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PERSPECTIVE

Thrombotic thrombocytopenia associated with COVID-19 infection or vaccination: Possible paths to platelet factor 4 autoimmunity

Michel Goldman [1]*, **Cédric Hermans** [2]

1 Institute for Interdisciplinary Innovation in Healthcare, Université libre de Bruxelles (ULB), Brussels, Belgium, **2** Division of Hematology, Hemostasis and Thrombosis Unit, Saint-Luc University Hospital, Université catholique de Louvain (UCLouvain), Brussels, Belgium
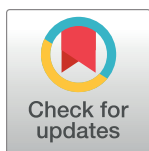
* mgoldman@i3health.eu

Introduction

Thrombotic thrombocytopenia mimicking heparin-induced thrombocytopenia has been observed in patients with severe Coronavirus Disease 2019 (COVID-19) or after immunisation with adenoviral vector-based vaccines against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Herein, we discuss the pathogenesis of the autoimmune response to platelet factor 4 (PF4) that underlies these disorders.

There is convincing evidence that autoimmunity is involved in the pathogenesis of COVID-19 [1,2]. Regarding the severe forms of the disease in which thromboinflammation is prominent, both endothelial cells and platelets might be affected by autoimmune reactions in addition to direct viral infection and cytokine-mediated activation [3,4]. Firstly, multiple anti-phospholipid antibodies have been detected in the blood of hospitalized patients in relation with the severity of the disease and the formation of neutrophil extracellular traps known to contribute to thrombotic events [5]. A recent study further established that among anti-phospholipid autoantibodies detected in COVID-19 patients, immunoglobulin G (IgG) to cardiolipin and phosphatidylserine/prothrombin might be the ones driving endothelial cell activation [6]. In addition, anti-annexin A2 autoantibodies found in critically ill patients were suggested to contribute to small vessel damage in the lungs [7].

Besides endothelial cell damage, activation of platelets is the other cornerstone of the pro-thrombotic state characteristic of COVID-19 [4]. Several factors are involved including mitochondrial disturbances caused by hypoxia, mediators of inflammation, and other stressors, leading to platelet hyperactivation and apoptosis [4,8]. Furthermore, infection of platelets by the SARS-CoV-2 virus might also contribute to their activation via angiotensin converting enzyme 2 (ACE2)-dependent [9] as well as non-ACE2 mechanisms involving heparan sulfate [10] or CD147 [11]. Following viral entry, SARS-CoV-2 ssRNA might trigger intracellular Toll-like receptor 7–dependent activation pathways as in the case of influenza infection [12]. Antibody-mediated mechanisms involving engagement of the FcγRIIA receptor on platelets were also shown to contribute to procoagulant activity in severe COVID-19 [13,14]. Although the antigenic specificity of these antibodies could not always be defined, antibodies to PF4 were shown to be involved in certain cases [15–22].

PF4, also called CXCL4, is a tetrameric chemokine stored in platelet alpha-granules [23]. Upon platelet activation, PF4 is released and binds polyanions with high affinity [24]. Indeed, PF4 was shown to play a critical role in heparin-induced thrombocytopenia [25]. Below, we

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Abbreviations: ACE2, angiotensin converting enzyme 2; COVID-19, Coronavirus Disease 2019; IgG, immunoglobulin G; PF4, platelet factor 4; PG, proteoglycan; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

summarize the key features of heparin-induced thrombocytopenia before proposing that COVID-19 and adenovirus-vectored COVID-19 vaccines can on rare occasions cause autoimmune thrombotic thrombocytopenia mimicking heparin-induced thrombocytopenia.

PF4 autoimmunity in heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia is a severe prothrombotic condition that occurs in less than 5% of patients receiving heparin. Anti-PF4 antibodies are key biomarkers of heparin-induced thrombocytopenia [25]. They recognize an epitope exposed on PF4 tetramers upon conformational changes induced by their interaction with heparin or other long polyanions [26]. Indeed, injection of heparin has been shown to induce the release of PF4 [27], resulting in the assembly of PF4/heparin complexes, which activate complement and bind circulating B lymphocytes in a complement-dependent manner [28]. B cells responsible for the synthesis of PF4 autoantibodies display unique characteristics that enable them to rapidly mount an IgG response following a first exposure to heparin [29]. Indeed, B cells, which are able to produce anti-PF4 antibodies, are present in healthy individuals but in an anergic state that normally prevents their activation. This B cell tolerance might be broken upon heparin exposure and under some inflammatory conditions [30]. In these situations, anti-PF4 IgG antibodies elicit thrombus formation and thrombocytopenia via multiple mechanisms. Immune complexes assembled with PF4 bound to heparin induce platelet activation and aggregation by cross-linking FcγRIIA receptors [25]. Anti-PF4 antibodies also activate the procoagulant activity of monocytes by cross-linking their FcγRI receptors and of endothelial cells via the recognition of PF4 firmly attached to surface proteoglycans (PGs) [31]. Thrombocytopenia results from enhanced apoptosis and clearance of antibody-coated platelets in addition to consumption in the coagulation process [8].

A prothrombotic syndrome with all the features of heparin-induced thrombocytopenia has been reported in the absence of heparin exposure [32]. These observations led to the definition of a so-called "spontaneous heparin-induced thrombocytopenia" caused by anti-PF4 autoantibodies elicited by polyanions reproducing the conformational changes induced in PF4 tetramers by heparin [33]. Potential polyanions triggering "spontaneous heparin-induced thrombocytopenia" include bacterial wall components, nucleic acid materials, or endogenous PGs released by damaged cells.

Thrombotic thrombocytopenia during COVID-19: An autoimmune reaction induced by SARS-CoV-2?

The high incidence of thrombotic and thromboembolic events during severe COVID-19 results in the frequent administration of heparin in affected patients [34]. Thrombosis can develop in unusual locations such as cerebral venous sinuses [35]. When thrombocytopenia develops in this setting, heparin-induced thrombocytopenia must be considered as a possible cause [18]. Indeed, several studies report the presence of anti-PF4/heparin antibodies in COVID-19 patients. However, these antibodies sometimes occur in absence of heparin administration [18]. Furthermore, they do not always activate platelets in presence of heparin/PF4 complexes [36], although they do so in presence of PF4 alone [14], suggesting that they were induced by another mechanism than classical heparin-induced thrombocytopenia [26]. There is indeed clinico-biological evidence that infection with SARS-CoV-2 by itself can elicit antibody-mediated thrombotic thrombocytopenia. IgG antibodies present in the serum of severe COVID-19 patients were found to induce platelet apoptosis and procoagulant activity via FcγRIIA receptor-dependent mechanisms [13]. The antigenic specificity of these antibodies was not defined, but one can speculate that at least some of them are directed against PF4.
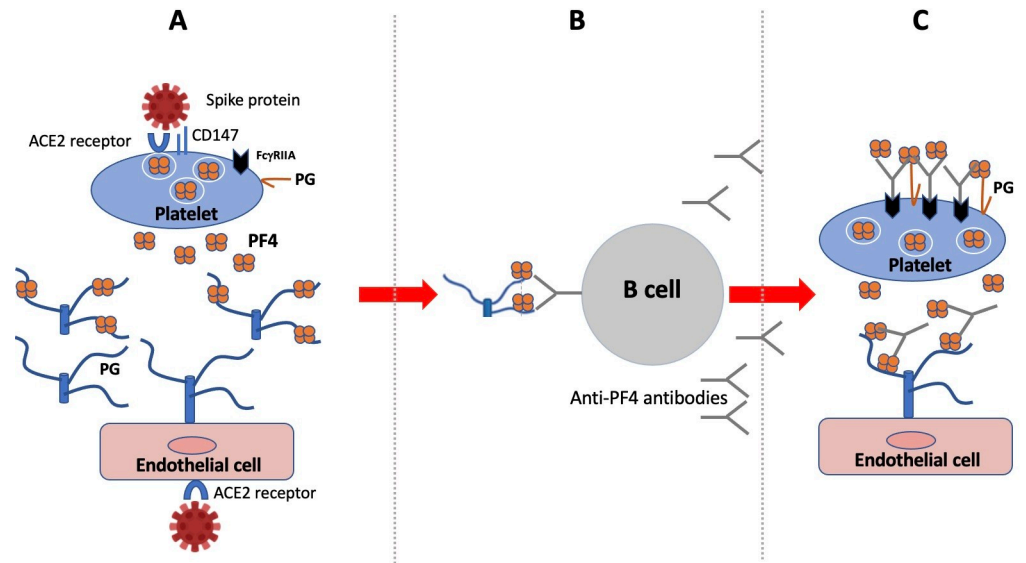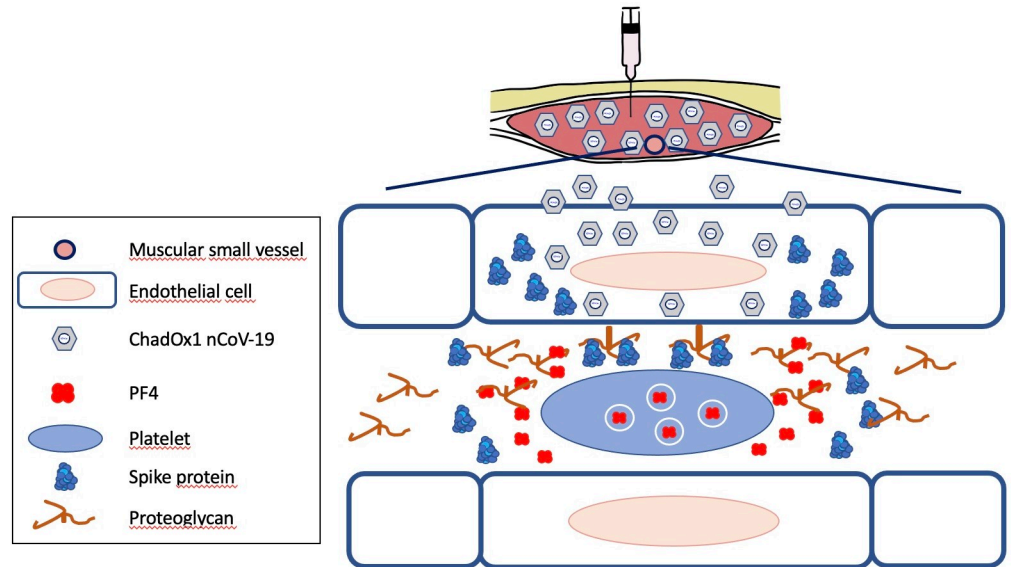
Fig 1. Hypothetical model for thrombotic thrombocytopenia during COVID-19. (A) SARS-CoV-2 induces the release of PF4 by activated platelets and of polyanionic PG by endothelial cells (e.g., syndecan and endocan). (B) Complexes of PF4 and PG expose PF4 immunogenic epitopes, which activate extrafollicular B lymphocytes secreting PF4 autoantibodies. (C) PF4 autoantibodies bind complexes of PF4 and PG on platelets and endothelial cells and stimulate their procoagulant activities. Cross-linking of FcγRIIA receptors also promote apoptosis and clearance of antibody-decorated platelets. COVID-19, Coronavirus Disease 2019; PF4, platelet factor 4; PG, proteoglycan; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

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The model that we are proposing in Fig 1 is first based on the hyperactivation of platelets during COVID-19, resulting in the release of PF4 in the circulation [37]. Circulating PF4 could form complexes with endogenous polyanionic PGs released by damaged endothelial cells. Syndecan-1 and endocan are potential PG candidates since their serum levels are increased in severely ill COVID-19 patients in association with other markers of endothelial injury [38–40]. Complexes formed between PF4 and endothelial cell-derived polyanionic PG would then stimulate extrafollicular B cells producing anti-PF4 antibodies. Indeed, autoimmune responses elicited by extrafollicular B cells were previously suggested to be involved in the pathophysiology of severe COVID-19 [41]. Anti-PF4 antibodies would then recapitulate the sequence of events responsible for heparin-induced thrombocytopenia. Besides anti-PF4 autoantibodies, anti-phospholipid antibodies could also contribute to platelet activation as well as antiviral antibodies as observed in other infections [42,43].

Thrombotic thrombocytopenia following COVID-19 vaccination

Several observations of prothrombotic thrombocytopenic events following vaccination with the adenovirus-vectored vaccine ChAdOx1 nCoV-19 vaccine (Vaxzevria, Oxford/AstraZeneca) were reported in European countries [44–46]. The incidence of these events is very low (around 1 in 100,000 recipients) but still significant by comparison with the background rate. As the clinical presentation is often reminiscent of heparin-induced thrombocytopenia, the hypothesis of a vaccine-induced autoimmune response to PF4 was put forward. Indeed, Greinacher and colleagues, Schultz and colleagues, and Scully and colleagues reported the detection of platelet-activating anti-PF4 antibodies in sera of patients suffering from unusual thrombotic events associated with thrombocytopenia within 4 to 16 days after injection of the ChAdOx1 nCoV-19 vaccine [44–46]. Shortly after these observations, 17 cases of

Fig 2. Hypothetical model for thrombotic thrombocytopenia after adenoviral vector-based COVID-19 vaccines.
After intramuscular injection, vaccine adenoviruses infect endothelial cells, inducing their production of the SARS-CoV-2 Spike protein. Heparan sulfate PG could bind the spike protein on the luminal side of endothelial cells or be released by damaged cells. Spike proteins would activate platelets via ACE2-dependent and ACE2-independent mechanisms. PF4 released by activated platelets would become immunogenic after binding heparan sulfate PG shed from endothelial cells. ACE2, angiotensin converting enzyme 2; COVID-19, Coronavirus Disease 2019; PF4, platelet factor 4; PG, proteoglycan; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

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thrombocytopenic thromboses affecting cerebral venous sinuses were reported in the United States after administration of the Ad26.COV2.S vaccine (Janssen/Johnson & Johnson), another adenoviral vector-based COVID-19 vaccine [47]. Strikingly, serum anti-PF4 antibodies were present in the 11 patients in whom they were searched for [47]. So far, there is no evidence for an increased incidence of similar events after administration of mRNA vaccines, suggesting a role for the adenoviral vectors in the induction of the anti-PF4 autoimmune response.

Indeed, Greinacher and colleagues recently reported that ChAdOx1 nCoV-19 vaccine-induced anti-PF4 antibodies do not cross-react with the SARS-CoV-2 spike protein, excluding a phenomenon of molecular mimicry between the viral protein and PF4 [48]. The same group formulated several hypotheses about the vaccine components that could be involved, including adenovirus-derived substances [49]. As adenoviruses are known to activate platelets [50], it is plausible that the replication-deficient adenoviral vector could be directly responsible for the release of platelet-derived PF4. However, this hypothesis implies that significant amounts of vaccine particles would reach the bloodstream after intramuscular injection, which seems unlikely. An alternative scenario depicted in Fig 2 would involve endothelial cells. Indeed, endothelial cells are efficiently transduced upon intramuscular injection [51]. Transduced endothelial cells might be directly damaged by the spike protein that they synthesize, as suggested by in vitro and in vivo observations [52,53]. Furthermore, endothelial cells might expose the spike protein on their luminal side, possibly bound to PG of the glycocalyx as heparan sulfate PGs were shown to be attachment factors for the spike protein [54]. Platelets might then be recruited and activated by the spike protein bound to endothelial cells [9]. PF4 released by activated platelets could combine with anionic PGs shed from endothelial cells. In such a scenario, both the adenovirus and the spike protein would contribute to the formation of immunogenic PF4 following vaccination with adenoviral vector-based COVID-19 vaccines.

Concluding remarks

Autoantibodies to PF4 contribute to thrombotic thrombocytopenia, which occasionally occurs during COVID-19 or after vaccination with adenoviral vector-based vaccines against SARS-CoV-2. We propose that heparan sulfate PG shed from damaged endothelial cells contribute to making PF4 immunogenic. As far as postvaccine thrombotic events are concerned, it will be important to specify the role of the adenoviral vector in view of the current developments of other vaccines based on the same technology. Finally, further research is needed to identify the risk factors, which predispose rare individuals to these severe complications.

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