Comment on Nicolai et al, page 478

Platelets get particular

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In this issue of *Blood*, Nicolai et al[1] provide insight into the mechanism by which vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with an adenoviral vector can lead to the development of autoantibodies associated with vaccine-induced thrombocytopenia/thrombosis (VITT) and immune thrombocytopenia (ITP).

Approximately 1/10[5] to 1/10[6] recipients of adenovirus-based vaccines against SARS-CoV-2 develop an autoantibody-mediated hematologic disorder, most commonly VITT and ITP. Although the incidence of VITT and ITP is low and management strategies have evolved to reduce morbidity, the mechanism by which a foreign antigen, in this case a vaccine containing an adenoviral vector, promotes the appearance of autoantibodies is unknown. Insight into this process may have important implications for vaccine development and gene therapy in the future as well as understanding the initiation of autoimmune disorders more broadly.

These investigators identified antibodies reactive with platelet glycoproteins in the sera of 8 of 27 patients with clinically diagnosed and serologically confirmed platelet factor 4 (PF4)-dependent platelet-activating antibodies implicated in VITT and 11 of 26 patients with isolated thrombocytopenia but suspicion for VITT after vaccination with ChAdOx1 nCov-19 (possibly ITP), but not in 52 healthy vaccine recipients. The authors point out that the true prevalence of platelet antibodies is likely to be higher had platelet eluates been available for study. To simulate the potential sequence of pathologic events in a mouse model, they demonstrated that IV injection of vaccine containing ChAdOx1 nCov-19 particles activated platelets and formed platelet-adenoviral aggregates that were cleared by splenic macrophages within a couple of hours. Platelet remnants in the marginal zone and follicles, the place of rapid B-cell response to blood-borne antigens,[2] were followed 6 days later by the presence of platelet-reactive immunoglobulin G (IgG) and IgM in the serum.

These interesting findings raise the important question of how adenoviral-platelet complexes might lead to the production of both anti-PF4 and diverse platelet antiglycoprotein antibodies. It is important to gain a deeper understanding of the next steps in autoantibody development because a similar process might underlie the development of "primary" ITP and other immune hematologic disorders that occur after natural viral infections or immunizations against other pathogens.[3-5]

The next steps in antibody production are a matter of conjecture as of now. Intense platelet activation occurs in diverse settings seemingly without formation of similar antibodies or clinical sequelae. Rather, the results reported here appear to focus attention on the adenoviral particle itself, as noted by the authors. Neither supernatants from heat-inactivated ChAdOx1 nCov-19 vaccine, nor ADV-004 particles, nor BNT162b2 messenger RNA (mRNA) vaccine caused thrombocytopenia. The requirement for the adenoviral particle is consistent with clinical experience showing patients with VITT do not typically experience a relapse after subsequent exposure to an mRNA-based vaccine and with the persistence of platelet-activating anti-PF4 antibodies in many patients with VITT in the absence of clinical sequelae.[6]

How might the viral particle contribute to autoantibody production? Perhaps antibodies that cause VITT can be induced by PF4 bound to anionic adenoviral hexons.[7] However, the lack of obvious structural similarities between PF4 and the various platelet glycoprotein targets lessens the likelihood that molecular mimicry and epitope spread of anti-PF4 antibodies explain the range of antibodies detected in this paper. Cloning and sequencing of VITT and HIT vs ITP antibodies and identification of variable light and heavy chain usage might provide insights into this possibility.

In theory, phagocytosis and proteolysis of viral-platelet complexes within the proteosome of antigen-presenting cells might generate novel immunogenic peptides that differ from processing of secreted or membrane proteins themselves. This possibility would be more attractive if major histocompatibility complex restriction on disease incidence was discovered. Rather, the range of antigenic targets might indicate that the inflammatory milieu that occurs in response to vaccination disrupts regulation of autoantibody formation. Natural IgM reactive with PF4 is found in the "naive" population.[8,9] The viral particle might serve as a template that sustains antigen presentation on these preexisting B-lymphocyte populations that express cognate receptors. This might occur if the adenovirus provides a costimulatory danger signal that lowers the threshold of B-cell receptors for antigens that bind with low affinity. However, this neither fully accounts for the marked predilection for VITT to develop after initial exposure to adenoviral vaccine nor, as the authors point out, does it explain how ITP might develop following exposure to mRNA-base vaccines. It would be also of interest to know if the immunized mice in this study developed sufficiently high-affinity IgG antibodies to cause thrombocytopenia to simulate passive murine models of ITP or if they also developed anti-mouse PF4 antibodies.

This article also has important clinical implications. First, the results may suggest the need for improved techniques and vigilance against inadvertent IV injection of vaccine. This must be accomplished without loss of local immune responses important in host protection. Second, the observation that a second adenovirus (ADV-004) did not induce antibody formation despite binding to platelets holds out the potential for rational design of an adenoviral vector capable of inducing a robust protective immune response without causing platelet activation and its sequelae.

Conflict-of-interest disclosure: L.R. receives research funding from AstraZeneca. D.B.C. declares no competing financial interest. ■

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DOI 10.1182/blood.2022016773

Comment on Jain et al, page 491, and Cherng et al, page 504

Tumor-intrinsic causes of CAR-T failure

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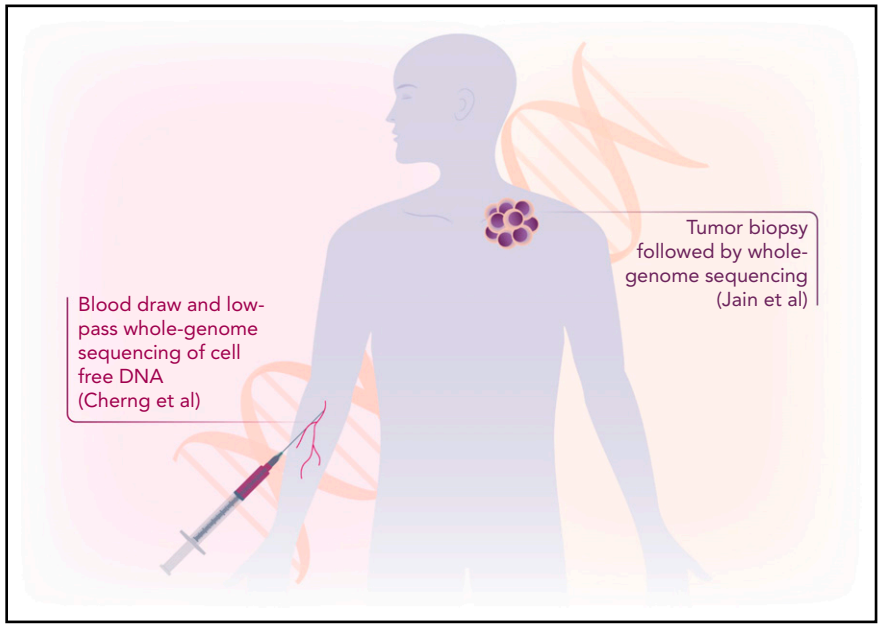
In this issue of *Blood*, two reports delineate tumor-specific factors associated with clinical responses to infusion of approved T-cell products genetically modified with a chimeric antigen receptor (CAR) targeting CD19 in patients with diffuse large B-cell lymphoma (DLBCL)[1,2] (see figure). Jain et al comprehensively characterized the genomes of lymphoma tissue from patients who received CD19 CAR-T cells and report that genome-wide mutational signatures and patterns of structural alterations are associated with CAR-T cell response. Cherng et al evaluated circulating tumor DNA and show that a high focal copy number alterations (CNAs) score, denoting genomic instability, was the most significant pretreatment variable associated with poor response to CAR-T therapy or subsequent relapse.

Autologous CD19 CAR-T cells have significant therapeutic benefit for patients with relapsed/refractory DLBCL, and 3 products have obtained Food and Drug Administration approval. Since the first approvals in 2017, the therapy has been used in several thousand patients, and "real-world" analyses confirmed the initial response rates of 50% to 80% reported in the studies that led to licensure.[3-7] However, more than half of recipients experience relapse and disease progression, with prolonged disease responses in only ~40%.[3,7] Therefore, predictive factors for subsequent progression or relapse would be useful to identify patients who may benefit from combination therapies or additional consolidation strategies.

A number of studies have investigated the infused cell product or host immune system to identify factors associated with poor outcomes. These reports correlated serum inflammatory markers and T-cell



Two papers interrogate the lymphoma genome to learn causes of CAR-T failure in patients with diffuse B-cell lymphoma. Jain et al evaluate tumor biopsies with WGS, whereas Cherng et al perform low-pass WGS on circulating tumor DNA derived from peripheral blood. Professional illustration by Somersault18:24.