VACCIREVIEW

Adenoviral core protein pVII, somatic hypermutation, and the mechanistic basis of VITT

Bibliography

Wang JJ, Schönborn L, Warkentin TE, et al. Adenoviral inciting antigen and somatic hypermutation in VITT. N Engl J Med. 2026;394(7):669-683.

Summary

This mechanistic study investigates why a tiny fraction of recipients of adenoviral vector–based COVID-19 vaccines (ChAdOx1-S or Ad26.COV2. S), and some patients with natural adenovirus infection, develop vaccine-induced immune thrombocytopenia and thrombosis (**VITT**), a severe prothrombotic syndrome mediated by platelet-activating antibodies against platelet factor 4 (PF4). The authors hypothesized that VITT arises from a misdirected, boosted immune response against an adenoviral antigen that cross-reacts with PF4 in genetically predisposed individuals. They combined antibody proteomics and genomics to characterize anti-PF4 antibodies from 21 patients with VITT, sequenced immunoglobulin light-chain genes from 100 patients, and systematically mapped antibody binding to adenoviral structural proteins, especially the highly conserved core protein VII (pVII), which binds viral DNA and shares biochemical features with PF4.

Mass-spectrometric sequencing of purified anti-PF4 antibodies showed a strikingly stereotyped molecular signature: virtually all pathogenic VITT antibodies used the same immunoglobulin lambda light-chain family, IGLV3-21, specifically alleles *02 or *03, and displayed a conserved acidic motif (DDSD) in LCDR2 plus a recurrent somatic hypermutation at position 31 of the light-chain CDR1, substituting germline lysine (K) with glutamic acid (E) or, less often, aspartic acid (D). In the heavy chain, different IGHV families were used, but HCDR3 length and the presence of an acidic ED motif were highly conserved, generating a strongly negatively charged paratope that can bind the positively charged PF4. Germline sequencing in 100 patients confirmed that position 31 is lysine in all cases and that E/D31 is a somatic mutation rather than a germline polymorphism.

To test functional causality, the authors reverse-engineered two human recombinant VITT antibodies (CR22046 and CR23004) from patient sequences. These antibodies bound PF4 but not PF4–heparin complexes, and in vitro they activated platelets in a PF4-dependent fashion. In a human PF4/human FcγRIIa transgenic mouse model, CR22046 caused profound thrombocytopenia (≈80% platelet drop) and thrombosis at typical VITT sites (cerebral venous sinus, splanchnic veins, pulmonary embolism). When the key light-chain mutation was reverted to germline (E31K), both recombinant

antibodies largely lost PF4 binding, required very high concentrations to activate platelets in vitro, and induced thrombosis in only a minority of mice, with platelet counts similar to controls. A second engineered variant that replaced the DDSD motif (alleles *02/*03) with the YDSD motif encoded by IGLV3-21*01/*04 abolished PF4 binding and platelet activation, underscoring the requirement for both the specific allele and the K31E/D mutation for full pathogenicity.

To identify the adenoviral trigger, the authors purified antibodies from VITT sera using immobilized ChAdOx1 virions and individual adenoviral proteins (penton, pIIIa, pV, pVI, and pVII). Antibodies against intact virions did not show clonotypes matching the pathogenic anti-PF4 fingerprint and did not cross-react with PF4, arguing against a surface capsid antigen as inciting epitope. In contrast, IgG purified against recombinant pVII contained clonotypic species whose light- and heavy-chain CDR3 "barcodes" matched those of anti-PF4 antibodies from the same patient. These anti-pVII antibodies cross-reacted with PF4, and conversely, purified anti-PF4 antibodies bound pVII. Importantly, anti-pVII antibodies from a healthy vaccine recipient lacked this cross-reactivity, indicating that cross-reactive clones are specific to VITT.

Using a library of overlapping 15-mer peptides spanning ChAdOx1 pVII, they mapped the shared epitope to a highly basic linear sequence (RYARAKSRRRRIARR) in the central region of pVII. Recombinant VITT antibodies bound strongly to this peptide, whereas the back-mutated E31K variant bound the peptide more strongly than PF4, supporting the idea that the germline antibody is primarily anti-pVII and only with K31E/D mutation acquires high-affinity PF4 binding. The epitope sequence is nearly identical in Ad26 pVII, providing a structural explanation for VITT after both ChAdOx1-S and Ad26.COV2.S. Structural modeling showed that the acidic paratope formed by the DDSD motif and E/D31 faces the basic PF4 surface, enabling high-avidity binding and formation of large PF4–IgG immune complexes capable of clustering PF4 and cross-linking platelet FcγRIIa receptors, the hallmark of VITT pathophysiology.

The authors integrate these findings into a two-hit model. First, in genetically predisposed individuals (carrying IGLV3-21*02/*03), natural adenovirus infection primes B cells specific for the basic epitope on pVII, generating a polyclonal anti-pVII response with germline K31. Second, during adenoviral vector vaccination (or reinfection), booster stimulation and somatic hypermutation in rare B-cell clones introduce K31E/D, converting some anti-pVII antibodies into high-avidity anti-PF4 antibodies that still weakly recognize pVII but now preferentially bind PF4, forming pathogenic immune complexes and triggering VITT. The rarity of the required genetic background plus the specific somatic event explains the extremely low incidence of VITT. The authors

emphasize that other anti-PF4 disorders (heparin-induced thrombocytopenia, CMV-associated anti-PF4 disease) likely involve different viral or drug epitopes and may or may not share similar genetic constraints. They propose that redesigning adenoviral vectors to alter pVII or replace it with a non-mimicking analogue could eliminate this upstream trigger, potentially preserve the platform's advantages while reducing VITT risk.

Comment

Clinically, this work does not change acute VITT management, but it clarifies why VITT is tightly linked to adenoviral vectors, why it is so rare, and why anti-PF4 antibodies in most vaccinated individuals are non-pathogenic. For future products, it provides a roadmap for rational adenoviral vector reengineering and suggests that, in principle, genetic or serologic risk stratification might one day identify individuals at highest VITT risk—though such testing is far from ready for routine use.

*Brought to you by Chief Editor **Joe Schmitt** – Supported by AI*