Perspective

Proposed mechanism for thrombotic thrombocytopenia induced by adenovirus-based COVID-19 vaccines

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Abstract

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare and often deadly complication of adenovirus-based vaccination against SARS-CoV-2. It is caused by the appearance of autoreactive antibodies against platelet factor 4 (PF4) protein in platelets, leading to thrombosis and rapid platelet degradation. It is unkown how vaccination triggers the generation of highly autoreactive B cells. We propose that VITT arises when the adenovirus vectors come into contact with blood (e.g. when the syringe ruptures a blood vessel), and a B cell with week affinity for PF4 binds to and phagocytoses platelets to which high amounts of inocculated virus stick. The B-cell can get strong co-stimulatory signals from T_h cells recognizing viral antigens presented on its MHC-II complexes. By affinity maturation it will give rise to daughter cells with much stronger binding to PF4. We suggest measures for minimizing the risk for VITT.

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Background

Vaccination against Covid-19 with adenovirus vector-based vaccines ChAdOx1 nCoV-19 from Oxford-AstraZeneca and Ad26.COV2.S from Johnson & Johnson/Janssen has been associated to rare cases (10 in 1 million) of life-threatening cases of thrombotic thrombocytopenia.[1,2,3] Patients developed VITT around 5 to 20 days after vaccination, the mortality is around $30\% - 40\%$. The complication affects mostly young patients of median age 37. No statistical association of VITT with mRNA-based vaccinations against SARS-CoV-2 has been observed.[4,2]

Most tested patients had IgG antibodies against PF4, a cytokine protein expressed only in blood platelets, making antibodies that bind to and activate platelets the likely cause of the condition.[3,5] In contrast to the well-studied case of heparin-induced thrombocytopenia,[6] the antibodies bound to non-complexed PF4 alone, and binding affinity was even decreased in the presence of heparin.[3] Various hypotheses for VITT have been advanced,[7] but most cannot explain the often quite short time for symptoms to appear nor why autoimmunity is directed mostly against PF4.[8,9]

The role of PF4 and weak anti-PF4 autoantibodies in innate immunity

Healthy mice and humans of all ages possess preexisting PF4-specific IgM^+ B cells.[10] This might be an evolutionary adaptation linked to a function of PF4 besides promoting blood coagulation: its role in innate immunity. The negative surface charge that many pathogens posses is a reliable indicator of non-self. Positively charged PF4 binds to these polyanionic surfaces. The anti-PF4 IgM antibodies are too weak to bind scattered PF4, but when the PF4 density on the pathogen's surface is sufficient, cooperative interactions

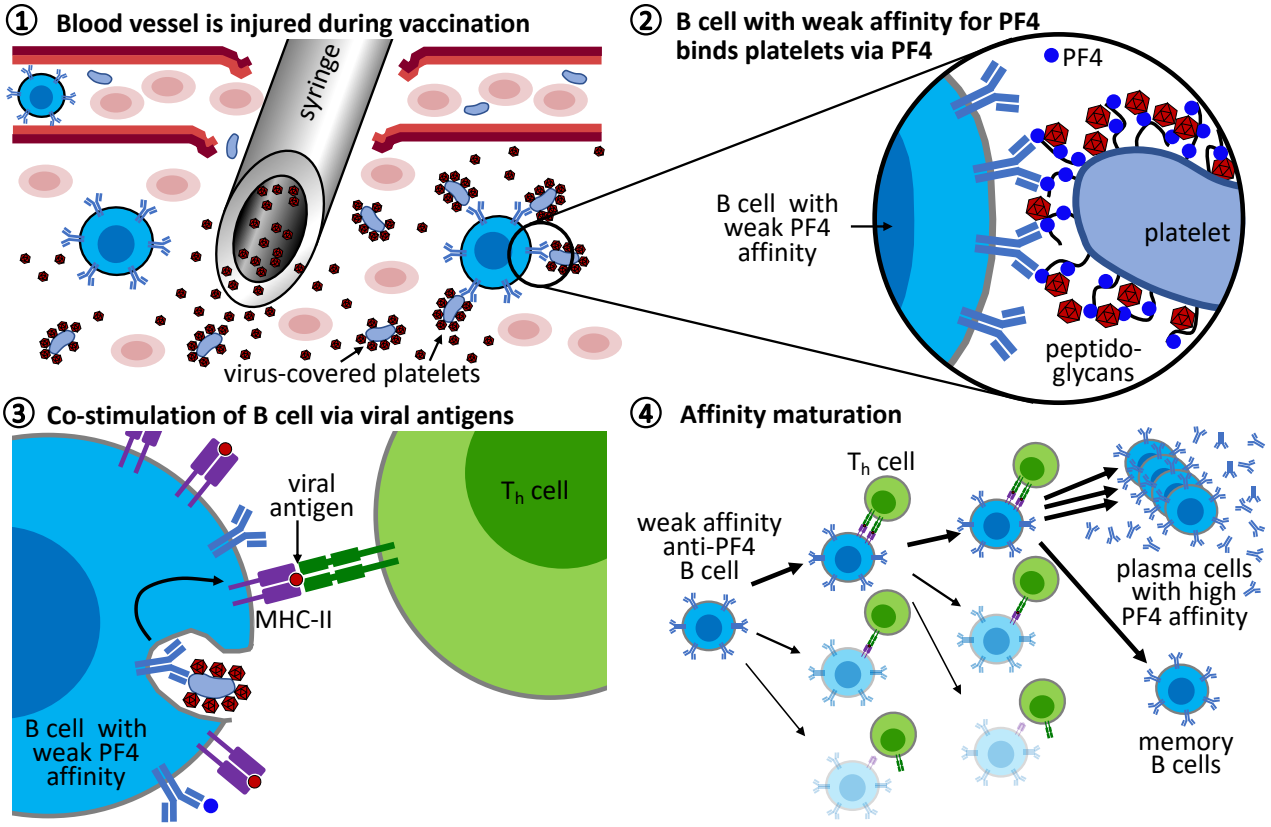

Figure 1: Model for generation of anti-PF4 platelet-activating antibodies in VITT. ① During vaccination, blood may come into contact with high concentrations of viral vector. Platelets become covered by large amounts of virus particles. ② VITT is triggered when a IgM^+ B cell with weak B cell receptor affinity to platelet factor 4 (PF4) binds to platelets via PF4. ③ The B cell phagocytoses several platelets with bound viruses and presents the peptide antigens on its MHC-II complexes to T_h cells in a lymph node. Some T_h cells recognize the presented viral antigens and co-stimulate the B cell. ④ The IgM^+ B cell undergoes class-switching to IgG and affinity maturation, helped by the same T_h cells, and gives rise to memory B cells and to plasma cells that produce IgG antibodies with high affinity to PF4, resulting in VITT.

can lead to opsonization of pathogens, facilitating pathogen clearance and complement activation.[11,12,13] Indeed, the concentration of anti-PF4 IgM antibodies seems to be tightly regulated.[14] Heparin-induced thrombocytopenia might similarly be caused by spatial clustering of PF4 on heparin polymers, which allow anti-PF4 antibodies to form antibody-PF4-heparin aggregates.

Mechanism of vaccine-induced generation of anti-PF4 platelet-activating antibodies

We first note that some adenoviruses can bind and activate platelets.[15,16,17] This affinity can be mediated by viral receptors that bind specific ligands on the platelets[18] such as fibronectin receptor.[15] Alternatively, PF4 protein also has some binding affinity to various viruses as part of its role in innate immunity,[13] and viruses may therefore stick to the activated platelets via PF4 bound to their proteoglycans.

The model consists of four steps (Figure 1). ① If during the vaccine injection a small blood vessel was injured, platelets and B cells could seep into the muscle tissue with high amounts of inoculated virus. To trigger blood clotting, the platelets excrete PF4 protein, which binds to polyanionic proteoglycans sticking out from the platelet surface, neutralizing their negative charge and enabling platelets to stick together. Platelets also become covered by large amounts of virus particles sticking to PF4 or directly to the platelets. ② We posit that VITT is triggered when an IgM^+ B cell with weak receptor affinity to PF4, binds to platelets via PF4. ③ The B cell would then phagocytose its own B cell receptors with the bound platelets and with the viruses sticking to it. It would migrate to the draining lymph node and wait there in an attempt to receive a co-stimulatory signal by a CD4 T_h cell that might recognize one of the antigens

presented on its MHC-II complexes. Since the B cell phagocytosed the entire platelet with bound viruses, it will present both platelet and virus antigens on its MHC type-II complexes. To co-stimulate the B cell, it then suffices for a T_h cell to recognize an MHC-II-presented virus antigen. Such activated T_h cells will be present after a few days as a result of the vaccination. ④ The B cell will undergo class switching to IgG and affinity maturation, helped by highly activated T_h cells specific for viral antigens. This will give rise to memory B cells and plasma cells that churn out antibodies against PF4, resulting in VITT.

Model predictions and implications for safer anti-viral vaccines

The model explains why the autoimmunity is directed mostly against PF4 and not against other proteins expressed on platelets.[3] It also explains why it takes around 5 to 20 days from vaccination to first symptoms of VITT. Since most humans harbor IgM^+ B cells whose B cell receptors have a weak binding affinity to PF4,[10] proliferation, affinity maturation, and class switching can be as fast as 5 days or as slow as 20 days, depending on how much help the B cells get from T_h cells. It further explains why the autoreactive antibodies in patients' plasma are of class IgG: B cells were helped by T_h cells that recognized viral antigens, which therefore instructed the IgM^+ B cells to switch to IgG class antibodies most active against viruses. In fact, autoantibodies in systemic autoimmune diseases are often class-switched and display high somatic hypermutation indicative of affinity maturation.[19]

The model makes the following predictions. (1) VITT should only be caused by vaccines containing recombinant, attenuated, or inactivated virus particles that have some binding affinity to platelets. Therefore, vaccines not containing virus particles such as mRNA-based or subunit vaccine formulations should be safe in this regard. This prediction is so far borne out by statistical analyses.[4,2] (2) The model predicts a risk of VITT for all vaccines based on adenovirus vectors of serotypes 5 and 26 (Ad5 and Ad26), since Ad5 is known to bind and activate platelets[17,16] and Ad26 is used in Ad26.COV2.S by Johnson & Johnson. This applies to Sputnik V, which is based on Ad26 followed by Ad5, and to AD5-nCOV by CanSino Biologics. (3) Vaccination-induced hematomas should substantially increase the risk for VITT, since they indicate that high concentrations of virus particles could have come into contact with blood. (4) Since the generation of autoreactive antibodies requires a high concentration of virus particles to get into direct contact with blood, it is very unlikely that a natural infection with SARS-CoV-2 would trigger VITT-like autoimmunity. It is also very unlikely that VITT is caused by cross-reaction of antibodies directed against the CoV-2 spike proteins with PF4. Indeed, this is confirmed by recent experiments.[5] (5) The model predicts that VITT patients do not usually possess T_h cells specific for PF4 antigens, since the autoreactive B cells instead received their co-stimulatory signals from T_h cells recognizing viral antigens. (6) The concentration and affinity of BCRs of anti-PF4 IgM^+ cells in patients' blood should be positively correlated with VITT risk and is therefore expected to be higher with young age and in women.

If true, the model has several important clinical implications. First, switching to mRNA or subunit vaccines should abolish the risk of VITT. Second, during vaccine injection, rupturing of blood vessels needs to be avoided by all means. Third, vaccine additives and modifications of viral proteins such as PEGylation could be explored that can reduce the affinity of virus vectors to platelets after inoculation.[20,21] Fourth, a recent intruiging study showed that the thrombocytopenic effect upon intravenous administration of Ad5 vectors for gene therapy depends on the shaft of the adenovirus fiber protein and that substituting the Ad5 fibre shaft with the one from serotypes Ad3 or Ad35 could reduce the thrombocytopenia.[22] This suggests that recombinantly replacing the fiber protein shaft of Ad5 and possibly further suppressing platelet interaction using protein engineering of the shaft might minimize the risk of VITT.

Vaccine-induced immune thrombocytopenic purpura is a rare side effect (1:40 000) of vaccinations of children with live attenuated measles-mumps-rubella vaccine,[23] and also, more rarely, with other live virus vaccines.[24] In contrast to COVID-19 vaccine-induced VIIT, this condition is generally milder, and adverse effects are mostly from thrombocytopenia that usually resolves within months rather than life-threatening thrombosis. We propose that vaccine-induced immune thrombocytopenic purpura originates through the same mechanism as VITT, and the same measures that reduce risk for VITT could be taken. This would also imply that immune thrombocytopenic purpura is only associated with vaccines using recombinant, attenuated or inactivated viral particles, a prediction that might be verifiable using available data.

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