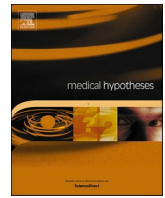




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Letter to Editors

Models for SARS-CoV-2 associated thrombocytopenia associated with hemophagocytic histiocytes



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ABSTRACT

A subset of COVID-19 patients is experiencing secondary immune thrombocytopenia, also called immune thrombocytopenic purpura (ITP) or secondary hemophagocytic lymphohistiocytosis (HLH). The pathogenesis of SARS-CoV-2 associated thrombocytopenia is unknown. Very rare cases of vaccine induced prothrombotic immune thrombocytopenia (VIPIT) are occurring associated with COVID-19 vaccines. COVID-19 VIPIT is associated with autoantibodies targeting platelet factor 4 (PF4) for COVID-19 adenovirus vaccines. Herein, four models for hemophagocytic histiocytes contributions to the etiology of thrombocytopenia associated with SARS-CoV-2 are proposed. One of the models proposes potential involvement of hemophagocytic histiocytes targeting platelets bound by autoantibodies consistent with observed PF4 autoantibodies in COVID-19 VIPIT.

Introduction

A study of 1,099 COVID-19 patients detected 36.2% with thrombocytopenia (abnormally low levels of platelets) [1]. Platelets, also called thrombocytes, are blood cells that aggregate in response to bleeding injuries to form blood clots together with coagulation factors. COVID-19 causes a spectrum of disease; some patients develop a unique coagulopathy associated with microthrombotic events, elevation of D-dimer levels, and disseminated intravascular coagulopathy (DIC). Platelets can also act like immune cells by binding and internalizing viruses [2,3]. A hemophagocytic histiocyte is a bloodstream phagocyte (e.g., macrophage). A subset of COVID-19 patients experiencing secondary immune thrombocytopenia (ITP) [4–6] also called secondary hemophagocytic lymphohistiocytosis (sHLH) [7,8], reactive HLH, or cerebral venous sinus thrombosis (CVST). ITP overlaps macrophage activation syndrome (MAS). Very rare cases of thrombocytopenia are also associated with two COVID-19 adenoviral vaccines AZD1222 ChAD0x1 (Oxford-AstraZeneca) [9] and Ad26.CoV2.S (Janssen/Johnson & Johnson) [10,11]. Herein, etiology models for possible hemophagocytic histiocyte contributions to both COVID-19 associated thrombocytopenia and COVID-19 vaccine associated thrombocytopenia are proposed.

The etiology of SARS-CoV-2 associated thrombocytopenia is unknown. Multiple mechanisms are possible including widespread microthrombi formation, immune dysregulation, and autoantibodies binding to platelets. At sites of disrupted endothelium, platelets bind, activate (change shape, secrete chemical messengers, and turn on receptors), followed by aggregation associated with activation of the coagulation cascade associated with fibrin deposition and linking to form microthrombi. Platelets levels can be reduced by formation of multiple microthrombi and also phagocytosis of platelets by macrophages named hemophagocytic histiocytes. Hemophagocytic histiocytes can be visualized with immunohistochemistry combined with in situ hybridization on paraffin-embedded tissue sections from lymph nodes, spleen, liver, and bone marrow [12]. Enrichment of hemophagocytic histiocytes is observed in COVID-19 patients and only rarely observed in

non-COVID-19-related acute respiratory distress syndrome (ARDS) patients [12]. Observations of scattered hemophagocytic lymphohistiocytes vary between studies with 4 cases associated with spleen but not liver of bone marrow [12], 16 of 17 cases associated with bone marrows [13], and 19 of 19 bone marrows [14]. Scattered macrophages with engulfed erythrocytes, erythroblasts, or lymphocytes are observed [12,13]. Macrophages containing hemosiderin (suggestive of red blood cell phagocytosis) were also frequently found [12,13]. Occasionally, multinucleate histiocytes are observed [13]; SARS-CoV-2 infected cells expressing the Spike protein can form multinucleated cells (syncytia) facilitated by cell surface Spike proteins [15]. Platelets can be hyperactivated in COVID-19 [16]. Severe COVID-19 infection is associated with platelet apoptosis induced by antibodies cross-linking Fc γ receptor IIA [17]. Platelet-monocyte aggregate formation is observed in severe COVID-19 patients [18]. Some studies, but not all [19], detect angiotensin-converting enzyme 2 (ACE2) associated with platelets [16,20]. The SARS-CoV-2 Spike protein has been shown to bind to ACE2 receptors, including platelet ACE2 receptors [20]. In addition, SARS-CoV-2 RNA has been detected within platelets [16]. RNA-seq was performed on RNA from highly purified platelets from 6 non-ICU and 4 ICU SARS-CoV-2 patients and 5 matched healthy donors to find 3,090 differentially expressed genes between non-ICU patients compared to healthy donors and 2,256 differentially expressed genes between ICU patients and health donors [19]. Platelet aggregation in response to low-dose agonists was significantly increased in COVID-19 patients compared to healthy donors [19]. SARS-CoV-2 induces altered platelet gene expression, platelet-leukocyte interactions, platelet-platelet interactions, and increased platelet reactivity [19]. Hemophagocytic histiocytes have been observed in COVID-19 patients [13] and autopsies [12–14,21]. Hemophagocytic histiocytes are associated with rare, and often fatal, hemophagocytic syndrome (hemophagocytic lymphohistiocytosis), secondary to other infections (e.g., Epstein-Barr [22] or Dengue Fever [23]), immunodeficiencies [24], cancer [25] or other major events.

Recently, rare cases of thrombocytopenia have been identified

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following vaccination with encoded SARS-CoV-2 Spike protein. Immune thrombocytopenia has been reported in a case report following COVID-19 vaccination [26]. A man with preexisting thrombocytopenia flared two days after COVID-19 vaccination [27]. Thrombocytopenia is very rare following Pfizer and Moderna SARS-CoV-2 vaccination [28]. In at least 5 countries, at least 13 patients (ages 20 to 50) have symptoms related to widespread blood clots, low platelet counts, and internal bleeding; seven of these patients have died [29,30]. An association of unusual thrombotic events and thrombocytopenia with autoantibodies targeting platelet factor 4 (PF4) has been advanced in association with the AstraZeneca (AZD1222) ChAdOx1 SARS-CoV-2 vaccine encoding the Spike protein with the suggested name of vaccine induced pro-thrombotic immune thrombocytopenia (VIPIT) [9,31,32]. A 73% mortality rate was observed for COVID-19 VIPIT patients with intracranial hemorrhage and platelet counts below 30,000 per cubic millimeter [33]. VIPIT is also been reported in association with both mRNA COVID-19 Spike vaccines [34]. PF4, also called CXCL4, is a tetrameric chemokine stored in platelet alpha-granules. Activated platelets release PF4 which binds polyanions with high affinity [35] and surface proteoglycans. PF4 plays an important role in heparin-induced thrombocytopenia (HIT) [36,37]. For COVID-19 adenovirus vaccines, activation of platelets is possible by adenovirus [38]. Greinacher et al. recommend non-heparin anticoagulants and proposed evaluation of high-dose immunoglobulin as possible treatments [9].

Herein, I propose four models for hemophagocytic histiocytes contributions to the etiology of thrombocytopenia associated with SARS-CoV-2.

1. The first model proposes that histiocytes are targeting platelets with ACE2 externally bound virions (Fig. 1-A).
2. The second model proposes that histiocytes are targeting platelets expressing viral proteins (Fig. 1-B).
3. A striking loss of germinal centers in lymph nodes and spleen was observed in COVID-19 patients [39]; this may contribute to expansion of possible autoantibodies that bind platelets. The third model proposes that hemophagocytic histiocytes target platelets bound by autoantibodies (Fig. 1-C). Thrombocytopenia associated with PF4-autoantibodies is consistent with this model (Fig. 1-C).
4. Most monocytes and macrophages do not express the ACE2 protein. I previously proposed expanded cellular tropism to phagocytic cells binding SARS-CoV-2 with Fc receptors [40]. This has been observed in COVID-19 patients with infected macrophages [41,42] and monocytes [43]. SARS-CoV-2-infected cells express the Spike protein on their surface [15]. The fourth model proposes that infected monocytes and macrophages express Spike proteins on their surface and target platelets by binding ACE2 to become hemophagocytic histiocytes (Fig. 1-D).

These proposed models can be evaluated to determine thrombocytopenia pathogenesis in COVID-19 patients. To test the first model, isolated hemophagocytic histiocytes can be evaluated for histiocyte phagocytosis of platelets alone (little or no phagocytosis of platelets) compared to platelets mixed with SARS-CoV-2 virions (phagocytosis of platelets with SARS-CoV-2 virions binding to platelet ACE2 receptors); essentially, comparing histiocyte phagocytosis of COVID-19 patient platelets compared to platelets from non-COVID-19 controls. An

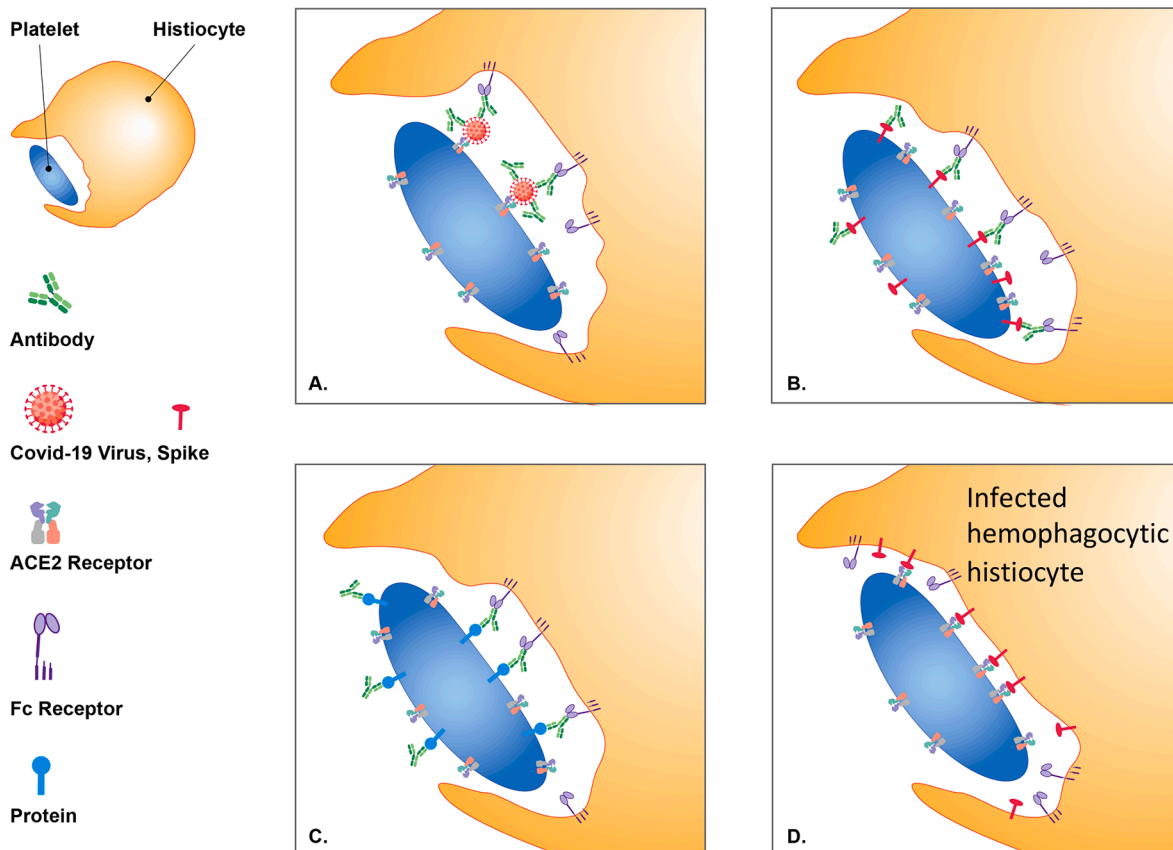


Fig. 1. Thrombocytopenia Models (A) Hemophagocytic histiocytes engulfing platelets with SARS-CoV-2 viruses bound to ACE2 receptors by Fc receptor binding to SARS-CoV-2 antibodies; (B) Hemophagocytic histiocytes engulfing platelets with surface expressed SARS-CoV-2 proteins by Fc receptor binding to SARS-CoV-2 antibodies; (C) Hemophagocytic histiocytes engulfing platelets by Fc receptor binding to platelet bound autoantibodies (amplified in COVID-19); and (D) Hemophagocytic histiocytes engulfing platelets by surface expressed Spike proteins binding to platelet ACE2 receptors with either SARS-CoV-2 infection of hemophagocytic histiocytes or vaccine encoded Spike proteins.

antibody to the Spike protein may be sufficient to evaluate the second model for platelets expressing viral proteins. Enzyme-linked immunosorbent assays (ELISA) [9,31] or an antibody to the Fc or other constant region of antibodies could detect autoantibodies binding platelets to evaluate the third model. The fourth model can be evaluated with electron microscope image analysis of hemophagocytic histiocytes for coronavirus-like particles [13,44]. Blocking the binding of Spike with ACE2 may provide therapeutic benefits against model 1 (blocking platelet ACE2 binding virion) and model 4 (blocking platelet ACE2 binding histiocyte expressed Spike); the ability to block at therapeutic dosages is unknown. Intravenous gamma globulin (IVIG) may provide therapeutic benefits against models 1, 2, and 3 [9]; essentially diluting Fc γ receptor mediated binding of antibodies and Fc γ receptor mediated activation of platelets observed in VIPIT [9].

Summary

Four etiology models for hemophagocytic histiocytes contributions to thrombocytopenia associated with SARS-CoV-2 are proposed. In addition, one of these models is consistent with hemophagocytic histiocytes contributing to VIPIT associated with SARS-CoV-2 adenoviral vaccines AZD1222 ChAdOx1 and Ad26.CoV2.S (Janssen/Johnson & Johnson).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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