A double-edged sword: antibody-mediated procoagulant platelets in COVID-19

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The past year has demonstrated the power of collaborative scientific efforts in rapidly developing vaccines to curb the SARS-CoV-2 pandemic and repurposed drugs to improve patient outcomes in severe COVID-19 disease. However, the morbidity and mortality associated with COVID-19 remains significant. A better understanding of COVID-19 pathogenesis will aid future therapeutic advances. Viral infection first triggers the innate immune system, with stimulation of natural killer cells and dendritic cells, secretion of interferons and presentation of viral antigens to the adaptive immune system, which mounts a specific antibody and T lymphocyte response toward the virus and develops memory against it. However, a recent study published in Blood by Althaus et al. shows that in COVID-19, the antibody arm of the adaptive immune response also triggers platelet procoagulant activity (Figure 1), thereby exacerbating the disease.

Early in the pandemic, it became clear that COVID-19 is associated with thromboinflammation. Patients with severe COVID-19 have high incidences of venous thromboembolism, arterial thrombosis leading to myocardial infarction or stroke, and pulmonary microvascular thrombosis [1–3]. In thromboinflammation, highly complex interactions between the immune and hemostatic systems drive thrombin generation and thrombosis under inflammatory conditions, with platelet and immune cell activation, endothelial cell dysfunction and the plasma coagulation, complement and contact systems all contributing. How each of these systems is disrupted in COVID-19 is subject of intense research. The study by Althaus et al. establishes a new role for procoagulant platelets, a subpopulation of activated platelets that expose phosphatidylserine (PS) and enhance thrombin generation [4]. This insightful study was conducted during the first peak of the pandemic and though it involved a small cohort of patients, its results clearly link platelet procoagulant activity to thromboinflammation in COVID-19. During the 30-day follow-up of the study, 33% of the COVID-19 patients requiring intensive care unit (ICU) support sadly died and 57% experienced thromboembolic complications, underscoring the severity of this disease. The percentage of platelets exposing PS was increased in COVID-19 patients in ICU compared to hospitalized COVID-19 patients not requiring ICU, or patients requiring ICU for non-COVID-19 reasons. Although the percentage of platelets exposing PS was highly variable, it positively correlated with D-dimer levels and sequential organ failure assessment (SOFA) score. PS exposure was accompanied by elevated cytosolic Ca^{2+} concentration ([Ca^{2+}]_c) and depolarized mitochondrial membrane potential (ΔΨ_m), both key drivers of procoagulant platelet formation [5]. Cleaved caspase-9, an activated apoptotic protease, was also increased in COVID-19 ICU patient platelets. Furthermore, PS exposure and [Ca^{2+}]_c were greater in COVID-19 ICU patients who experienced thrombotic events and in non-survivors. Procoagulant platelet formation appears to be triggered by antibodies. Heat-inactivated sera from COVID-19 ICU patients induced PS exposure, ΔΨ_m depolarization, raised [Ca^{2+}]_c and caspase-9 cleavage in platelets from healthy donors. Isolated IgG from COVID-19 ICU sera had the same effects, and could be blocked by IV3, a monoclonal antibody that blocks FcγRIIa signaling. Together, the picture painted by these results shows that circulating antibodies activate platelet FcγRIIa, triggering procoagulant activity, thrombin generation and thromboinflammation (Figure 1). Antibodies play a central role in the immune response to viral infection, but this part of the body’s own defense response may be a double-edged sword, exacerbating thromboinflammation in COVID-19.

This study has several implications for the underlying pathogenesis of COVID-19, and in turn possible future therapeutic options. Here, Althaus et al. were the first to demonstrate the role of antibody-mediated platelet activation through FcγRIIa in COVID-19. This has now been replicated by Nazy et al., in this rapidly developing field [6], although Nazy et al. did not directly examine platelet PS exposure in their study. Nazy et al. also found that immune complexes were responsible for FcγRIIa-mediated platelet activation, whereas Althaus et al. found no evidence of IgG aggregates or immune complexes. Despite these differences, these studies highlight similarities between COVID-19 and diseases in which antibody-mediated platelet activation is central to their pathogenesis, including heparin-induced thrombocytopenia (HIT), immune thrombocytopenia (ITP) and lupus [7]. Indeed, some patients develop ITP secondary to COVID-19 [8]. A greater understanding of the antibody-mediated platelet activation through FcγRIIa will have wide-ranging implications. Signaling downstream of FcγRIIa involves Src, Syk and PI3-K [9]. Syk is a potential therapeutic target in HIT. Fostamatinib, a Syk inhibitor, and duvelisib, a Src inhibitor, are currently being tried in COVID-19 [10,11].

To date, understanding platelet FcγRIIa biology has been difficult as mouse platelets do not express FcγRIIa and IgG responses differ significantly between mice and humans [12]. Progress has been made with humanized mouse models and larger
model animals, though further understanding of FcγRIIA biology will likely also require pharmacological approaches in human platelets and in vitro models. Moreover, FcγRIIA varies between people. FcγRIIA expression is higher in patients with stroke and relatively common FcγRIIA polymorphisms are associated with increased disease severity in patients with lupus and HIT [13]. Whether such differences also contribute to the differing severity of COVID-19 between individuals is an interesting question for future studies. Another important question to address is why only some IgG antibodies activate platelet FcγRIIA, apparently without forming immune complexes, while others do not. The glycosylation state of IgG is critical in determining immune effector responses and differences in IgG glycome have been associated with COVID-19 disease severity and levels of immune cell activation through other FcγR subtypes [14,15]. The effect of the IgG glycosylation state in COVID-19 on platelet FcγRIIA has not been reported to date but would be an interesting research question.

The study by Althaus et al. also highlights the need to further understand the diverse stimuli and signaling pathways that trigger platelet PS exposure. In many cells, PS exposure is triggered by caspases and is often considered a hallmark of apoptosis. Caspase-dependent PS exposure can also be triggered in platelets by BH3 mimetics [16]. Here, Althaus et al. showed that caspase-9 was activated, and caspase inhibition partially inhibited PS exposure induced by IgG from COVID-19 ICU patients, leading the authors to conclude that apoptosis plays a role. However, platelet procoagulant formation in thrombosis is non-apoptotic but resembles regulated necrosis, with ΔΨm depolarization and high sustained Ca2+ signals, and can be inhibited by the cyclophilin D inhibitor, cyclosporin A (CsA) [17]. Here, CsA completely inhibited IgG-induced PS exposure and ΔΨm depolarization, suggesting that antibody-induced PS exposure more closely resembles non-apoptotic platelet procoagulant activity. The interpretation of in vitro experiments is complicated by the absence of phagocytic scavengers, however, allowing apoptotic and procoagulant platelets to progress to secondary necrosis [18]. Furthermore, other regulated cell death (RCD) pathways have been shown to be active in platelets including ferroptosis [19], pyroptosis [20] and necroptosis [21] but their signaling is less well characterized, particularly in terms of their effects on platelet PS exposure, Ca2+ signals and ΔΨm. Characterization of RCD pathways in platelets will allow distinct biomarkers of each to be recognized and may reveal new therapeutic targets across a range of conditions.

Finally, this study also hints at the relationship between platelet RCD pathways and thrombocytopenia. Thrombocytopenia is now well established as a consequence of COVID-19 in some patients and is associated with more severe disease and increased mortality [22], although the thrombocytopenia observed is often relatively mild. Other viral infections have been associated with antibody-induced thrombocytopenia including dengue, influenza and hepatitis C [23]. In this study, 62% of the patients developed thrombocytopenia. Lower circulating platelet counts can be due to reduced platelet production but also to increased platelet death and clearance [24]. Exposed PS is an ‘eat-me’ signal for dying cells. Megakaryocytes have not been extensively studied in COVID-19 though there have been suggestions of increased young reticulated platelets circulating in COVID-19 to compensate for increased platelet consumption [25]. Strikingly, within the COVID-19 ICU group, lower platelet count was correlated with higher PS exposure and higher Ca2+ signals.

Together, these results in this study suggest that in COVID-19, antibodies trigger platelet death, leading to PS exposure and thrombosis, along with platelet clearance and thrombocytopenia. Recently, very rare thrombotic and thrombocytopenic events linked to the Oxford/AstraZeneca vaccine AZD1222 have been proposed to be due to antibody-mediated platelet activation through a mechanism similar to HIT [26,27,28], suggesting a link between COVID-19 pathogenesis and potential vaccination-related adverse events. Indeed, thrombocytopenia has previously been linked to other vaccines [29]. However, the rarity of the adverse events reported for AZD1222 compared...
to the extensive occurrence of thromboembolism in COVID-19 indicates that there is much more to be resolved about antibody-mediated platelet activation. Understanding how antibodies activate platelets, and how RCD pathways in platelets contribute to procoagulant activity, thrombosis and thrombocytopenia may open up new opportunities for treating COVID-19 and other antibody-mediated thrombotic disorders.

Acknowledgements

The authors were supported by the British Heart Foundation project grants PG/17/45/33071 and PG/20/12/34882.

Funding

This work was supported by the British Heart Foundation [PG/17/45/33071, PG/20/12/34882].

Declaration of Interest

The authors have no financial or scientific conflicts of interest related to this work.

Funding

This work was supported by the British Heart Foundation [PG/17/45/33071, PG/20/12/34882].

References

1. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, Merdji H, Clere-Jehl R, Schenck M, Fagot Gandet F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med 2020 May;46:1–10. DOI: 10.1007/s00134-020-06662-x
2. Malas MB, Naezie IN, Elsayed N, Mathlouthi A, Marmor R, Clary B. Thromboembolism risk of COVID-19 is high and associated with a higher risk of mortality: a systematic review and meta-analysis. ECLinicalMedicine 2020;29:100639. DOI: 10.1016/j.eclinm.2020.100639
3. Boonyawat K, Chantrathammachart M, Numthavaj P, Nanthatani N, Phusanti S, Phuphuakrat A, Niparuck P, Angchaisukprasiri P. Incidence of thromboembolism in patients with COVID-19: a systematic review and meta-analysis. Thrombosis J 2020;18(1):34. DOI: 10.1186/s12999-020-00234-5
4. Althaus K, Marini I, Zlamal J, Pelzl L, Singh A, Häberle H, Mehrländer M, Hammer S, Schulze H, Bützer M, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. Blood 2021;137(8):1061–1071. DOI: 10.1182/blood.2020008762
5. Millington-Burgess SL, Harper MT. Cytosolic and mitochondrial Ca2+ signaling in procoagulant platelets. Platelets 2021 Feb;18:1–8. DOI: 10.1080/09537104.2021.1881951
6. Nazy I, Jevtic SD, Moore JC, Huyahn A, Smith JW, Kelton JG, Arnold DM. Platelet-activating immune complexes identified in critically ill COVID-19 patients suspected of heparin-induced thrombocytopenia. J Thrombosis Haemostasis: JTH 2021 Feb 27. DOI: 10.1111/jth.15283
7. Patel P, Michael JV, Naik UP, McKenzie SE. Platelet FcγRIIA in immunity and thrombosis: adaptive immunothrombosis. J Thrombosis Haemostasis [accessed 2021 Mar 12]. https://onlinelibrary.wiley.com/doi/abs/10.1111/jth.15265
8. Bhattacharjee S, Banerjee M. Immune thrombocytopenia secondary to COVID-19: a systematic review. Sc Compr Clin Med 2020 Sep;19:1–11. DOI: 10.1007/s42399-020-00521-8
9. Qiao J, Al-Tamimi M, Baker RL, Andrews RK, Gardiner EE. The platelet Fc receptor, FcγRIIA. Immuno Rev 2015;268(1):241–252. DOI: 10.1111/irm.12370
10. Washington University School of Medicine. A pilot study of duxelisib to combat COVID-19. clinicaltrials.gov; 2021. Report No.: NCT04372602. https://clinicaltrials.gov/ct2/show/NCT04372602
11. National Heart, Lung, and Blood Institute (NHLBI). A phase II study evaluating fostamatinib for hospitalized adults with COVID-19. clinicaltrials.gov; 2021. Report No.: NCT04579393. https://clinicaltrials.gov/ct2/show/NCT04579393