


PERSPECTIVE

Vaccine-induced immune thrombosis and thrombocytopenia syndrome following adenovirus-vectored severe acute respiratory syndrome coronavirus 2 vaccination: a novel hypothesis regarding mechanisms and implications for future vaccine development

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Keywords

Hypothesis, SARS-CoV-2 vaccines, thrombocytopenia, thrombosis

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Received 9 August 2021;

Revised 4 October 2021;

Accepted 4 October 2021

doi: 10.1111/imcb.12505

Immunology & Cell Biology 2021; **99**:
 1006–1010

Abstract

We hypothesize that thrombosis with thrombocytopenia syndrome recently described after administration of adenovirus-vectored vaccines for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) occurs as a result of the unique properties of the adenovirus vectors, which can have widespread biodistribution throughout the body. The antigen is delivered to megakaryocyte cells, which act as part of the primary immune system and distribute the antigen within progeny platelets, also a key component of the immune system. The interaction of the antigen induces preformed antiplatelet factor 4 (PF4) antibodies to bind to PF4–heparan sulfate complexes in the absence of exogenous heparin, at sites where the heparan sulfate concentration in the vascular glycocalyx is optimal for complex formation, causing thrombosis and thrombocytopenia as observed clinically. This hypothesis is testable in cell culture and animal models, and potentially *in vivo*, and if proven correct has significant implications for vaccine development and our understanding of the links between the coagulation and immune systems.

The coronavirus disease 2019 (COVID-19) pandemic presents the greatest global health challenge in over 100 years with more than 180 million infections, 4 million deaths and devastating health, societal and economic impacts. Public health nonpharmacological interventions helped contain viral transmission. The rapid development of highly effective vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was largely based on the emergence of two new technologies: messenger RNA (mRNA) and recombinant adenovirus–vectored vaccines. These vaccines have led to a large-scale reduction

in the incidence of infection and the associated impact of the COVID-19 disease. In recent months, the rare but significant complication of thrombosis thrombocytopenia syndrome (TTS) or vaccine-induced immune thrombosis and thrombocytopenia has almost exclusively been reported following the administration of adenovirus-based vaccines of AstraZeneca (Vaxzevria), and to a lesser extent, the Janssen COVID-19 vaccine.^{1–3} There is only a single case report of potential TTS/vaccine-induced immune thrombosis and thrombocytopenia following an mRNA vaccine, although the patient may have in fact had

heparin-induced thrombocytopenia (HIT).⁴ Interestingly, TTS/vaccine-induced immune thrombosis and thrombocytopenia cases have a pathophysiology similar to that of autoimmune HIT, with most cases being associated with positive antiplatelet factor 4 (PF4) in ELISAs or functional HIT testing.⁵ The majority of papers considering the pathophysiology of this entity have considered the downstream antibody interactions.^{6,7} We propose a hypothesis that accounts for a number of features of this rare and novel thrombotic complication that, if proven correct, may have significant implications for other postvaccine autoimmune diseases and future vaccine development strategies.

ADENOVIRUS-VECTORED VACCINES

Replication-incompetent human adenoviruses, especially serotype 5 (HAdV-C5), have been extensively used in research as delivery vehicles for engineered genomic materials both *in vitro* and *in vivo*.⁸ However, the utility of these vectors as potential vaccines has been limited because of pre-existing immunity within human populations that may significantly reduce the immunogenicity and subsequent efficacy,⁹ although this has not been problematic for adenovirus-vectored COVID vaccines.¹⁰ Pre-existing immunity to adenoviruses varies widely across geographically distinct populations related to the prevalence of community adenovirus infections. Simian adenovirus vectors can circumvent pre-existing immunity to human adenoviruses.¹¹ Conceptually, the aim of vaccination is to trigger recognition and uptake of antigen into local draining lymph nodes from the injection site. However, adenovirus vectors are known to have more extensive biodistribution to multiple organs including the liver, spleen, lung and bone marrow,¹² a feature that is theoretically likely to be higher using adenoviral vectors against which pre-existing immunity does not exist.

Currently, there are four adenovirus-vectored vaccines that have been developed against SARS-CoV-2, all of which encode the spike glycoprotein of SARS-CoV-2:

- 1 Recombinant chimpanzee adenoviral (ChAdOx1-S) vector, Oxford-AstraZeneca's [ChAdOx1/AZD1222 COVID-19 (Vaxzevria)]
- 2 Recombinant human adenovirus type 26 vector, Johnson & Johnson's INJ-7843735/Ad26.COV2.s (Janssen)
- 3 Recombinant human adenovirus type 26 and type 5, Gamelaya—Sputnik V (GAM COVID vaccine)
- 4 Recombinant human adenovirus type 5 (AD5-nCOV) (CanSino Convidecia)

Based on the prevalence of pre-existing immunity to vector type, one would predict that the biodistribution

for ChAdOx1-S would be the most extensive followed by Ad26.COV2.S. Lack of immunity against the vector, which may improve effectiveness,^{9,11} likely increases the biodistribution beyond the injection site. The frequency of TTS observed with each vaccine may reflect the varying biodistribution. Pre-existing immunity may also underlie the dramatic difference in TTS observed after the first dose of ChAdOx1-S (approximately 1 in 50 000 doses) compared with the second dose (approximately 1 in 600 000–700 000 doses)^{13,14} if immunity to the vector develops after the first dose. Thus, the first concept in our hypothesis is that TTS (and potentially other autoimmune complications) may be intimately linked to the systemic biodistribution of vaccine beyond the lymphoid drainage of the immediate injection site.

PLATELETS AND MEGAKARYOCYTES AS IMMUNE CELLS

Blood coagulation is primarily conceptualized as a hemostatic process to stop vascular bleeding. However, clotting is a component of innate immunity that developed earlier than the adaptive immune system from an evolutionary perspective.¹⁵ Renewed interest in this concept for platelets and megakaryocytes is specifically driven by their pathological roles in immune-related thrombotic mechanisms.¹⁶

Megakaryocytes and their progeny platelets maintain significant immune functioning capacity, including the capacity for viral antigen presentation and defence.^{17,18} Both express receptors that confer immune sensing including Toll-like receptors, Fc γ receptors and CD40 ligand, and have the ability to migrate toward chemotactic stimuli.¹⁸

Megakaryocytes regulate proliferation of hematopoietic cells, facilitate neutrophil exit from marrow¹⁹ and possess the capacity to cross-present antigen and promote systemic inflammation through microparticles rich in interleukin-1. Megakaryocytes directly respond to viral infections by secreting interferons and upregulating IFITM3.¹⁸ When positioned close to marrow sinusoids, megakaryocytes monitor blood-borne pathogen entry to bone marrow.¹⁷ Megakaryocytes can also egress directly into the circulation. In human venous blood, megakaryocytes appear at a concentration of 110 cells mL⁻¹ with most of the blood-borne megakaryocytes migrating to the lungs, approximately 100 000 to over 1 000 000 megakaryocytes per hour, suggesting a potential immunological role for megakaryocytes in monitoring pathogen entry via the lungs.¹⁷

Platelets, the anucleate derivatives of megakaryocytes, possess a broad array of receptors including Toll-like receptors, a key component of innate immune cells, as

well as interact with other immune cells including dendritic cells, lymphocytes and myeloid leukocytes.¹⁶ Prior studies consider platelets as a single population; however, more recent refined analyses have characterized differences in circulating platelets with respect to their (1) size, (2) surface receptor expression, (3) glycosylation, (4) granule content, (5) response to agonist stimulation and (6) participation in thrombus formation.²⁰ Heterogeneity in circulating platelets may correspond to distinct platelet subpopulations with specialized functions, similar to the dedicated roles of subsets of immune cell.²⁰

The second concept in our hypothesis is that megakaryocytes and progeny platelets may have a significant immunologic role.

PATHOPHYSIOLOGY OF HIT

The pathophysiological basis of HIT is the formation of an immunocomplex consisting of an auto-antibody against PF4, which binds to the surface of platelets and monocytes, provoking activation by cross-linking Fcγ receptors. PF4 is not immunogenic in its primary form.²¹ However, when PF4 is complexed with negatively charged molecules, especially heparin and other glycosaminoglycans (GAGs), conformational changes expose a neo-antigen.²¹ The size

and the charge of the PF4–heparin complexes play a central role in the pathogenicity of HIT, which depends on the relative amounts of PF4 and heparin.²¹

Glycosaminoglycans including heparin sulfate comprise a major part of vascular endothelial antithrombotic activity, and vary in amount in different vascular beds.²² Thus, the third component of the hypothesis is that, in the absence of exogenous heparin, the site of thrombosis relates to the distribution of endogenous heparin sulfate in vascular beds to maximize complex formation.

THE HYPOTHESIS

We hypothesize that adenovirus-based COVID-19 vaccines, with biodistribution beyond local lymphoid tissues, have the potential to transduce megakaryocytes, leading to SARS-CoV-2 S protein expression within megakaryocytes and their platelet progeny. In a proportion of patients this may lead to substantial, sustained production of platelets expressing S protein, which are destroyed as part of the acquired immune response, resulting in immune thrombocytopenia (vaccine-induced immune thrombocytopenic purpura). Thus, postinfectious or postvaccine immune thrombocytopenic purpura may not be related to molecular mimicry *per se*, as usually suggested,²³ but rather to direct expression of viral

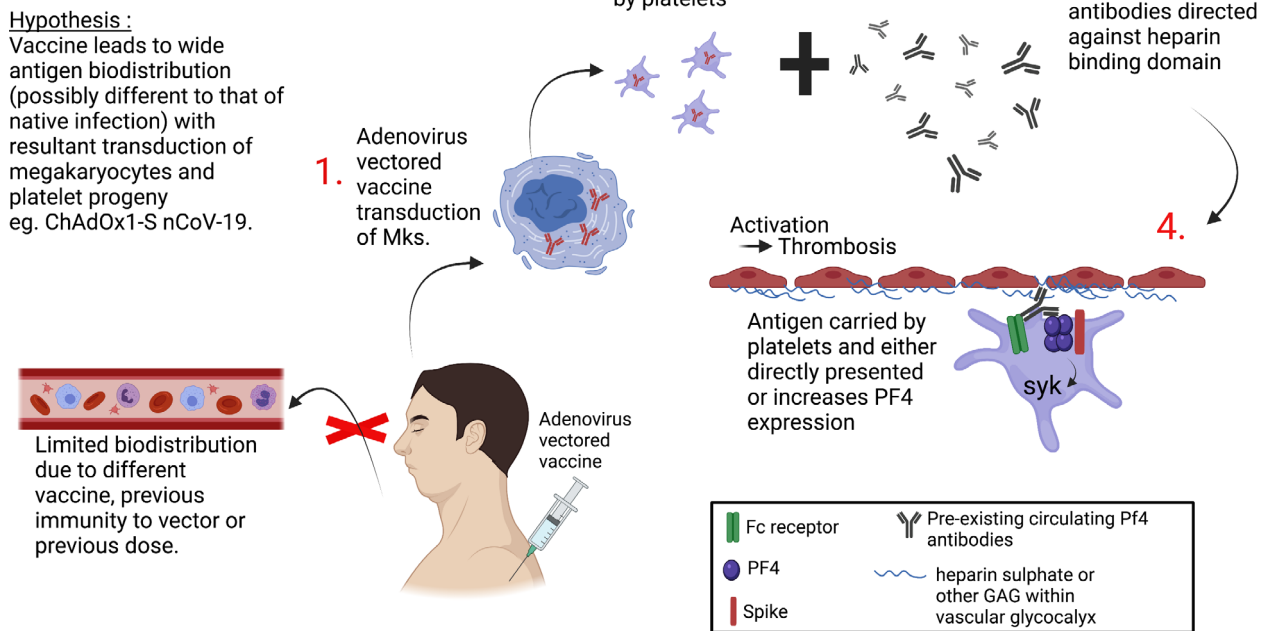


Figure 1. Hypothesis regarding the pathophysiology of thrombosis with thrombocytopenia syndrome (TTS) or vaccine-induced thrombosis and thrombocytopenia (VITT). GAG, glycosaminoglycan; MKs, megakaryocytes; PF4, platelet factor 4; spike, SARS-CoV-2 spike protein with trimeric heparin-binding domains; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; syk, any of the pathways to platelet activation by FcγRIIA including Src, Syk and Btk kinases.

protein by megakaryocytes/megakaryocyte progenitor cells, and platelet progeny.

For the phenomenon of TTS/vaccine-induced immune thrombosis and thrombocytopenia, we postulate that the incorporation of vaccine-encoded spike protein into circulating platelets leads to an activating antibody response that drives thrombosis as well as thrombocytopenia. This may occur in susceptible individuals immunologically primed to produce PF4 antibodies.¹⁸ Whether detection using anti-PF4 (HIT-like antibodies directed against the heparin-binding domain of PF4²⁴) reflects colocalization of the spike with PF4 within the platelet, exposing the HIT neoepitope, or whether the antibody binding the SARS-CoV-2 spike activates the Fcγ receptors leading to activation and release of large amounts of PF4, thereby precipitating a secondary HIT-like phenomenon, warrants investigation.

The site of thrombosis is determined by the distribution of endogenous heparin sulfate within the vascular tree, either venous or arterial (Figure 1).

In terms of proving this hypothesis, initial adenovirus-vectored transduction of megakaryocytes in cell culture could demonstrate the production of the spike protein in megakaryocytes and their progeny platelet-like particles.¹⁹ The relationship with PF4 in the platelet-like particles could likewise be determined. An animal model could confirm the process *in vivo*, including the distribution of the vector and antigen after intramuscular injection, although a key factor will be the proportion of transduced megakaryocyte cells. Studies in affected humans will likely be very difficult, as the transduced megakaryocytes and hence affected platelet population may be a small subpopulation of the overall platelet mass, and platelets positive to PF4 antibodies may represent the initial activation of a subsequent cascading process.

Conceptually, if this hypothesis is proven for adenovirus-vectored vaccines against SARS-CoV-2, there are potential implications for the future development of vaccines, with more emphasis on targeting the vaccine to the specific immune system, to avoid such complications. The delivery mechanism may lead to a biodistribution of antigen that is not usually observed in native infection. The possibility that a similar mechanism of antigen uptake by off-target cells can lead to autoimmune consequences in other organs or cells would also require further consideration and assessment in preclinical studies.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Paul Monagle: Conceptualization; Writing-original draft. **Ashley Ng:** Conceptualization; Writing-review & editing. **Matthew Linden:** Validation; Visualization; Writing-review & editing. **Vera Ignjatovic:** Validation; Writing-review & editing. **Alison Farley:** Validation; Visualization; Writing-review & editing. **Samir Taoudi:** Validation; Writing-review & editing. **Sant Rayn Pasricha:** Validation; Writing-review & editing. **Joseph Torresi:** Conceptualization; Writing-review & editing.

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