

Polyethylene glycol allergy: risks of skin testing and complement mediated anaphylaxis

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Many severe immediate-type allergic reactions, including anaphylaxis, to Coronavirus Disease 2019 (COVID-19) vaccines have been attributed to excipient allergies, such as polyethylene glycol (PEG) found in Fosun-Pharma Pfizer BioNTech vaccine (BNT). Classically, immediate-type hypersensitivity reactions such as anaphylaxis have been attributed as immunoglobulin (Ig) E-mediated reactions. However, complement-mediated reactions, such as complement activation-related pseudoallergy (CARPA), have been postulated with PEG and COVID-19 vaccine associated allergies. These reactions are triggered by activation of the complement cascade, producing C3a, C4a and C5a anaphylatoxins.[1] However, evidence for CARPA following PEG exposure has been scarce. We describe a case of severe anaphylaxis elicited by skin testing with PEG, and subsequent in-vitro workup supporting a complement-mediated process.

A 48-year-old male was referred to our Vaccine Allergy Safety Clinic for evaluation of suspected PEG prior to COVID-19 vaccination. He had a history of suspected immediate-type hypersensitivity after ingestion of Klean-Prep (Helsinn-Birex Pharmaceutical, Ireland) which contains PEG-3350. He had no other known drug allergies and there was no history of atopy. Around nine years ago, he developed generalised urticaria around 15 minutes after Klean-Prep ingestion prior to an elective colonoscopy. His symptoms were self-limiting and there were no features of systemic involvement.

The patient consented for allergy testing with PEG, as per departmental protocol. Skin testing was performed with PEG 3350 (1/10 concentration). Ten minutes after intradermal skin testing (IDT) was performed, the patient complained of generalised pruritus and chills. There was no rash or other mucocutaneous manifestations and his IDT to PEG 3350 was unequivocally negative. He then experienced dizziness and severe hypotension (systolic BP of 55mmHg). He was given two doses of intramuscular adrenaline and intravenous fluids without improvement, and intravenous adrenaline was required before his blood pressure returned to normal.

Following stabilization, investigations showed significantly elevated tryptase of 22.7ng/mL (baseline level 4.8ng/mL), confirming the diagnosis of anaphylaxis. Soluble C5b-9 complex (Sc5b-9), also known as the terminal complement complex, was also checked as per recommendation by the Centers for Disease Control and Prevention[2] and was significantly elevated to 257.2ng/mL (reference range 75-219ng/mL), indicating activation of the complement system. IgG against PEG was detected in the patient's serum by enzyme-linked immunoassay (ELISA) with an antibody activity threshold index of 4.0 (positive antibody activity defined as values >1.0).

Six weeks after the event, basophil activation tests (BAT) were performed using the patient's basophils with PEG and the BNT vaccine. Flow cytometry showed significant upregulation of CD63c and CD203c after stimulation with BNT, however basophils remained non-responsive to stimulation with multiple molecular weights of PEG (PEG2000, 3350, and 4000). (Figure 1). The patient was advised to avoid all PEG-containing compounds in the future and received a non-PEG containing COVID-19 vaccine (CoronaVac, SinoVac) without any reaction.

Immediate-type hypersensitivity reactions such as anaphylaxis are traditionally defined as IgE-mediated reactions. However, non-IgE mediated mechanisms, such as complement activation-related pseudoallergy (CARPA) have been postulated with PEG and COVID-19 vaccine allergies.[3] The wide heterogeneity of clinical presentations and laboratory workup of PEG-associated allergies may be

explained by differing proportions of IgE and complement-mediated mechanisms contributing to the reaction.

To the best of our knowledge, we are the first to report a confirmed case of PEG anaphylaxis (by clinical criteria and significantly elevated serum tryptase) following skin testing with evidence of CARPA by significantly elevated Sc5b-9. Elevated tryptase or positive BAT cannot distinguish between IgE-mediated or non-IgE-mediated causes of mast cell degranulation.[4][5] On the other hand, Sc5b-9 is a by-product of complement cascade activation that is elevated in complement-mediated reactions,[6] as exemplified by this case. Anti-PEG IgG antibodies are thought to play a role in the pathogenesis, and they were present in the patient's serum. The anti-PEG IgG ELISA not only detects the presence of antibodies, but measures anti-PEG activity, which combines the antibody concentration and avidity for the PEG antigen. These antibodies activate the complement cascade upon binding to PEG or PEGylated substances with formation of immune complexes, leading to activation of the complement cascade via the classical pathway, producing C3a, C4a and C5a anaphylatoxins, also inducing degranulation of basophils and mast cells.[1, 7]

Various conjugations and formulations of PEG are used for the workup of suspected PEG allergies. The BAT performed on our patient yielded positive results to BNT, but not to purified PEG, even in various molecular weights. This finding has been well-documented and is likely due to the augmented immunogenicity when PEG is conjugated with lipid nanoparticles.[8, 9] The anti-PEG antibody ELISA also utilizes PEG conjugated to bovine serum albumin, and not purified PEG as the target antigen. This may also explain the persistent negative skin testing sites throughout the allergic reaction.

However, the patient was only exposed to PEG in both episodes of allergic reactions—once after ingestion of Klean-Prep and once after skin testing with PEG-3350. This may suggest that purified PEG undergoes changes in conformation or chemical structure after conjugation with organic substances,

and is able to induce allergic reactions *in-vivo*. This phenomenon warrants further research and investigation.

We report a case of severe anaphylaxis induced by skin testing with purified PEG. Although elevated tryptase levels and positive BAT results support the diagnosis of an immediate-type hypersensitivity reaction, comprehensive workup including soluble C5b-9 complex (Sc5b-9), and positive IgG antibodies against PEG suggests the role of complement-mediated mechanisms. Our report also highlights the importance of choosing conjugated forms of PEG, such as PEG-containing vaccines, for *in-vitro* testing for more accurate workup of suspected cases. Furthermore, *in-vivo* allergy investigations may also carry considerable risk. We therefore suggest cautious skin testing by starting with skin prick tests first, and only consider IDT (at progressive dilutions) if skin prick tests were negative. All investigations should be conducted in a specialist drug allergy centre equipped with trained personnel, adequate resuscitation equipment and securing venous access as a precaution against severe reactions.

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Conflict of interest

The authors have no conflict of interest in relation to this work.

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Figure 1: Basophil Activation Tests to BNT Vaccine and PEG