Lessons from vaccine-induced immune thrombotic thrombocytopenia

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Adenoviral vector vaccines are effective against SARS-CoV-2 but have been associated with a rare side effect termed vaccine-induced immune thrombotic thrombocytopenia (VITT). Here, we discuss our understanding of how vaccine-induced antibodies to platelet factor 4 (PF4) form immune complexes that activate platelets and trigger the thrombotic events seen in VITT.

The isolation of the human adenovirus in the early 1950s was an important step in our understanding of this common pathogen, which is typically associated with mild, self-limiting infections. Advances in molecular and cellular biology in the 1990s permitted the use of human and non-human adenoviruses as non-replicating vectors for the delivery of vaccines and therapeutic genes. The advantages of adenoviral vector vaccines include their proven ability to drive immune activation, low production costs and high stability, particularly for long-term storage. These vaccines have been widely evaluated in cancer trials and also used for vaccination against human infections, such as HIV, Ebola virus and Zika virus. Given their history of efficacy and safety, two adenoviral vector vaccines were rapidly developed for SARS-CoV-2: one produced by Johnson and Johnson using HAdV26 and another by AstraZeneca/COVISHield using the chimpanzee-based ChAdOx1 vector. Meta-analysis documented the clinical effectiveness of these vaccines as well as their ability to induce high antibody titres and T cell reactivity against SARS-CoV-2 (REF.¹). Although the safety data from clinical trials were reassuring, given the limited sample size, these trials would not have identified very rare serious adverse effects (SAEs) such as vaccine-induced immune thrombotic thrombocytopenia (VITT).

COVID-19-related thrombosis

Shortly after the emergence of SARS-CoV-2, it became apparent that individuals infected with the virus showed a high risk of thromboembolic events. In a meta-analysis of some 8,000 patients with COVID-19, arterial and venous thromboembolic complications were observed in 2% and 21% of patients, respectively, which significantly increased the risk of death². It is noteworthy that many of the arterial and venous thrombi associated with VITT are anatomically unusual. However, other clots resemble the thromboembolic complications observed in unvaccinated patients with COVID-19. The mechanistic basis of thromboembolic events in patients with COVID-19 is still unclear, but there is evidence of generalized

activation of the coagulation cascade. In a study of ten patients with acute COVID-19, we found that a number of the patients had immune complexes in their plasma that activated platelets³. Some of these patients also had elevated levels of antibodies to platelet factor 4 (PF4)³.

Vaccine-related thrombosis

The widespread use of adenoviral vector vaccines for COVID-19 began in late 2020 and had an immediate positive impact in reducing severe illness and death. However, by early April 2021, the European Medicines Agency (EMA) had received reports of atypical thrombi following vaccination and concluded that there was a signal of disproportionality. Initial reports described atypical cerebral thrombi, especially in young women and occurring days to several weeks after vaccination with COVID-19 adenoviral vector vaccines.

The reports, often in young people, of unexpected venous or arterial clots post vaccination were distinct from the thromboses observed in patients with COVID-19 infection. These events were accompanied by low levels of platelets and were highly reminiscent of the early reports describing heparin-induced thrombocytopenia (HIT)⁴. Heparin, a long-chain anionic glycosaminoglycan, can bind to PF4 — a small positively charged protein — causing at-risk individuals to form antibodies to the PF4–heparin complexes⁴. These antibodies can crosslink and form immune complexes capable of binding to platelet FcγRIIa receptors, which leads to activation of platelets and other cells and triggers coagulation.

Towards an understanding of VITT

Very few clinical conditions cause both arterial and venous thrombi in unusual locations, accompanied by thrombocytopenia and generalized activation of the coagulation cascade. VITT mirrors HIT with regard to these clinical and laboratory characteristics⁴. Within weeks of the first VITT reports, three different groups all reported the presence of antibodies to PF4 in patients with VITT, but not in vaccinated persons without VITT^{5–7}. But a major difference between HIT and VITT

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Fig. 1 | **Overview of vaccine-induced immune thrombotic thrombocytopenia.** In most people, administration of an adenovirus-based COVID-19 vaccine induces antibodies to the spike protein of SARS-CoV-2 and protects the recipient against COVID-19 (steps 1–4). In rare cases (step 5), vaccine-induced immune thrombotic thrombocytopenia (VITT) antibodies are induced that can bind to platelet factor 4 (PF4) and form immune complexes that activate platelets (steps 5–7). This leads to pathological activation of clotting cascades and a fall in platelet counts (steps 8–9). VITT-associated clots have been described in the brains, lungs, abdomen, liver and legs (step 10). Image courtesy of Christy Groves, adapted by S. Bradbrook/Springer Nature Limited.

was apparent — specifically, that patients with VITT had not received heparin. To date, it is not possible to determine whether the two currently available adenoviral vector COVID-19 vaccines pose a difference in the risk of developing VITT. Complexities in reporting and lack of serological confirmation adds to the uncertainty. In our laboratory, the majority of patients who are suspected of having VITT turn out to be serologically negative and do not have VITT. This means that at least some clinically diagnosed episodes of VITT are not causally related to the vaccination. Adding to the difficulties in understanding VITT and its association with adenoviral vector vaccines is a patient who had VITT following an mRNA vaccine⁸. It remains uncertain whether this was a causal or a coincidental association.

Our laboratory has studied the epitopes on PF4 that are targeted by the antibodies in patients with HIT. Using alanine scanning mutagenesis to identify

antibody-binding sites, we found that patients with HIT have a polyclonal response targeting multiple amino acids on the PF4 tetramer⁹. We used a similar approach to study the epitopes on PF4 that constitute the VITT antibody-binding sites and identified a unique and conserved VITT antibody-binding site on the PF4 tetramer made up of eight amino acids, within the heparin binding site¹⁰. In contrast to what is seen in HIT, the VITT antibodies did not require heparin for immune complex formation and FcγRIIa-dependent platelet activation. Consistently, we found that therapeutic doses of heparin inhibited the reaction, which can be explained by the overlapping binding region on PF4 between VITT antibodies and heparin¹⁰. The question then became: is it possible that the PF4 antibodies in patients who develop VITT crosslink PF4 tetramers on their own, thereby forming immune complexes capable of activating platelets? Using bio-layer interferometry, we

documented that, first, patients with VITT antibodies have a strong antibody response to PF4 (a measure of the amount of antibody produced to an antigen) and, second, VITT-associated antibodies show a similar dissociation rate from PF4 to HIT-associated antibodies¹⁰. We also noted that VITT-associated antibodies show higher binding to PF4 than to PF4–heparin complexes. These observations demonstrate that VITT antibodies have sufficient binding strength (avidity) to form immune complexes with PF4 and to crosslink FcγRIIa receptors on platelets and consequently activate platelets, producing procoagulant-rich microparticles and activating the coagulation cascade (FIG. 1).

These observations provided a framework for better understanding the clotting disorder of VITT following vaccination with an adenoviral vector vaccine. The rapid formation, literally within days, of the IgG autoantibodies suggests an anamnestic response. The mechanism by which the vaccines induce VITT-associated antibodies remains unknown. One possibility is that PF4 might complex with electro-negative surface charges on the adenoviral vector rendering it immunogenic, in a similar manner to how it can bind other polyanions, such as heparin, DNA and bacteria.

Adenoviral vaccines: future strategies

It is likely that adenoviral vector vaccines will continue to be needed around the world. Several steps should be taken. First, our data emphasized that VITT is frequently clinically overdiagnosed, making a rare disorder appear more common. Standard serological tests should be required to confirm the diagnosis. Second, until we

have developed more effective treatments for VITT, the focus should shift to modification of the vaccine to prevent VITT. Just as pharmacological strategies modifying heparin molecules progressively reduced the risk of HIT, it is likely that modifications to the adenoviral vector vaccines can lead to even safer vaccines. A better understanding of the components of the vaccine that trigger the formation of autoantibodies to PF4 should be an early focus.

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Competing interests

The authors declare no competing interests.