CORRESPONDENCE

Antibody Fingerprints Linking Adenoviral Anti-PF4 Disorders

TO THE EDITOR: Disorders caused by antibodies against platelet factor 4 (PF4) gained major attention during the pandemic, when platelet-activating anti-PF4 antibodies were found to explain vaccine-induced immune thrombocytopenia and thrombosis (VITT) after coronavirus disease 2019 (Covid-19) vaccination with two adenoviral vector–based vaccines, ChAdOx1 nCoV-19 (Oxford–AstraZeneca) and Ad26.COV2.S (Johnson & Johnson–Janssen).[1] Affected patients often have multiple or unusual sites of thromboses, such as the cerebral venous sinus and splanchnic veins, with high levels of D-dimer.[2]

VITT antibodies feature an oligoclonal or monoclonal antibody profile with a remarkable degree of clonal identity of the light chains,[3,4] which results in a strikingly similar structure of the hypervariable region of these anti-PF4 antibodies. All the patients with VITT who have been evaluated have expression of the IGLV3-21*02 allele and a heavy-chain motif, resulting in at least eight acidic (negatively charged) amino acids of the paratope (antigen-binding region), by which anti-PF4 antibodies bind to their antigen. These negatively charged regions mediate strong binding of the antibodies to the positively charged arginine and lysine residues on PF4.[5]

Recently, the same VITT-like clinical and laboratory features were reported in patients after they had been infected with adenovirus (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). We performed proteomic analysis to characterize the affinity-purified anti-PF4 antibodies in samples obtained from four of these patients (Fig. 1A).[3] We found highly similar amino acid fingerprints of their antigen-binding sites, which led to superimposable molecular structures of these VITT-like anti-PF4 antibodies and anti-PF4 antibodies that had been identified in patients with VITT (Fig. 1B). Figure 1C shows the resulting paratope.

We conclude that the antibodies induced by adenoviral vector–based Covid-19 vaccination (classic VITT) and the VITT-like antibodies induced by natural adenovirus infection are extremely similar. Such an extraordinary level of autoantibody fingerprint identity between two disorders — at the level of patient-derived antibodies — strongly indicates that VITT and the anti-PF4 disorder that is associated with adenoviral infection are a distinct class of adverse immune responses associated with viral (presumably, adenoviral) structures.

Our findings indicate that the anti-PF4 disorder that was first recognized as VITT is essentially identical to the disorder caused by adenovirus infection that occurs sporadically. Thus, the clinical lessons learned from VITT remain relevant: thrombosis associated with thrombocytopenia with greatly elevated D-dimer levels — particularly after a viral infection — may be investigated and treated as an anti-PF4 disorder. Specific laboratory testing includes screening for

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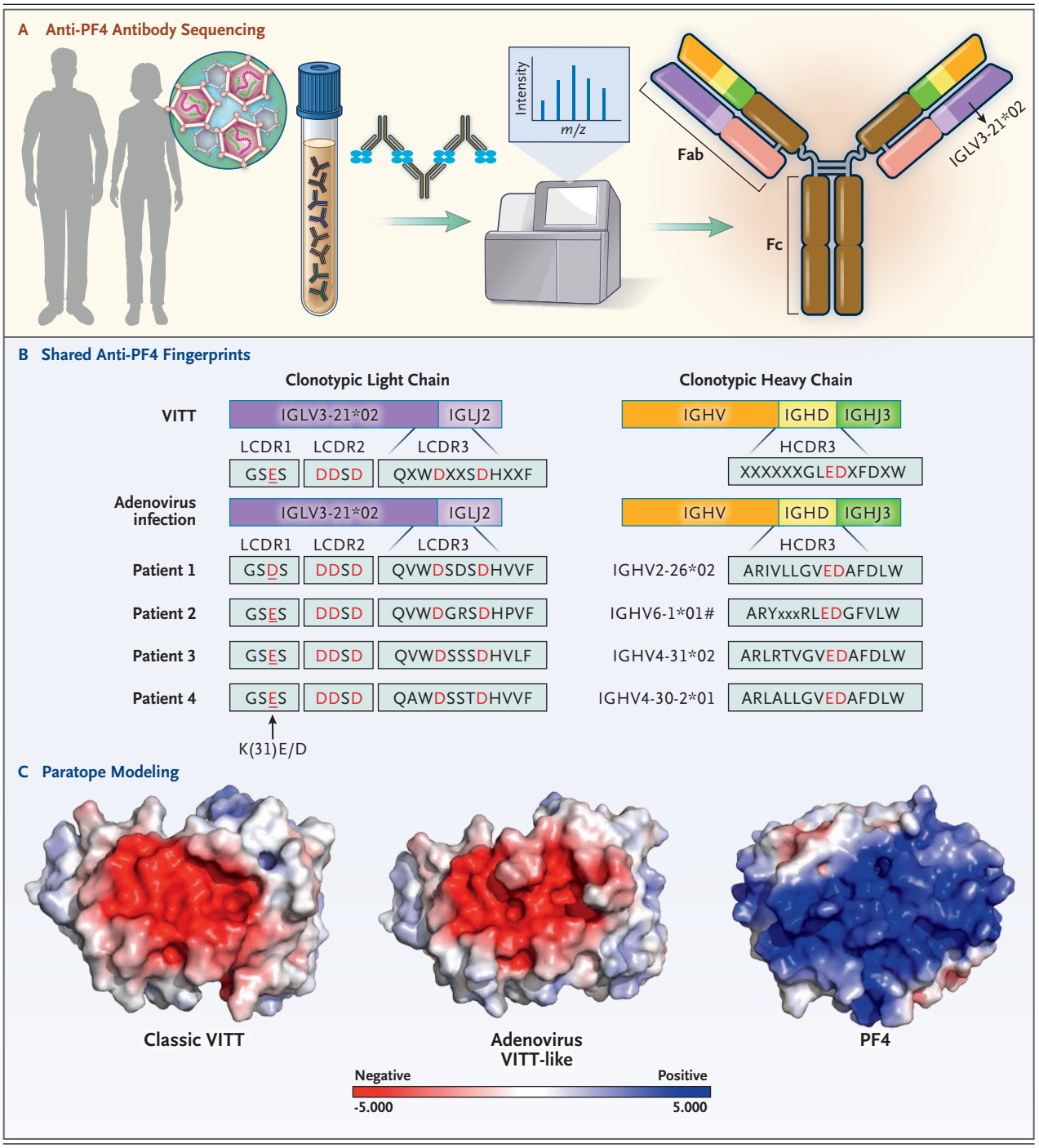

anti-PF4 antibodies by enzyme-linked immunosorbent assay, confirmatory testing by platelet-activation assays, and combined treatment with therapeutic-dose anticoagulation and high-dose immune globulin. Our data indicate that adenovirus, rather than other vaccine constituents, directly or indirectly induces the formation of platelet-activating anti-PF4 antibodies, findings that provide important implications for vaccine development. Additional work is required to identify the antigen.

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Figure 1 (facing page). Shared Fingerprints of Anti-PF4 Antibodies in VITT and VITT-like Disorders after Adenovirus Infection.

Panel A shows proteomic profiling of serum antibodies against platelet factor 4 (PF4) generated after adenovirus infection. Resulting anti-PF4 antibodies are purified from patient serum (or plasma) with the use of PF4 protein–coupled Dynabeads and are digested with enzymes to generate peptides for liquid chromatography–tandem mass spectrometry. Peptide sequences in immunoglobulin heavy- and light-chain variable regions are collated to identify amino acid fingerprints.[3] Panel B shows the matching of anti-PF4 fingerprints in vaccine-induced immune thrombocytopenia and thrombosis (classic VITT) and adenovirus VITT-like disorders. Consensus anti-PF4 antibody fingerprints from patients with classic VITT are shown at the top of the panel. These fingerprints are distinguished by a single immunoglobulin lambda variable 3-21*02 (IGLV3-21*02) light chain paired with a single heavy chain that expresses a shared motif in the heavy-chain third complementarity-determining region (HCDR3). Below are shown serum anti-PF4 antibody fingerprints obtained from four patients with adenovirus-associated (VITT-like) anti-PF4 disorder. The anti-PF4 signatures in these cases are remarkably similar to those of classic VITT with conserved acidic residues shown in red (with D denoting aspartic acid and E glutamic acid). All four patients express IGLV3-21*02 light chains with a strongly acidic DDSD motif, a basic lysine (K) to acidic E or D (E/D) mutation at position 31 (underlined) in light-chain complementarity-determining region 1 (LCDR1), along with identical LCDR3 lengths with two equally spaced D residues combined with an immunoglobulin lambda joining 2 (IGLJ2). As in classic VITT, these heavy chains have identical HCDR3 lengths (a marker of clonal sharing) with a contiguous amino acid ED motif rearranged with an immunoglobulin heavy joining 3 (IGHJ3). Anti-PF4 antibodies express distinct immunoglobulin heavy variable (IGHV) subfamilies, as observed in classic VITT. The acidic amino acid residues (in red) from heavy and light chains form the PF4-binding paratopes. (In Patient 2, a full HCDR3 sequence was not available because of a limited sample volume.) Panel C shows representative paratopes modeled by AlphaFold, a machine-learning structural-prediction method. The structures represent the heavy- and light-chain variable domains of classic VITT and adenovirus VITT-like disorders. The antibody variable domains and PF4 tetramer (Protein Data Bank code, 1RHP; www.rcsb.org/structure/1RHP) were visualized with PyMoL. The scale bar indicates surface electrostatic potential and corresponds to acidic (red) and basic (blue) amino acid residues. Anti-PF4 antibodies that were obtained from patients with classic VITT and adenovirus VITT-like disorders show highly similar acidic (negatively charged) paratopes (in red) that electrostatically match their highly positively charged binding sites (in blue) on the PF4 tetramer.

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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;384:2092-101.
2. Pavord S, Scully M, Hunt BJ, et al. Clinical features of vaccine-induced immune thrombocytopenia and thrombosis. N Engl J Med 2021;385:1680-9.
3. Wang JJ, Armour B, Chataway T, et al. Vaccine-induced immune thrombotic thrombocytopenia is mediated by a stereotyped clonotypic antibody. Blood 2022;140:1738-42.
4. Kanack AJ, Bayas A, George G, et al. Monoclonal and oligoclonal anti-platelet factor 4 antibodies mediate VITT. Blood 2022;140:73-7.
5. Wang JJ, van der Neut Kolfschoten M, Rutten L, et al. Characterization of reverse-engineered anti-PF4 stereotypic antibodies derived from serum of patients with VITT. Blood 2024;143:370-4.

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