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PERSPECTIVE

Vaccine Induced Immune Thrombosis and thrombocytopenia (VITT) Syndrome following adenovirus vectored SARS-CoV 2 vaccination: a novel hypothesis regarding mechanisms and implications for future vaccine development

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Abstract

We hypothesize that thrombosis with thrombocytopenia syndrome recently described after adenovirus vectored vaccines for Sars CoV 2 occurs due to the unique properties of the adenovirus vectors, which can have widespread bio distribution 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 anti PF4 antibodies to bind to PF4 - heparan sulphate complexes in the absence of exogenous heparin, at sites where the heparan sulphate 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 COVID-19 pandemic presents the greatest global health challenge in over 100 years with over 180 million infections, 4 million deaths and devastating health, societal and economic impact. Public health non pharmacological interventions helped contain viral transmission. The rapid development of highly effective vaccines against SARS-CoV 2 was largely based on emergence of two new technologies, namely

mRNA and recombinant adenovirus vectored vaccines. These vaccines have led to large-scale reduction in the incidence of infection and the associated impact of COVID 19 disease. In recent months, the rare but significant complication of Thrombosis Thrombocytopenia Syndrome (TTS) or Vaccine induced Immune thrombosis and thrombocytopenia (VITT) has almost exclusively been reported following administration of adenovirus-based vaccines of AstraZeneca [Vaxzevria] and to a lesser extent the Janssen Covid-19 Vaccine [1-3]. To date, there is only a single case report of potential TTS/VITT following an mRNA vaccine, although the patient may have in fact had heparin induced thrombocytopenia[4]. Interestingly, TTS/VITT cases have a pathophysiology similar to autoimmune heparin induced thrombocytopenia (HIT), with most cases associated with positive anti platelet factor 4 (PF4) ELISA assays 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 post-vaccine 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 material both *in vitro* and *in vivo*[8]. However, the utility of these vectors as potential vaccines has been limited due to 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 node groups from the injection site. However, adenovirus vectors are known to have more extensive biodistribution to multiple organs including 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.

There are four current adenoviral 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 Vac)
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 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 to the second dose (approximately 1 in 600,000–700,000 doses)[13,14] if immunity to the vector develops after first dose. Thus, the first concept in our hypothesis is that TTS (and potentially other autoimmune complications) may be intimately linked to the systemic bio-distribution of vaccine beyond the immediate injection site lymphoid drainage.

Platelets and megakaryocytes as immune cells

Blood coagulation is primarily conceptualised as a haemostatic process to stop vascular bleeding. However, clotting forms 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 specifically is 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 towards chemotactic stimuli [18].

Megakaryocytes regulate proliferation of hematopoietic cells, facilitate neutrophil exit from marrow[19], 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 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 derivates 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 characterised differences in circulating platelets with respect to their (a) size, (b) surface receptor expression, (c) glycosylation, (d) granule content, (e) response to agonist stimulation, and (f) 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, conformational changes exposed when PF4 is complexed with negatively charged molecules, especially heparin and other glycosaminoglycans (GAGs), expose a neo-antigen[21]. The size and the charge of the PF4 heparin complexes play a central role in pathogenicity of HIT, and depends on the relative amounts of PF4 and heparin[21].

Glycosaminoglycans including heparin sulphate 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 sulphate in vascular beds to maximise complex formation.

The hypothesis

We hypothesize that adenoviral based COVID 19 vaccines, with biodistribution beyond local lymphoid tissues, have the potential to transduce megakaryocytes leading to SARS-CoV2 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 thrombocytopaenia (vaccine induced ITP). Thus post infectious or post vaccine ITP may not be related to molecular mimicry *per se,* as usually suggested[23], but due to direct expression of viral protein by megakaryocytes/ megakaryocyte progenitor cells, and platelet progeny.

For the phenomenon of TTS/VITTS, 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 heparin binding domain of PF4[24]) reflects co-location of the spike with PF4 within the platelet, exposing the HIT neo epitope, or whether antibody binding the SARS-CoV2 spike activates the Fc γ receptors leading to activation and release of large amounts of PF4, precipitating a secondary HIT like phenomenon warrants investigation.

The site of thrombosis is determined by the distribution of endogenous heparin sulphate within the vascular tree, either venous or arterial (see 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 (PLPs)[19]. The relationship with PF4 in the PLPs 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 adenoviral 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 pre-clinical studies.

Author contributions

PM and JT conceived the hypothesis. AN, VI, ML, AF, ST, SRP assisted in refining the hypothesis and defining potential mechanisms to prove or disprove. PM drafted the manuscript and all authors reviewed and contributed to the manuscript and have read and approved the submitted version. There are no conflicts of interest for any author. There is no funding source. Ethics approval is not relevant for a hypothesis.

REFERENCES

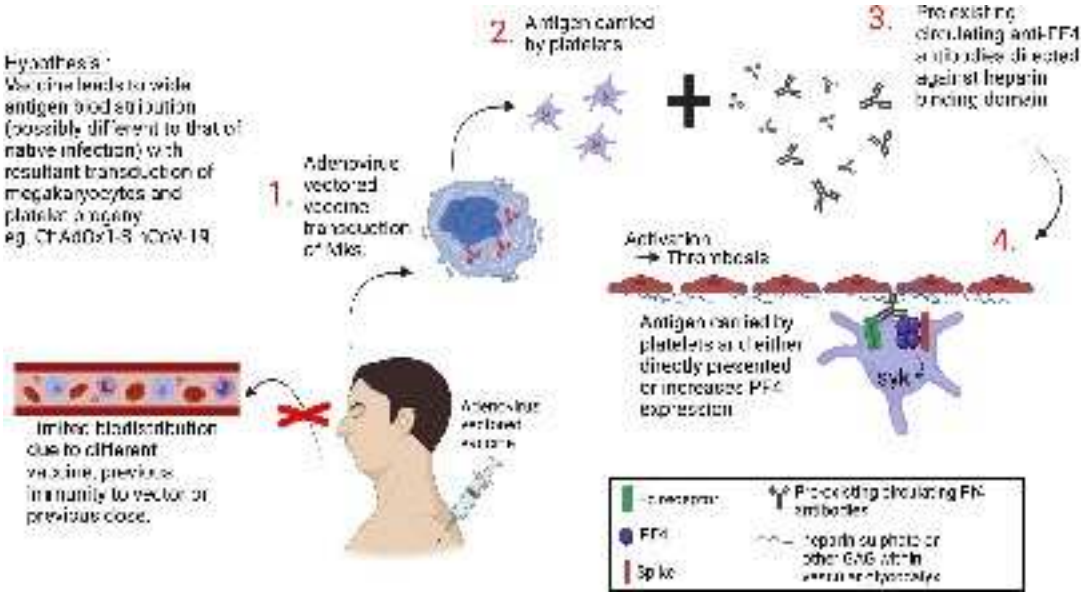
1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N Engl J Med* 2021; **384**: 2092-2101.
2. Schultz NH, Sørvoll IH, Michelsen AE, *et al*. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* 2021; **384**: 2124-2130.
3. Pavord S, Scully M, Hunt BJ, *et al*. Clinical Features of Vaccine-Induced Immune Thrombocytopenia and Thrombosis. *N Engl J Med* 2021. doi: 10.1056/NEJMoa2109908. Epub ahead of print.
4. Sangli S, Virani A, Cheronis N, *et al.* Thrombosis With Thrombocytopenia After the Messenger RNA-1273 Vaccine. *Ann Intern Med* 2021. doi: 10.7326/L21-0244. Epub ahead of print.
5. Vayne C, Rollin J, Gruel Y, *et al.* PF4 Immunoassays in Vaccine-Induced Thrombotic Thrombocytopenia. *N Engl J Med* 2021; doi: 10.1056/NEJMc2106383. Epub ahead of print.
6. Greinacher A, Selleng K, Palankar R, *et al*. Insights in ChAdOx1 nCov-19 Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT). *Blood* 2021: blood.2021013231. doi: 10.1182/blood.2021013231. Epub ahead of print.
7. Greinacher A, Selleng K, Mayerle J, *et al.* Anti-Platelet Factor 4 Antibodies Causing VITT do not Cross-React with SARS-CoV-2 Spike Protein. *Blood* 2021: blood.2021012938. doi: 10.1182/blood.2021012938. Epub ahead of print.

8. Daussy CF, Pied N, Wodrich H. Understanding Post Entry Sorting of Adenovirus Capsids; A Chance to Change Vaccine Vector Properties. *Viruses* 2021; **13**: 1221.
9. Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important? *Hum Vaccin Immunother* 2014; **10**: 2875-2884.
10. Zhu FC, Guan XH, Li YH, *et al.* Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2020; **396**: 479-488.
11. Morris SJ, Sebastian S, Spencer AJ, Gilbert SC. Simian adenoviruses as vaccine vectors. *Future Virol* 2016; **11**: 649-659.
12. Snook AE, Baybutt TR, Hyslop T, Waldman SA. Preclinical Evaluation of a Replication-Deficient Recombinant Adenovirus Serotype 5 Vaccine Expressing Guanylate Cyclase C and the PADRE T-helper Epitope. *Hum Gene Ther Methods* 2016; **27**: 238-250.
13. MHRA weekly yellow card reporting summary for week ending July 1 2021 https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting
14. Pottegård A, Lund LC, Karlstad Ø, *et al*. Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study. *BMJ* 2021; **373**: n1114.
15. Weyrich AS, Lindemann S, Zimmerman GA. The evolving role of platelets in inflammation. *J Thromb Haemost* 2003; **1**: 1897-1905.
16. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 2013; **13**: 34-45.
17. Cunin P, Nigrovic PA. Megakaryocytes as immune cells. *J Leukoc Biol* 2019; **105**: 1111-1121.
18. Campbell RA, Schwertz H, Hottz ED, *et al.* Human megakaryocytes possess intrinsic antiviral immunity through regulated induction of IFITM3. *Blood* 2019; **133**: 2013-2026.
19. Ng AP, Kauppi M, Metcalf D, *et al*. Mpl expression on megakaryocytes and platelets is dispensable for thrombopoiesis but essential to prevent myeloproliferation. *Proc Natl Acad Sci USA* 2014; **111**: 5884-5889.
20. Blair TA, Michelson AD, Frelinger AL 3rd. Mass Cytometry Reveals Distinct Platelet Subtypes in Healthy Subjects and Novel Alterations in Surface Glycoproteins in Glanzmann Thrombasthenia. *Sci Rep* 2018; **8**: 10300.

21. Marchetti M, Zermatten MG, Bertaggia Calderara D, Aliotta A, Alberio L. Heparin-Induced Thrombocytopenia: A Review of New Concepts in Pathogenesis, Diagnosis, and Management. *J Clin Med* 2021; **10**: 683. doi.org/10.3390/ jcm10040683
22. Liu HQ, Li J, Xuan CL, Ma HC. A review on the physiological and pathophysiological role of endothelial glycocalyx. *J Biochem Mol Toxicol* 2020; **34**: e22571.
23. Perricone C, Ceccarelli F, Nesher G, *et al*. Immune thrombocytopenic purpura (ITP) associated with vaccinations: a review of reported cases. *Immunol Res* 2014; **60**: 226-235.
24. Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopenia. *Nature* 2021. doi: 10.1038/s41586-021-03744-4. Epub ahead of print.

Figure caption:

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



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