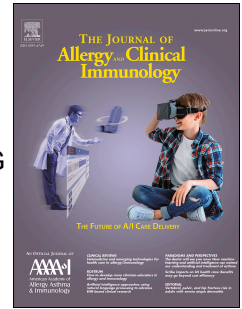


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Basophil reactivity to BNT162b2 is mediated by PEGylated lipid nanoparticles in PEG allergic patients

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**Basophil reactivity to BNT162b2 is mediated by PEGylated lipid nanoparticles in
PEG allergic patients**

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Conflicts of interest:

None of the authors declare any conflicts of interest.

Authorship:

Alexander Troelnikov and Griffith Perkins contributed equally to the manuscript in conception, design of the study, data analysis and writing of the manuscript and are co-first authors. Pravin Hissaria contributed to the conception, design and writing of the manuscript. Chino Yuson, Summaya Balouch, Aida Ahamdie and Griffith Perkins participated in acquisition of data. Plinio Hurtado contributed substantially to conception of the study and review of manuscript.

Capsule Summary:

Polyethylene glycol (PEG) allergic patients demonstrate positive skin and basophil activation tests to PEGylated liposomal drugs, BNT162b2 vaccine and doxorubicin, but not to PEG alone, suggesting that presentation of PEG on PEGylated liposomes facilitates hypersensitivity.

Words: 35

Clinical Implications:

- Skin testing with BNT162b2 (Comirnaty) vaccine itself, and not PEG-containing surrogates, is required for diagnosis of BNT162b2 hypersensitivity
- Basophil activation tests to PEGylated liposomal drugs may be useful for the assessment of BNT162b2-associated anaphylaxis

Words: 36

Abstract**Background:**

The mechanisms underpinning allergic reactions to the BNT162b2 (Pfizer) COVID-19 vaccine remain unknown, with polyethylene glycol (PEG) contained in the lipid nanoparticle suspected as being the cause.

Objective:

To evaluate the performance of skin testing and basophil activation testing to PEG, polysorbate 80, BNT162b2 and AZD1222 (Astra-Zeneca) COVID-19 vaccines in patients with a history of PEG allergy.

Methods:

Three known PEG allergic participants and three healthy controls were recruited, and evaluated for hypersensitivity to the BNT162b2 and AZD1222 vaccines and related compounds by skin testing and basophil activation measured by CD63 upregulation using flow cytometry.

Results:

We found that the BNT162b2 vaccine induced positive skin tests in PEG allergic patients, whereas traditional PEG skin testing was negative in two of three patients. One

patient was found to be co-sensitized to both the BNT162b2 and AZD1222 vaccines due to cross-reactive PEG and polysorbate allergy. The BNT162b2 vaccine, but not PEG alone, induced dose-dependent activation of all patients' basophils *ex vivo*. Similar basophil activation could be induced by PEGylated liposomal doxorubicin, suggesting that PEGylated lipids within nanoparticles, and not PEG in its native state, are able to efficiently induce degranulation.

Conclusions:

Our findings implicate PEG, as covalently modified within the vaccine lipid nanoparticle, as a potential trigger of anaphylaxis to BNT162b2.

Words: 210

Body Text**Introduction**

Early safety monitoring during the mass vaccination campaigns internationally reported an unexpectedly high rate of immediate hypersensitivity reactions to both the BNT162b2 (Comirnaty; Pfizer-BioNTech) and mRNA-1273 (Moderna) mRNA lipid nanoparticle vaccines (1,2).

Although adverse event reporting more recently indicates a lower rate of anaphylaxis of 4-5 per million for the BNT162b2 vaccine, in a well-characterised cohort of 64,900 health care workers, anaphylaxis was confirmed in 16 patients, or approximately 1 in every 4000 individuals vaccinated (3), outnumbering the expected rate of 1 per million(4).

Polyethylene glycol (PEG) conjugated to lipids, within the lipid nano-particle encapsulating the mRNA, is considered the likely culprit allergen (5). PEG driving the unanticipated frequency of anaphylaxis is surprising as this is otherwise an infrequent cause of anaphylaxis despite its widespread presence in many other drugs, foods and cosmetics (6). To assess the risk for allergic reactions to BNT162b2, major authorities have suggested skin testing protocols for patients with a history of anaphylaxis to vaccine components, in particular to PEG allergy and polysorbates (1,7,8). There have been reports of cross-reactivity between PEG and polysorbates, and the AZD1222 vaccine, the only alternative COVID-19 vaccine currently available to our patients in Australia, contains polysorbate 80 (1). This study aimed to evaluate a protocol for PEG, polysorbate and COVID-19 vaccine skin and basophil activation testing in patients with confirmed PEG hypersensitivity. The testing panel used is based on the presence of PEG 2000 in the BNT162b2 and mRNA-1273 vaccines, and polysorbate 80 in AZD1222.

Results and discussion

We recruited three adult patients known to our service with a history of PEG allergy (demographics and index reaction details provided in Table 1). In addition to PEG, Patient 1 had a history of allergy to polysorbates. Skin prick testing (SPT) and intradermal testing (IDT) to PEG and polysorbates was performed using a panel adapted from Banerji et al (1) (Table 1). The panel consisted of freshly thawed BNT162b2 (Comirnaty) and AZD1222 vaccines that were otherwise destined for discard, and readily available and validated skin testing reagents that are related to components of the vaccines licensed for in vivo use (relevant component specified in Table 1). For the study, three healthy individuals used as controls were simultaneously tested with the skin testing panel. All patients developed a positive skin test to the BNT162b2 vaccine, whilst only Patient 1 tested positive for AZD1222, in agreement with the previous positivity skin test to polysorbate. Surprisingly, Patient 2 and Patient 3 had otherwise negative skin testing to the panel, including to PEG containing steroid methylprednisolone acetate at 1:10 dilution, the recommended, non-irritating concentration(9).

To further explore the results of the skin testing, we performed a basophil activation test (BAT) with a range of PEG molecular weights (200 to 6000 g/mol) (Tokyo Chemical Industry, Japan) and with both BNT162b2 vaccine and AZD1222. Prior to skin testing, blood was drawn for basophil activation test (BAT) as previously described (10). In brief, IL-3 treated whole blood was incubated with varying concentrations of allergen to induce degranulation, and then stained with an antibody cocktail as described. Basophil degranulation following allergen exposure was determined by surface expression of CD63 and results compared to a positive (anti-IgE) and negative control (PBS). We observed no increase in CD63 expression on basophils with PEGs of any molecular

weight or with the AZD1222 vaccine (Table 2). However, we observed a dose-dependent increase in CD63 expression in the presence of varying concentrations of BNT162b2 in all three patients (figure 1), not observed in vaccinated nor unvaccinated, non-allergic controls (n=3).

Given that the PEG-containing vaccine, but not PEG alone, was able to induce basophil activation and skin test positivity, we repeated BAT with freshly prepared PEGylated liposomal doxorubicin, which contains PEGylated liposomal nanoparticles similar to BNT162b2(11). We found similar dose dependent CD63 upregulation, even in the presence of very low concentrations of PEGylated liposomal doxorubicin (Figure 2; Table 2) which was not observed in healthy controls, further supporting the contribution of the PEGylated liposomes to basophil activation.

This is the first study to demonstrate positive COVID-19 vaccine allergy skin testing in a well-characterized cohort of PEG allergic patients and validates specific vaccine skin testing for detection of PEG allergy. However, our study also demonstrates the limitations of current skin testing panels for macrogol and methylprednisolone acetate in PEG diagnosis and indeed do not substitute for vaccine specific skin testing. Although all of our patients had past positive PEG skin testing, current skin testing results suggested that in those patients with historical reactions, intradermal testing with drugs containing PEG excipients may yield false negatives. Intriguingly, BNT162b2 skin testing was able to identify PEG allergy in all three PEG allergic patients despite two having otherwise tested negative to PEG containing substances. A recent report also highlights this limitation, with a single patient suffering vaccine allergy demonstrating skin test positivity to only PEG 4000 but not to other molecular weight PEGs and PEG-containing

substances (12). Our results indicate a need to re-evaluate skin testing protocols for suspected PEG allergy and to include PEGylated liposomal compounds.

Current recommendations for PEG allergic patients suggest the use of an alternative vaccine, however, in Patient 1, who was co-sensitized to polysorbate 80 and PEG, cross-reactivity between BNT162b2 and AZD1222 vaccines was observed upon skin testing. Co-sensitization with polysorbates may be seen in as many as 30% of PEG allergic patients(13) and this would greatly limit COVID-19 vaccine options, given the presence of either excipient in all major vaccines in development(5). Given the urgent need for COVID-19 vaccination, it may be necessary to utilize a desensitization protocol for co-sensitized patients.

Ex vivo diagnosis of PEG allergy has historically been challenging and fraught with technical limitations (14,15) with no clear established protocols. Basophil activation testing is a powerful diagnostic and prognostic allergy tool, providing complementary diagnostic information to specific IgE and skin testing, with superior diagnostic accuracy for certain drug and food allergies(16). In this study, basophil activation testing using PEGylated liposomal nanoparticles was able to identify PEG allergic patients, even in the absence of concurrent positive skin testing to PEG alone. We were able to show dose-dependent basophil activation with BNT162b2 as well as PEGylated liposomal doxorubicin, but not to unconjugated PEG. Allergic reactions to PEGylated chemotherapeutics have long been known to cause hypersensitivity like reactions, although they have been purported to be non-IgE-mediated, complement activation related pseudo-allergy (CARPA) reactions (17). Some authors have suggested that CARPA may be responsible for COVID-19 PEGylated lipid nanoparticle vaccine related

allergy (1,18). In our study, the concurrent pattern of positive and negative skin and basophil activation tests suggest that the PEG conformation or arrangement on the surface of nanoparticles determines activation of basophils. This could be the result of increased avidity augmenting IgE crosslinking on the surface of basophils, or by facilitating oligomerization-dependent, IgG-mediated complement activation(19). Future studies are required to determine the exact mechanism underlying hypersensitivity in these patients. Our study also demonstrates that PEGylated doxorubicin can effectively activate these patients' basophils indicating that other PEGylated drugs could be surrogates to detect significant sensitization to PEGylated lipid nanoparticles. By contrast, AZD1222 did not induce *ex vivo* basophil activation in the patient with positive skin testing.

Small sample size is a limitation of our study, however we believe that our findings emphasize the importance of validating PEG allergy testing protocols in the assessment of suspected allergic reactions to PEGylated drugs, including COVID-19 vaccines.

This is the first report implicating PEGylated lipids as mediators of allergy to BNT162b2 vaccine, as demonstrated by intradermal and basophil activation tests. Our study indicates the indispensable requirement for specific vaccine skin testing in the evaluation of patients following BNT162b2 allergic reactions. Further studies are required to clarify whether these findings hold true in patients who experience allergic reactions following administration of the COVID-19 vaccines.

Words: 1299

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Table 1: Patient characteristics and summarized skin test results. All skin tests were carried out at as a series of three dilutions (1:1, 1:10 and 1:100) to a maximal, non-irritating concentration as outlined in the table.

Table 2: Summary of basophil activation test results. Results indicate the concentration at which the percentage of CD63⁺ basophils is greater than 25 percent of the positive control.

Figure 1: *Ex vivo* basophil activation in response to the BNT162b2 (Comirnaty) vaccine.

A: Gating strategy for basophils and basophil activation demonstrated for a positive response. CD63 positive gating set with fluorescence minus one (FMO).

B: Activation of basophils from three patients and a representative healthy control in response to varied concentrations of BNT162b2 vaccine (or a positive or negative control) presented as histograms. BNT162b2 was diluted in phosphate buffered saline to achieve testing concentrations.

C: Grouped comparison of three PEG allergic patients and three healthy controls.

Statistical significance identified by unpaired *t*-test for each concentration: **P* < 0.05.

Figure 2: Activation of basophils from three patients and a representative healthy, control in response to two dilutions of PEGylated liposomal doxorubicin (or a positive or negative control) presented as histograms and bar charts.

323 **Table 1.**

Patient characteristics		Patient 1	Patient 2	Patient 3
Age		51	55	22
Gender		Female	Male	Female
Preceding allergen triggers		Macrogol containing aperiens, polysorbate containing drugs, cosmetics and foods	Macrogol containing aperiens	Parenteral steroid preparation
Time since last reaction		3 months	3 months	5 years
Skin prick testing				
Drug	Component allergen			
Movicol 100mg/ml	PEG 3350	Positive 1 mg/mL	Negative 100 mg/mL	Negative 100 mg/mL
Optive ® advanced	Polysorbate 80 + Carmellose	Negative neat	Negative neat	Negative neat
Cellufresh ®	Carmellose	Negative neat	Negative neat	Negative neat
Methylprednisolone acetate 40mg/mL	PEG 3350	Negative 40 mg/ml	Negative 40 mg/mL	Negative 40 mg/mL
Methylprednisolone succinate 40mg/mL	Excipient free	Negative 40 mg/ml	Negative 40 mg/mL	Negative 40 mg/mL
Triamcinolone 10mg/mL	Polysorbate 80	Negative 10 mg/mL	Negative 10 mg/mL	Negative 10 mg/mL
BNT162b2 100µg/mL	PEGylated nanoparticle	Negative 100 µg/mL	Negative 100 µg/mL	Negative 100 µg/mL
AZD1222 neat	Polysorbate 80	Negative- neat	Negative- neat	Negative- neat
Intradermal testing				
Drug	Component allergen			
Methylprednisolone acetate 4 mg/mL	PEG 3350	Positive 0.4 mg/mL	Negative 4 mg/mL	Negative 4 mg/mL
Methylprednisolone succinate 4 mg/mL	Excipient free	Positive 0.04 mg/mL	Negative 4 mg/mL	Negative 4 mg/mL
Triamcinolone 1mg/mL	Polysorbate 80	Positive 0.1 mg/mL	-	-
Optive ® Advanced 1;10 dilution	Polysorbate 80	-	Negative 1:10 dilution	Negative 1:10 dilution
BNT162b	PEGylated nanoparticle	Positive- 1 µg/mL	Positive- 1 µg/mL	Positive- 1 µg/mL
AZD1222	Polysorbate 80	Positive 1:10	Negative 1:10	Negative 1:10

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325 **Table 2.**

Compound	Range of allergen concentrations tested	Basophil Activation Test Results		
		Patient 1	Patient 2	Patient 3
BNT162b2	0.05 µg/ml to 10 µg/mL	5 µg/mL	0.05 µg/mL	0.05 µg/mL
PEGylated Liposomal Doxorubicin	1µg/ml to 10 µg/mL	10 µg/mL	1 µg/mL	10 µg/mL
AZD1222	1:2000 to 1:10 dilution	Negative	Negative	Negative
PEG2000	0.05 µg/mL to 5 mg/mL	Negative	Negative	Negative
PEG 200	5 mg/mL	Negative	Negative	Negative
PEG 400	5 mg/mL	Negative	Negative	Negative
PEG 600	5 mg/mL	Negative	Negative	Negative
PEG 6000	5 mg/mL	Negative	Negative	Negative

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