, host and viral phenotypic, and environmental.[114] Recently, studies using the bronchoalveolar lavage of COVID-19 patients in intensive care, highlighted the presence of a 'lipid storm' but were unable to definitively demonstrate whether platelets or other cells are the cause of this altered lipid profile.[115] Currently, available studies suggest that the COVID-19 coagulopathy comprises a combination of localized pulmonary platelet consumption, low-grade disseminated intravascular coagulation, and thrombotic microangiopathy.

Of particular interests are the various circulating inflammatory coagulation biomarkers involved directly in clotting, with specific focus on fibrin/fibrinogen, D-dimers, P-selectin, von Willebrand factor multimers, soluble thrombomodulin, and tissue factor, which may amplify inflammation and hypercoagulability in patients with COVID-19. Central to the activity of these biomarkers are their receptors and signaling pathways on endothelial cells, platelets, monocytes, and erythrocytes. Altogether, these collective observations raise the question as to whether the virus acts directly on the hemostatic system or whether hemostatic activation is secondary to the upstream inflammatory process.

Currently, literature remains ambivalent regarding ACE2 expression on platelets. It would therefore be useful to explore whether SARS-CoV-2 directly binds platelets via ACE2 or through alternative pathways. These studies may give better insight into underlying pathways driving the "cytokine storm coagulation" that contributes to the multiple organ dysfunction associated with severe COVID-19. While therapeutic intervention targeting the cytokine storm in severe COVID-19 is gaining increasing attention,[116] the use of antiplatelet therapy also warrants further study. Aspirin administration has been associated with a reduced risk of mechanical ventilation, intensive care unit admission, and in-hospital mortality in 412 hospitalized COVID-19 patients.[117] A limitation to this study, however, is that it is a retrospective, observational cohort study, which limits its

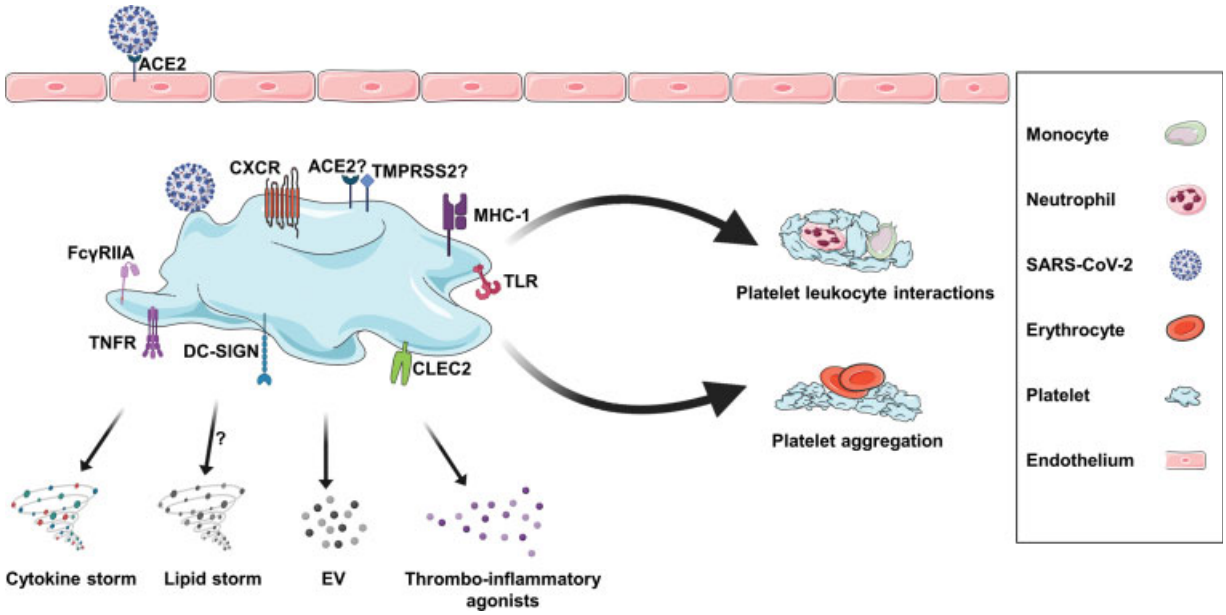

Fig. 1 Hypothetical model for platelet interaction with SARS-CoV-2. SARS-CoV-2 potentially depends on ACE2 receptor for viral entry through the endothelium and spread in the host. Then various platelet receptors can mediate binding to viral particles; however, SARS-CoV-2 binds to platelets probably via its potential receptor ACE2, and viral hemagglutinin is cleaved by TMPRSS2 to activate internalization of the virus. Such cleavage triggers platelet activation and downstream signaling events leading to cytokine overproduction, platelet aggregation, and leukocyte–platelet aggregate formation. The combination of the cytokine storm, platelet activation, microvesicle shedding, and immunothrombotic events have deleterious consequences such as cellular damage, acute lung injury, and thromboembolism (created with BioRender and Servier). ACE2, angiotensin-converting enzyme 2; CLEC, C-type lectin-like receptor; CXCR, chemokine receptor; DC-SIGN, dendritic cell-specific ICAM-grabbing nonintegrin; EV, extracellular vesicles; FcγRIIa, low-affinity immunoglobulin gamma Fc region receptor II-a; MHC-1, major histocompatibility complex 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TLR, toll-like receptor; TNFR, tumor necrosis factor receptor.

strength as clinical evidence. This study does demonstrate that further randomized controlled trials examining the efficacy of antiplatelet therapeutics in treating patients with severe COVID-19 are of clinical value. These future trials would be strengthened by complementary basic and translational studies dissecting the role of platelets in COVID-19 pathophysiology.

Ultimately, a cross-disciplinary approach drawing upon the expertise of biomedical and clinical communities is critical in developing a therapeutic arsenal to target not only the cytokine storm but also the coagulopathy related to SARS-CoV-2 infection. A deeper understanding of the contributions of platelets to viral immunity will not only allow for better treatment of COVID-19, but also help to be more prepared to manage future viral pandemics.

Authors' Contributions

A.A., A.A.K., O.B., M.N., M.L., F.G., and Y.Z. contributed to literature search and writing of this review. A.A.K., M.L., F. G., and Y.Z. designed the structure and content of this review. Y.Z. provided the figure. All authors approved the submitted version of the manuscript.

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Conflict of Interest

None declared.

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