

Potential mechanisms of anaphylaxis to COVID-19 mRNA vaccines

Kimberly A. Risma, MD, PhD,^a Kathryn M. Edwards, MD,^b Donna S. Hummell, MD,^c Frederic F. Little, MD,^d Allison E. Norton, MD,^c Amy Stallings, MD,^e Robert A. Wood, MD,^f and Joshua D. Milner, MD^g *Cincinnati, Ohio; Nashville, Tenn; Boston, Mass; Durham, NC; Baltimore, Md; and New York, NY*

Anaphylaxis to vaccines is historically a rare event. The coronavirus disease 2019 pandemic drove the need for rapid vaccine production applying a novel antigen delivery system: messenger RNA vaccines packaged in lipid nanoparticles. Unexpectedly, public vaccine administration led to a small number of severe allergic reactions, with resultant substantial public concern, especially within atopic individuals. We reviewed the constituents of the messenger RNA lipid nanoparticle vaccine and considered several contributors to these reactions: (1) contact system activation by nucleic acid, (2) complement recognition of the vaccine-activating allergic effector cells, (3) preexisting antibody recognition of polyethylene glycol, a lipid nanoparticle surface hydrophilic polymer, and (4) direct mast cell activation, coupled with potential genetic or environmental predispositions to hypersensitivity. Unfortunately, measurement of anti-polyethylene glycol antibodies *in vitro* is not clinically available, and the predictive value of skin testing to polyethylene glycol components as a coronavirus disease 2019 messenger RNA vaccine-specific anaphylaxis marker is unknown. Even less is known regarding the applicability of vaccine use for testing (*in vitro/vivo*) to ascertain pathogenesis or predict reactivity risk. Expedient and thorough research-based evaluation of patients

who have suffered anaphylactic vaccine reactions and prospective clinical trials in putative at-risk individuals are needed to address these concerns during a public health crisis. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: COVID-19 vaccine, mRNA vaccine, anaphylaxis, allergy, polyethylene glycol, PEGylated liposome, lipid nanoparticle, mast cells

On December 8, 2020, the world watched as the first dose of coronavirus disease 2019 (COVID-19) messenger RNA (mRNA) vaccine was given in the United Kingdom. The subsequent US Food and Drug Administration emergency use authorization of both Pfizer-BioNTech and Moderna mRNA vaccines was historic, because the use of the mRNA platform had never progressed beyond phase 1 to 2 trials.¹ Encouraging preclinical data for the COVID-19 mRNA vaccines and phase 1 to 3 trial data²⁻⁸ demonstrating 95% efficacy against COVID-19 had been published. Given the public health emergency and the worldwide death count of nearly 1.8 million, a vaccine to prevent COVID-19 was critically needed.

However, within 24 hours of the first vaccination, media reported that 2 individuals had developed anaphylaxis minutes after administration of the Pfizer-BioNTech COVID-19 vaccine. By December 23, 2020, 1,893,360 first doses of Pfizer-BioNTech COVID-19 vaccine had been administered in the United States and 21 cases of anaphylaxis had been reported.⁹ One month later, the Centers for Disease Control and Prevention reported that 10 anaphylactic events had occurred out of 4,041,396 first doses of Moderna COVID-19 vaccinations.¹⁰ At the time of this writing, the rates of anaphylaxis are calculated at 5.0 cases per million for the Pfizer-BioNTech and 2.8 cases per million for the Moderna vaccine, although a minority of the country has been vaccinated. If the current vaccine reactions remain constant, the rate of anaphylaxis from COVID mRNA vaccines will be 2 to 5 times the rate of other commonly administered vaccines such as Tdap (0.51 per million) and the trivalent inactivated flu vaccine (1.35 per million) (reviewed in McNeil and DeStefano¹¹).

Previous investigations into the immune mechanisms of vaccine-associated anaphylaxis have focused on the presence of gelatin, latex, egg protein, and more recently on a widely used surfactant, polysorbate 80, present in numerous vaccines.¹² However, because none of these excipients were included in the Pfizer-BioNTech COVID-19 mRNA vaccine (Table I and Fig 1) and no cases of anaphylaxis had been observed in the large phase 2/3 clinical trials,^{7,8} this occurrence was unexpected.

The public reports of these reactions and early precautionary guidance in patients with a history of severe allergic reactions substantially alarmed our patients. It is incumbent on the allergy community to respond to these concerns. Recommendations for

From ^aCincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati; ^bthe Division of Infectious Diseases, Department of Pediatrics, Vanderbilt University Medical Center, Nashville; ^cthe Division of Pediatric Allergy, Immunology, and Pulmonary Medicine, Department of Pediatrics, Monroe Carell Jr. Children's Hospital at Vanderbilt, Vanderbilt University School of Medicine, Nashville; ^dthe Division of Pulmonary, Allergy, Sleep and Critical Care Medicine, Department of Pediatrics, Boston University School of Medicine, Boston; ^ethe Division of Pediatric Allergy and Immunology, Duke University Medical Center, Durham; ^fthe Division of Pediatric Allergy and Immunology, Johns Hopkins University School of Medicine, Baltimore; and ^gthe Department of Pediatrics, Columbia University Irving Medical Center, New York.

Disclosure of potential conflict of interest: All authors have served as consultants to the Centers for Disease Control and Prevention (CDC)-sponsored Clinical Immunization Safety Assessment (CISA) Project. This work was not supported by CISA. K. M. Edwards has grant funding from the CDC and is a consultant for IBM and Bionet and a member of the data safety and monitoring boards for Pfizer, Sanofi, Moderna, Seqirus, CEPI, Merck, X-4 Pharma, and Roche. A. Stallings has support from Regeneron Pharmaceuticals. R. A. Wood, F. F. Little, K. M. Edwards, and J. D. Milner have grant funding from the National Institutes of Health. J. D. Milner is providing expert witness testimony on trauma and hypersensitivity. R. A. Wood receives research support from Aimmune, Astellas, DBV, HAL-Allergy, Genentech, Regeneron, and Sanofi, and royalties from Up To Date. A. E. Norton and K. A. Risma have no external funding sources to declare.

Received for publication February 12, 2021; revised March 24, 2021; accepted for publication April 1, 2021.

Corresponding author: Kimberly A. Risma, MD, PhD, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229. E-mail: Kimberly.Risma@cchmc.org.

0091-6749/\$36.00

© 2021 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaci.2021.04.002>

Abbreviations used

BAT:	Basophil activation test
BST:	Basal serum tryptase
C3a:	Complement component 3a
C5a:	Complement component 5a
COVID-19:	Coronavirus disease 2019
DC:	Dendritic cell
Fc:	Fragment crystallizable region
HMW:	High-molecular-weight
LNP:	Lipid nanoparticle
mRNA:	Messenger RNA
PEG:	Polyethylene glycol
PEGylated:	Polyethylene glycol conjugated

reasonable clinical management have been published¹³ at a time when we have very limited understanding of the nature of these reactions; there also emerge a series of research-based questions that are critical to answer.

IMMEDIATE REACTIONS TO COVID-19 mRNA LIPID NANOPARTICLE VACCINES—PSEUDOALLERGIC OR ALLERGIC?

The occurrence of anaphylaxis on first exposure to the COVID-19 vaccine implies either preexisting, antibody-mediated immunity (allergic) or a pseudoallergic response independent of previous exposure. Although anaphylaxis related to known allergens is best understood via the classic paradigm of cross-linking IgE bound to fragment crystallizable region (Fc) ϵ receptors on mast cells and basophils, nonclassical pathways such as antibody-dependent activation of complement or IgG-mediated mast cell/granulocyte/platelet/basophil activation via Fc γ receptors have been described in animal models and in allergic responses to medications in humans.^{14–18} In addition, various pseudoallergic mechanisms that lead to direct activation/degranulation of mast cells (through G protein-coupled receptors or complement activation) or mast cell-independent mechanisms (stimulation of bradykinin production) causing vascular leak have been described.^{14,15} These mechanisms are summarized in Fig 2 and discussed in consideration of the COVID-19 vaccines based on what is known about the components of the vaccine.

Contact system reactions to mRNA

Naked RNA is inherently proinflammatory due to its ability to bind pathogen-associated molecular pattern receptors, and by its negative charge, RNA may directly activate proteins in the contact system.^{19,20} Exogenous nucleic acids activate factor XII of this system and lead to the subsequent production of bradykinin, causing angioedema and/or anaphylactoid reactions. To decrease reactivity and protect the nucleic acid from degradation, the mRNA in the COVID-19 vaccines have been chemically modified and packaged in “stealth” lipid nanoparticles (LNPs)¹ (Fig 1). Because the LNPs encapsulate the mRNA and are rapidly endocytosed into phagocytic cells, the mRNA payload is less likely to be the primary stimulus for the injection reactions, unless the stability of the LNP vesicle has been disrupted. The latter may occur during freeze/thaw cycles before vaccination. By design,

the LNP is disrupted when the vaccine payload is phagocytosed to the endosome, allowing the mRNA to escape to the cytoplasm.

To further our understanding of vaccine reactions, the extent to which the mRNA may be liberated acutely on injection should be examined. Measuring intact and cleaved high-molecular-weight (HMW) kininogen in blood samples after a vaccine reaction may help determine whether the contact system pathway is activated during these acute events.²¹ Assessments will require a prospective approach to capture rare events, although mild reactions may also be informative. Animal models would certainly be useful.

Direct activation of mast cells by the LNP

The direct activation of mast cells or basophils leads to degranulation via various receptors including opioid receptors, Mas-related G protein-coupled receptor X2 receptors, and other yet-to-be-defined receptors for contrast agents.^{14,15} Because mast cells are poised to respond to pathogen danger signals, it is feasible that connective tissue mast cells in the muscle may degranulate in response to interaction with the LNP. A recent publication described efficient transfection of human mast cells using an LNP delivery system,²² presumably via phagocytosis, suggesting that the mast cells may take up the COVID mRNA vaccines. After phagocytosis of the LNP, a dispersed component of the vaccine may directly stimulate mast cell degranulation. Alternatively, the disruption of the mast cell endosome by the phagocytosed LNP may also lead to mast cell activation. Precedent to support the latter hypothesis comes from observations noted during intracellular listeria monocytogenes infection of mast cells. *In vitro* experiments demonstrated that incubation of listeria with mast cells led to measurable degranulation, potentially related to disruption of the phagolysosome and/or the direct activity of the listeriolysin O toxin.²³ To our knowledge, neither the Pfizer-BioNTech vaccine nor the Moderna mRNA vaccine has been tested *in vitro* for its ability to degranulate mast cells, platelets, or other granulocytes.

Complement-mediated reactions to LNP

The LNP is composed of an ionizable lipid bearing a positive charge at low pH that neutralizes the negative charge of the mRNA (Fig 1 and Table I) (reviewed in Pardi et al¹ and Cullis and Hope²⁴). In addition, the LNP includes neutral lipids and cholesterol that self-assemble into a core lipid structure with a surface layer that mimics a cell membrane. Finally, the LNP incorporates a phospholipid conjugated to polyethylene glycol (PEG) to increase the hydrophilicity of the LNP surface and to provide stability to the mRNA carrier. Historically, PEG has been used to decrease the immunogenicity of proteins and nucleic acids administered as pharmaceuticals.²⁵

Doxorubicin was the first pharmaceutical delivered in a PEGylated liposome (Doxil) to be approved by the US Food and Drug Administration in 1995. Liposomal preparations containing doxorubicin without PEG were rapidly cleared by the reticular endothelial system, limiting utility.²⁶ Inclusion of 5% molar PEG led to substantially improved stability. However, reports of immediate hypersensitivity reactions to Doxil followed in 1996.²⁷ Pseudoallergic reactions to Doxil were also subsequently demonstrated in porcine models, and were labeled as complement activation-related pseudoallergic reactions.²⁸ Doxil

TABLE I. Components of mRNA vaccines

Description	Pfizer-BioNTech COVID-19 vaccine	Moderna COVID-19 vaccine
mRNA	Nucleoside-modified mRNA encoding the receptor-binding domain of viral spike (S1) glycoprotein and encoding T4 fibrin to achieve trimerization	Nucleoside-modified mRNA encoding the viral spike (S2) glycoprotein
Lipids		
PEGylated	2[(polyethylene glycol)-2000]- <i>N,N</i> -ditetradecylacetamide	PEG 2000 dimyristoyl glycerol
Ionizable	(4-Hydroxybutyl)azanediy(bis(hexane-6,1-diyl)bis(2-hexyldecanoate)	SM-102 (Proprietary)
Neutral	1,2-Distearoyl-sn-glycero-3-phosphocholine Cholesterol	

Neither vaccine contains eggs, gelatin, latex, or preservatives.

	LNP component	Predicted Immunogenicity	µg/dose Pfizer-BioNtech	µg/dose Moderna
	PEG-lipid	Preexisting Anti-PEG IgM, IgG and/or IgE	50 µg	Total lipid dose is 1930 µg
	Ionizable lipid	Pathogen-associated molecular pattern receptors	430 µg	
	neutral lipid (DSPC)	Complement activation	90 µg	
	cholesterol		200 µg	
	mRNA	Pathogen-associated molecular pattern receptors and Factor XII activation	30 µg	100 µg

FIG 1. Components of COVID-19 mRNA LNP listed in Pfizer-BioNTech and Moderna COVID-19 vaccine package inserts. DSPC, 1,2-Distearoyl-sn-glycero-3-phosphocholine.

infusions led to the production of anaphylatoxins complement component 3a (C3a) and complement component 5a (C5a), which activated mast cells, resulting in severe hypotension and pulmonary hypertension in pigs. Humans experiencing infusion reactions to Doxil also showed evidence of complement activation, assessed by measurement of sC5b-9 in patient serum 10 minutes after infusion.²⁹ These patients were not known to have preexisting antibodies against PEG,³⁰ suggesting that the Doxil liposomes directly triggered their alternative pathway of complement.

Measurement of the intravascular production of complement split products could provide information about the involvement of complement in postvaccine hypersensitivity responses. To reflect the production of these mediators *in vivo*, specimens for sC5b-9, C3a *desArg*, and/or C5a *desArg* should be collected in EDTA tubes, which prevents ongoing activation of complement. Although these assays may certainly be useful as a research tool, because of the inherent instability of the complex, they require flash freezing of plasma on dry ice and storage at -60°C to -80°C for shipment, thus limiting clinical utility.

NONCLASSICAL ALLERGIC REACTIONS TO THE LNP

Allergic reactions to LNPs are also possible if there has been previous formation of antibodies (IgM, IgG, or IgE) against a component of the LNP. To date, the only anti-LNP antibodies that have been identified in animal models or humans are directed toward the PEG polymer shielding the LNP surface (reviewed by Yang and Lai³¹). The repeating structural elements of PEG on the surface of the LNP would certainly create an ideal immunogen for anti-PEG IgM-binding complement and/or IgE/IgG crosslinking Fc receptors on mast cells, neutrophils, or platelets (Fig 3).

The first documentation that antibody could form against PEG in humans came from the observation in 2005 that polyethylene glycol conjugated (PEGylated) uricase (pegloticase) administered in phase 1 trials was associated with the subsequent development of anti-PEG IgM and IgG antibodies.^{32,33} Anti-PEG antibodies have also been identified in individuals given PEG asparaginase for chemotherapy, and high-titer, preexisting antibodies have been associated with adverse reactions on first infusions in children with leukemia.^{34,35} The proposed mechanism

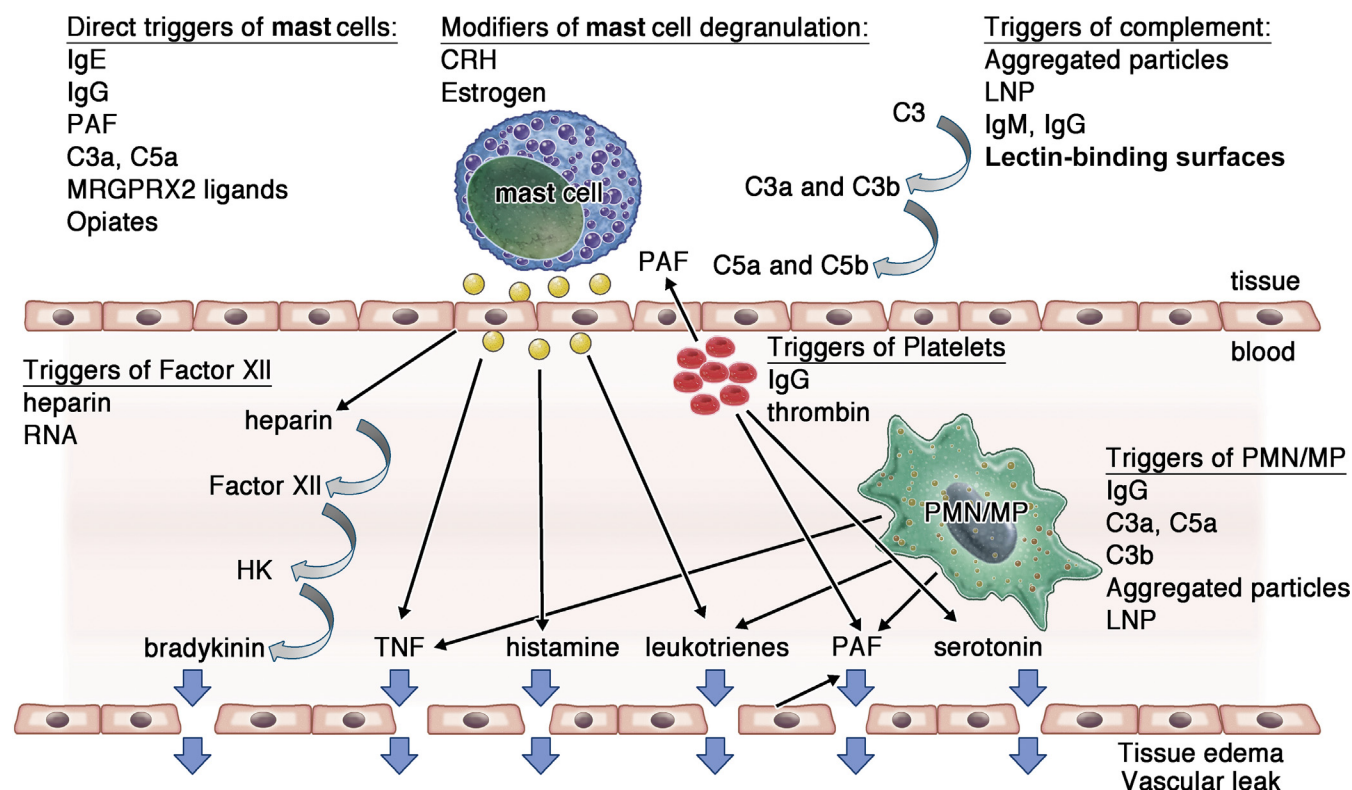


FIG 2. Potential allergic and pseudoallergic triggers and modifiers of anaphylaxis. CRH, Corticotropin-releasing hormone; HK, high-molecular-weight kininogen; MP, macrophage; MRGPRX2, Mas-related G protein-coupled receptor X2; PAF, platelet-activating factor; PMN, polymorphonuclear cell.

is a nonclassical pathway whereby IgM (or potentially IgG) activates complement and mast cells degranulate in response to C3a and/or C5a anaphylatoxins. Alternatively, IgG could bind to Fcγ receptors on granulocytes and/or platelets, leading to secretion of serotonin, cytokines, and platelet-activation factor, with subsequent vascular leak. Mast cells may degranulate in response to crosslinked IgG as demonstrated *in vitro*.³⁶ It is also possible that these infusion reactions are IgE-mediated, although anti-PEG IgE were not evaluated in these trials.

Infusion reactions reported for other PEG-containing liposomes have limited clinical usage. For example, PEGylated liposomes were evaluated for delivery of RNA aptamers, but phase 2/3 trials were halted because of an unacceptably high rate of anaphylaxis occurring on first exposure, associated with preexisting anti-PEG antibody.^{37,38} Both IgM and IgG anti-PEG antibodies were documented in these patients; tryptase was elevated in 6 of 11 patients with severe reactions, and complement C3a was also elevated at 90 minutes. Unfortunately, the authors did not report whether both the C3a and tryptase elevation occurred in the same patients.³⁷

Studies are urgently needed that prospectively and retrospectively measure antibodies (IgM, IgG, and IgE) against PEG. Unfortunately, anti-PEG antibody (IgG, IgM, and IgE) measurements are not yet available for routine clinical testing. A criterion standard ELISA has not been established,³⁹ which likely explains the reported differences in measurement of anti-PEG antibodies in healthy volunteers, ranging from 5% to 70% depending on the assay and the cutoffs used by individual research laboratories.^{34,35,37,40,41}

As a side note, although the existence of preexisting IgM and/or IgG antibodies against the LNP may adversely lead to nonclassical allergic reactions, they may also lead to enhanced efficacy of the vaccine. Preexisting IgG and IgM may enhance dendritic cell uptake of LNPs through Fc receptors or complement receptors on dendritic cells (Fig 3), leading to increased delivery of mRNA to the cytoplasm, increased spike protein expression, and the capacity for enhanced presentation to T cells. The data from phase 2/3 trials of COVID mRNA vaccines reveal remarkable efficacy, preventing 94% to 95% of infections.^{7,8} If preexisting, low-titer, anti-PEG antibodies are as high as 70% in the general population, as reported by some investigators,⁴¹ these antibodies may potentially contribute to immunogenicity/effectiveness.

CLASSICAL ALLERGIC REACTIONS: CAN PEG STIMULATE IgE PRODUCTION?

Recent evidence suggests that reactions to PEG may also be IgE-mediated. An increasing number of case reports of individuals suffering anaphylaxis after exposure to PEG in bowel preparations and injectable products have documented positive skin prick test results to HMW PEG or structurally related polysorbates.⁴²⁻⁴⁷ In addition, both skin prick testing and intradermal testing have led to systemic allergic symptoms (see Table E1 in this article's Online Repository at www.jacionline.org), strongly pointing to an IgE-mediated mechanism. The estimated prevalence of IgE-mediated reactions to PEG is unknown. One recent study by Stone et al reviewed 25,905 reports of anaphylactic events to the US Food and Drug Administration and found

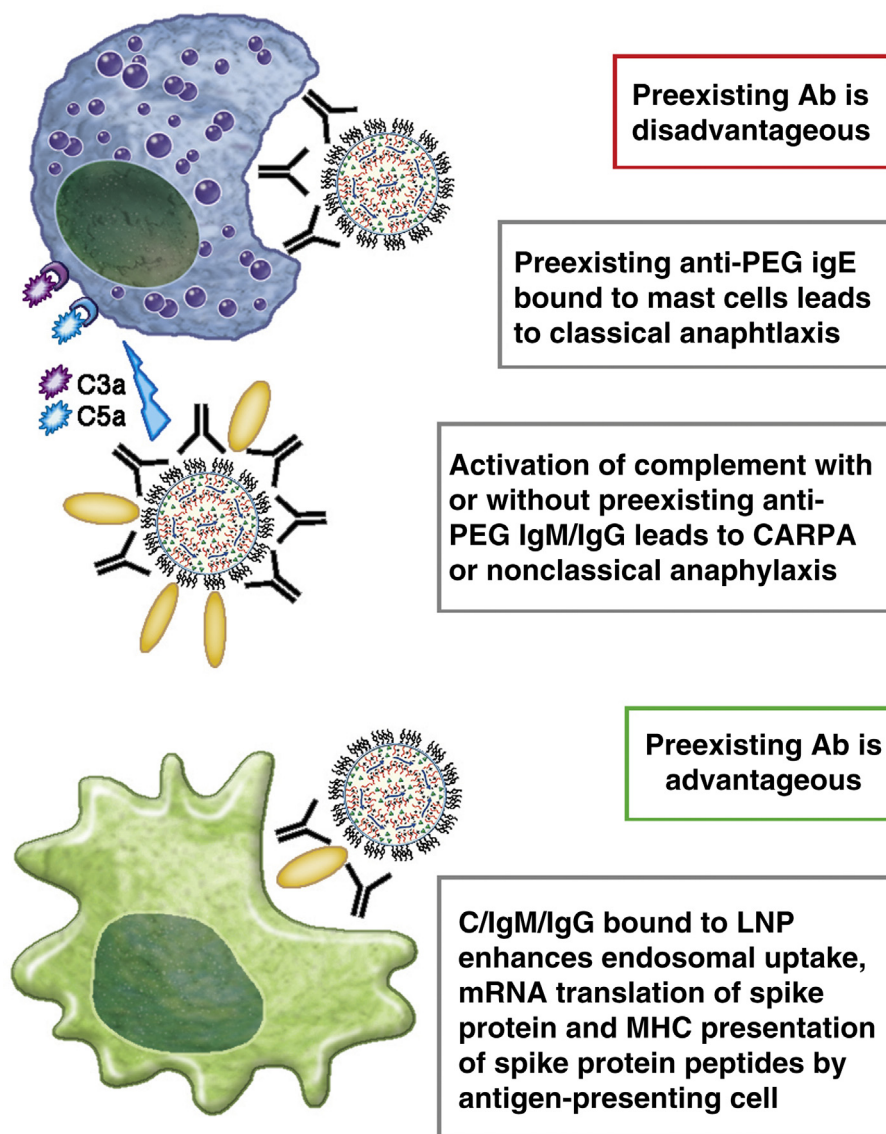


FIG 3. Preexisting antibodies against PEG—a threat for anaphylaxis and an immunologic advantage for vaccine efficacy. Yellow ovals represent complement (C) deposition. Ab, Antibody; CARPA, complement activation-related pseudoallergic reactions.

53 reports with unique case identifiers for anaphylaxis and PEG-containing products, with an estimate of 4 per year during the range 2005 to 2017 (range, 2-8 per year).⁴⁴

In consideration of classical allergic reactions to the COVID-19 mRNA vaccines, it is critical to evaluate the sensitivity and specificity of skin prick test to both HMW PEG and the vaccines themselves. Skin prick testing with PEG with molecular weight 3350 has been suggested by Banerji et al¹³ as a starting point for evaluation of anaphylactic reactions to the mRNA COVID-19 vaccines because PEG 3350 is readily available in the United States. Although the exact threshold of reactivity based on molecular weight of PEG is not exactly known, skin testing with PEGs in ranges of molecular weights from 400 to 20,000 has demonstrated reactivity in those with documented anaphylaxis to PEG 3350.⁴⁵

Skin testing to the vaccine is ideal and should be performed by prospectively and retrospectively testing individuals who have

reacted and those who have not reacted to the COVID-19 mRNA vaccines when the vaccine becomes available for testing.

The finding of IgE directed against PEG in individuals who have not previously received PEGylated proteins or PEGylated liposomes is quite surprising because class switching to IgE implies T-cell engagement. Because of its “inert” biochemical properties,²⁵ environmental exposure to unconjugated PEG would not be expected to lead to immunogenic, PEG-hapten-carrier proteins. As such, most anti-PEG antibodies are theorized to arise from T-independent B-cell production of IgM and IgG³¹ and the formation of IgE would be predictably rare. This uncommon immunologic occurrence could certainly account for the rarity of the current vaccine reactions, whereas pseudoallergic or nonclassical allergic reactions may be anticipated to occur more frequently.

Most recently, IgE against PEG was detected in the blood of a patient who suffered immediate reactions to each of 3 different

medications containing PEG—Definity liposomes, oral bowel prep, and steroid injection.⁴⁷ Anti-PEG IgE was measured by 2 independent immunoassays—chemiluminescent-based and dual cytometric bead assays.^{40,47} Zhou et al⁴⁰ showed that 6 additional patients with a history of reactions to HMW PEG had detectable IgE.⁴⁷ Interestingly, IgE was also identified in 2 of 2091 serum samples when screening healthy controls, suggesting that allergic sensitization may be more common than expected⁴⁰; however, these blood samples were not tested for their capacity to trigger basophil or mast cell activation.

Previous case reports of individuals with a history of PEG allergy have shown variable results with the basophil activation test (BAT), using HMW PEG or polysorbate 80 as an allergen.^{43,48,49} Although the BAT can be an extremely helpful flow cytometry assay to document both the reactivity and sensitivity of basophils to allergens *in vitro*, it is not yet available as a clinical test.⁵⁰ The BAT is certainly useful in research studies, with 2 main limitations—the need for testing fresh blood and the finding of nonreactive basophils in up to 20% of individuals. These limitations can be overcome using a mast cell activation test, more recently described by applying patient serum or plasma to healthy donor blood–derived mast cells or immortalized human mast cells and measuring degranulation by flow cytometry.^{51,52} The advantage of the mast cell activation test is that blood samples can be frozen and shipped to a research laboratory and the cultured mast cells may be confirmed to degranulate before experimentation.

A key set of experiments for evaluating COVID-19 mRNA vaccine reactions is the use of the BAT and/or mast cell activation test assays to determine whether the vaccine activates patient basophils or donor mast cells directly as outlined above or activates only in the presence of serum from the affected individual, the latter implying a mechanism of IgE-mediated degranulation, readily tested by blocking IgE.

Host factors leading to mast cell hyperresponsiveness

Genetic and environmental modifiers of mast cell activation in patients with vaccine reactions may also be considered. It should be noted that the individuals experiencing anaphylactic reactions to the COVID-19 mRNA vaccines have been strikingly female.^{9,10} Drug allergy and drug-induced anaphylaxis is more common in adult females than in males, with this difference emerging after puberty (reviewed by Eaddy Norton and Broyles⁵³). Few studies have examined these differences in drug allergy. The skewing of the allergic response to the COVID mRNA vaccine toward the female sex may be secondary to estrogen effects in promoting a T_H2 response, or conversely, testosterone and progesterone's known role in diminishing T_H2 responses.^{54,55} In addition, sex hormones may influence mast cell degranulation; although estrogen is thought to be stimulatory, studies demonstrate that progesterone suppresses histamine release from mast cells.^{55,56} Estrogen has also been demonstrated to increase endothelial nitric oxide synthase activity, enhancing the severity of anaphylaxis in murine studies.⁵⁷ An investigation into the discrepant role of sex hormones in this setting is critical for understanding the pathogenesis and potentially developing tools to screen for or prevent reactions.

An interesting observation is that atopic individuals also appear to be overrepresented in those suffering anaphylaxes to the

COVID mRNA vaccines.^{9,10} The common past histories of allergic reactions in those who have COVID-19 vaccine anaphylaxis need to be carefully curated to determine the type of reaction and associated with triggers. This inquiry might point to a predisposition for hyperresponsiveness to direct mast cell activation via these pathways.

Another host factor that may impact the likelihood of anaphylaxis is stress, particularly relevant during a global pandemic. Corticotropin-releasing hormone and neurotensin are secreted by neurons in response to acute and chronic stress and they lower the threshold for mast cell degranulation.⁵⁸ Substance P is also released by neurons adjacent to mast cells and leads to degranulation during a stress response.⁵⁹ Finally, the use of opiates or nonsteroidal anti-inflammatory drugs may enhance mast cell activation and/or vascular responsiveness,^{14,15} thus emphasizing the importance of a detailed history of medications taken before vaccination.

In addition to evaluating mechanisms and modifiers of anaphylaxis, predisposing disease conditions should be explored. Mastocytosis and other forms of clonal mast cell expansion can present with anaphylaxis alone, without any other associated comorbidities. This is best described in the context of hymenoptera venom hypersensitivity⁶⁰ but could be relevant for the vaccine reactions as well. Although a few patients with mastocytosis have tolerated the mRNA vaccine,⁶¹ this condition may still contribute to risk in some. Elevated basal serum tryptase (BST) can be a helpful clue and should be measured in all individuals with COVID-19 vaccine-related anaphylaxis. Although a normal BST does not exclude mastocytosis, establishing the pattern of BST in a critical mass of these patients would point to a need for further workup, including peripheral blood D816V KIT mutation measurement and, if clinically indicated, bone marrow biopsy examination.

Idiopathic mast cell activation syndrome refers to those with clinical and laboratory evidence of mast cell activation in the absence of mastocytosis.^{62,63} These patients can present with substantial histories of hypersensitivity and anaphylaxis, including to injectables. When possible, it should be determined whether individuals with severe immediate reactions to the vaccine have a clinical history of symptoms of mast cell activation and response to mediator blockade, along with documentation of elevated mediators during disease flares or reactions.

Genetic predisposition to anaphylaxis could provide another explanation for these cases. A common genetic trait—increased copy number of alpha tryptase at TPSAB1 causing hereditary alpha tryptasemia—is present in 5% of certain populations.⁶⁴ Hereditary alpha tryptasemia is significantly enriched among those with idiopathic anaphylaxis, severe hymenoptera reactions, mastocytosis, and even anaphylaxis within the context of mastocytosis.^{65,66} Genotyping can be performed to identify whether the vaccine anaphylaxis population is enriched for those with hereditary alpha tryptasemia, and a BST level of higher than 8 ng/mL can be highly suggestive as well. In addition, the recent report of a rare missense mutation in KARS provides an example of a rare monogenic predisposition to severe anaphylaxis.⁶⁷ Similar findings may be noted, whether in KARS or other rare, yet undiscovered variants, in this patient cohort. Whole-genome sequencing of individuals with reactions would be critical to identify known or novel rare variants associated with this unique hypersensitivity/anaphylaxis.

CONCLUSIONS

The high efficacy of mRNA LNP vaccination against COVID-19 in phase 2/3 clinical trials and the rapid successful mobilization of a useful vaccine suggests that the use of this technology is likely to revolutionize future vaccine approaches. The ability to generate a pandemic vaccine in less than a year for mass production is extraordinary, particularly when directed against RNA viruses, which undergo continuous mutation. Thus, it will be prudent to learn from the current worldwide vaccination efforts—not only to understand the mechanisms of anaphylaxis but also to develop strategies to identify risk factors for immediate reactions, identify sensitive and specific mechanisms for diagnosis, and risk stratification for future vaccination. Because of limited availability of clinical tools to assess for allergic responses to vaccines and the likelihood that nonclassical allergic responses and/or pseudoallergic responses contribute to COVID-19 mRNA vaccine reactions, research studies are imperative.

REFERENCES

- Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov* 2018;17:261-79.
- Walsh EE, Frencik RW Jr, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N Engl J Med* 2020;383:2439-50.
- Laczko D, Hogan MJ, Toulmin SA, Hicks P, Lederer K, Gaudette BT, et al. A single immunization with nucleoside-modified mRNA vaccines elicits strong cellular and humoral immune responses against SARS-CoV-2 in mice. *Immunity* 2020;53:724-32.e7.
- Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* 2020;586:567-71.
- Jackson LA, Anderson EJ, Roupheal NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. *N Engl J Med* 2020;383:1920-31.
- Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 2020;586:589-93.
- Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020;383:2603-15.
- Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021;384:403-16.
- Shimabukuro T, Nair N. Allergic reactions including anaphylaxis after receipt of the first dose of Pfizer-BioNTech COVID-19 vaccine. *JAMA* 2021;325:780-1.
- CDC COVID-19 Response Team; Food and Drug Administration. Allergic reactions including anaphylaxis after receipt of the first dose of Moderna COVID-19 vaccine — United States, December 21, 2020–January 10, 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:125-9.
- McNeil MM, DeStefano F. Vaccine-associated hypersensitivity. *J Allergy Clin Immunol* 2018;141:463-72.
- Stone CA Jr, Rukasin CRF, Beachkofsky TM, Phillips EJ. Immune-mediated adverse reactions to vaccines. *Br J Clin Pharmacol* 2019;85:2694-706.
- Banerji A, Wickner PG, Saff R, Stone CA Jr, Robinson LB, Long AA, et al. mRNA vaccines to prevent COVID-19 disease and reported allergic reactions: current evidence and suggested approach. *J Allergy Clin Immunol Pract* 2021;9:1423-37.
- Finkelman FD, Khodoun MV, Strait R. Human IgE-independent systemic anaphylaxis. *J Allergy Clin Immunol* 2016;137:1674-80.
- Jimenez-Rodriguez TW, Garcia-Neuer M, Alenazy LA, Castells M. Anaphylaxis in the 21st century: phenotypes, endotypes, and biomarkers. *J Asthma Allergy* 2018;11:121-42.
- Jonsson F, de Chaisemartin L, Granger V, Gouel-Cheron A, Gillis CM, Zhu Q, et al. An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis. *Sci Transl Med* 2019;11:eaat1479.
- Balbino B, Herviou P, Godon O, Stackowicz J, Goff OR, Iannascoli B, et al. The anti-IgE mAb omalizumab induces adverse reactions by engaging Fcγγ receptors. *J Clin Invest* 2020;130:1330-5.
- Beutier H, Hechler B, Godon O, Wang Y, Gillis CM, de Chaisemartin L, et al. Platelets expressing IgG receptor FcγγRIIA/CD32A determine the severity of experimental anaphylaxis. *Sci Immunol* 2018;3:eaan5997.
- Preissner KT, Fischer S, Deindl E. Extracellular RNA as a versatile DAMP and alarm signal that influences leukocyte recruitment in inflammation and infection. *Front Cell Dev Biol* 2020;8:619221.
- Bender L, Weidmann H, Rose-John S, Renne T, Long AT. Factor XII-driven inflammatory reactions with implications for anaphylaxis. *Front Immunol* 2017;8:1115.
- Sala-Cunill A, Bjorkqvist J, Senter R, Guilarte M, Cardona V, Labrador M, et al. Plasma contact system activation drives anaphylaxis in severe mast cell-mediated allergic reactions. *J Allergy Clin Immunol* 2015;135:1031-43.e6.
- Duguay BA, Huang KW, Kulka M. Lipofection of plasmid DNA into human mast cell lines using lipid nanoparticles generated by microfluidic mixing. *J Leukoc Biol* 2018;104:587-96.
- Garcia-Rodriguez KM, Bahri R, Sattentau C, Roberts IS, Goenka A, Bulfone-Paus S. Human mast cells exhibit an individualized pattern of antimicrobial responses. *Immun Inflamm Dis* 2020;8:198-210.
- Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. *Mol Ther* 2017;25:1467-75.
- Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. *Nat Rev Drug Discov* 2003;2:214-21.
- Barenholz Y. Doxil(R)—the first FDA-approved nano-drug: lessons learned. *J Control Release* 2012;160:117-34.
- Alberts DS, Garcia DJ. Safety aspects of pegylated liposomal doxorubicin in patients with cancer. *Drugs* 1997;54:30-5.
- Szebeni J, Muggia F, Gabizon A, Barenholz Y. Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: prediction and prevention. *Adv Drug Deliv Rev* 2011;63:1020-30.
- Chanan-Khan A, Szebeni J, Savay S, Liebes L, Rafique NM, Alving CR, et al. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. *Ann Oncol* 2003;14:1430-7.
- Moghimi SM, Andersen AJ, Hashemi SH, Lettieri B, Ahmadvand D, Hunter AC, et al. Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: the challenges ahead. *J Control Release* 2010;146:175-81.
- Yang Q, Lai SK. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2015;7:655-77.
- Ganson NJ, Kelly SJ, Scarlett E, Sundy JS, Hershfield MS. Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly(ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase. *Arthritis Res Ther* 2006;8:R12.
- Garay RP, El-Gewely R, Armstrong JK, Garratty G, Richette P. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. *Expert Opin Drug Deliv* 2012;9:1319-23.
- Liu Y, Smith CA, Panetta JC, Yang W, Thompson LE, Counts JP, et al. Antibodies predict pegaspargase allergic reactions and failure of rechallenge. *J Clin Oncol* 2019;37:2051-61.
- Khalil A, Wurthwein G, Golitsch J, Hempel G, Fobker M, Gerss J, et al. Pre-existing antibodies against polyethylene glycol reduce asparaginase activities on first administration of pegylated *E. coli* asparaginase in children with acute lymphocytic leukemia [published online ahead of print December 10, 2020]. *Haematologica*. <https://doi.org/10.3324/haematol.2020.258525>.
- Woolhiser MR, Okayama Y, Gilfillan AM, Metcalfe DD. IgG-dependent activation of human mast cells following up-regulation of FcγγRI by IFN-γγ. *Eur J Immunol* 2001;31:3298-307.
- Povsic TJ, Lawrence MG, Lincoff AM, Mehran R, Rusconi CP, Zelenkofske SL, et al. Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer. *J Allergy Clin Immunol* 2016;138:1712-5.
- Ganson NJ, Povsic TJ, Sullenger BA, Alexander JH, Zelenkofske SL, Sailstad JM, et al. Pre-existing anti-polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer. *J Allergy Clin Immunol* 2016;137:1610-3.e7.
- Kozma GT, Shimizu T, Ishida T, Szebeni J. Anti-PEG antibodies: properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals. *Adv Drug Deliv Rev* 2020;154:5:163-75.
- Zhou ZH, Stone CA Jr, Jakubovic B, Phillips EJ, Sussman G, Park J, et al. Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. *J Allergy Clin Immunol Pract* 2021;9:1731-3.e3.
- Yang Q, Jacobs TM, McCallen JD, Moore DT, Huckaby JT, Edelstein JN, et al. Analysis of pre-existing IgG and IgM antibodies against polyethylene glycol (PEG) in the general population. *Anal Chem* 2016;88:11804-12.
- Wylon K, Dolle S, Worm M. Polyethylene glycol as a cause of anaphylaxis. *Allergy Asthma Clin Immunol* 2016;12:67.

43. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. *Clin Exp Allergy* 2016;46:907-22.
44. Stone CA Jr, Liu Y, Relling MV, Krantz MS, Pratt AL, Abreo A, et al. Immediate hypersensitivity to polyethylene glycols and polysorbates: more common than we have recognized. *J Allergy Clin Immunol Pract* 2019;7:1533-40.e8.
45. Sellaturay P, Nasser S, Ewan P. Polyethylene glycol-induced systemic allergic reactions (anaphylaxis). *J Allergy Clin Immunol Pract* 2021;9:670-5.
46. Lu IN, Rutkowski K, Kennard L, Nakonechna A, Mirakian R, Wagner A. Polyethylene glycol may be the major allergen in depot medroxy-progesterone acetate. *J Allergy Clin Immunol Pract* 2020;8:3194-7.
47. Krantz MS, Liu Y, Phillips EJ, Stone CA Jr. Anaphylaxis to PEGylated liposomal echocardiogram contrast in a patient with IgE-mediated macrogol allergy. *J Allergy Clin Immunol Pract* 2020;8:1416-9.e3.
48. Giangrande N, Garcia-Menaya JM, Marcos-Fernandez M, Camara-Hijon C, Bobadilla-Gonzalez P. Anaphylaxis due to macrogol in a laxative solution with a positive basophil activation test. *Ann Allergy Asthma Immunol* 2019;123:302-4.
49. Jover Cerda V, Rodríguez Pacheco R, Domenech Witek J, Marco de la Calle FM, de la Sen Fernandez ML. Immediate hypersensitivity to polyethylene glycols in unrelated products: when standardization in the nomenclature of the components of drugs, cosmetics, and food becomes necessary. *Allergy Asthma Clin Immunol* 2019;15:9.
50. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: mechanisms and considerations for use in clinical trials and clinical practice [published online ahead of print January 21, 2021]. *Allergy*. <https://doi.org/10.1111/all.14747>.
51. Bahri R, Custovic A, Korosec P, Tsoumani M, Barron M, Wu J, et al. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. *J Allergy Clin Immunol* 2018;142:485-96.e16.
52. Santos AF, Couto-Francisco N, Becares N, Kwok M, Bahnson HT, Lack G. A novel human mast cell activation test for peanut allergy. *J Allergy Clin Immunol* 2018;142:689-91.e9.
53. Eaddy Norton A, Broyles AD. Drug allergy in children and adults: is it the double X chromosome? *Ann Allergy Asthma Immunol* 2019;122:148-55.
54. Trigunaite A, Dimo J, Jorgensen TN. Suppressive effects of androgens on the immune system. *Cell Immunol* 2015;294:87-94.
55. Fan Z, Che H, Yang S, Chen C. Estrogen and estrogen receptor signaling promotes allergic immune responses: effects on immune cells, cytokines, and inflammatory factors involved in allergy. *Allergol Immunopathol (Madr)* 2019;47:506-12.
56. Vasiadi M, Kempuraj D, Boucher W, Kalogeromitros D, Theoharides TC. Progesterone inhibits mast cell secretion. *Int J Immunopathol Pharmacol* 2006;19:787-94.
57. Hox V, Desai A, Bandara G, Gilfillan AM, Metcalfe DD, Olivera A. Estrogen increases the severity of anaphylaxis in female mice through enhanced endothelial nitric oxide synthase expression and nitric oxide production. *J Allergy Clin Immunol* 2015;135:729-36.e5.
58. Alysandratos KD, Asadi S, Angelidou A, Zhang B, Sismanopoulos N, Yang H, et al. Neurotensin and CRH interactions augment human mast cell activation. *PLoS One* 2012;7:e48934.
59. Theoharides TC. The impact of psychological stress on mast cells. *Ann Allergy Asthma Immunol* 2020;125:388-92.
60. Bonadonna P, Zanotti R, Muller U. Mastocytosis and insect venom allergy. *Curr Opin Allergy Clin Immunol* 2010;10:347-53.
61. Rama TA, Moreira A, Castells M. mRNA COVID-19 vaccine is well tolerated in patients with cutaneous and systemic mastocytosis with mast cell activation symptoms and anaphylaxis. *J Allergy Clin Immunol* 2021;147:877-8.
62. Akin C, Valent P, Metcalfe DD. Mast cell activation syndrome: proposed diagnostic criteria. *J Allergy Clin Immunol* 2010;126:1099-104.e4.
63. Picard M, Giavina-Bianchi P, Mezzano V, Castells M. Expanding spectrum of mast cell activation disorders: monoclonal and idiopathic mast cell activation syndromes. *Clin Ther* 2013;35:548-62.
64. Lyons JJ, Yu X, Hughes JD, Le QT, Jamil A, Bai Y, et al. Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. *Nat Genet* 2016;48:1564-9.
65. Lyons JJ, Chovanec J, O'Connell MP, Liu Y, Selb J, Zanotti R, et al. Heritable risk for severe anaphylaxis associated with increased alpha-tryptase-encoding germline copy number at TPSAB1. *J Allergy Clin Immunol* 2021;147:622-32.
66. Greiner G, Sprinzl B, Gorska A, Ratzinger F, Gurbisz M, Witzeneder N, et al. Hereditary alpha tryptasemia is a valid genetic biomarker for severe mediator-related symptoms in mastocytosis. *Blood* 2021;137:238-47.
67. Ribo P, Guo Y, Aranda J, Ainsua-Enrich E, Navines-Ferrer A, Guerrero M, et al. Mutation in Kars: a novel mechanism for severe anaphylaxis [published online ahead of print December 29, 2020]. *J Allergy Clin Immunol*. <https://doi.org/10.1016/j.jaci.2020.12.637>.

REFERENCES

- E1. Wylon K, Dolle S, Worm M. Polyethylene glycol as a cause of anaphylaxis. *Allergy Asthma Clin Immunol* 2016;12:67.
- E2. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. *Clin Exp Allergy* 2016;46:907-22.
- E3. Stone CA Jr, Liu Y, Relling MV, Krantz MS, Pratt AL, Abreo A, et al. Immediate hypersensitivity to polyethylene glycols and polysorbates: more common than we have recognized. *J Allergy Clin Immunol Pract* 2019;7:1533-40.e8.
- E4. Sellaturay P, Nasser S, Ewan P. Polyethylene glycol-induced systemic allergic reactions (anaphylaxis). *J Allergy Clin Immunol Pract* 2021;9:670-5.
- E5. Lu IN, Rutkowski K, Kennard L, Nakonechna A, Mirakian R, Wagner A. Polyethylene glycol may be the major allergen in depot medroxy-progesterone acetate. *J Allergy Clin Immunol Pract* 2020;8:3194-7.

TABLE E1. Reports of PEG skin testing associated with systemic allergic reactions

Author, date, country	Patients	Implicated drug(s)	Skin test results	Systemic symptoms on skin testing
Wylon et al, ^{E1} 2016, Germany	1 woman	<ul style="list-style-type: none"> ● DMPA contains 2.9% PEG 3350 and PS80 ● Joint injection of lidocaine, bupivacaine, triamcinolone (PEG 4000 and PS80) ● Oral medication, PEG 6000 ● Various HMW PEG products (3350-20,000), oral, vaginal, and injection 	DMPA: (–) on SPT 1% PS80: (–) on SPT/ID 10% PEG 3350: (–) on SPT 1% and 10% PEG 3350: (+) on ID	Patient developed systemic allergic symptoms during ID skin test
Wenande and Garvey, ^{E2} 2016, Denmark	14 women 23 men	<ul style="list-style-type: none"> ● Various HMW PEG products (3350-20,000), oral, vaginal, and injection 	Variety of HMW PEG products (3350-20,000): 19 of 22 patients tested (+) on SPT 0.0001%-10% HMW PEG: 4 of 5 patients tested (+) to ID	2 patients had systemic allergic reactions during SPT 3 patients had systemic allergic symptoms during ID skin test
Stone et al, ^{E3} 2019, United States	2 men	<ul style="list-style-type: none"> ● PEG 3350 bowel preparations ● MPA contains 2.8% PEG 3350 	First patient: 0.17%-17% PEG 3350: (+) on SPT PS80 in various preparations: (+) on ID Second patient: 0.17%-17% PEG 3350: (–) SPT MPA: (–) SPT/ID Triamcinolone acetonide preparation containing PS80: (+) on ID	First patient developed systemic allergic symptoms during ID skin test Second patient had systemic allergic symptoms to oral challenge PEG 3350 despite negative skin test results
Sellaturay et al, ^{E4} 2021, United Kingdom	4 women 1 man	<ul style="list-style-type: none"> ● Various HMW PEG products (3350-20,000), oral and injection 	Variety of HMW PEG products (3350-20,000): 3 of 5 patients tested (+) on SPT 1% PEG 20,000: 1 of 2 patients tested (+) on ID	1 patient developed systemic allergic symptoms during SPT 2 patients developed systemic allergic symptoms during ID skin test
Lu et al, ^{E5} 2020, United Kingdom	15 women	<ul style="list-style-type: none"> ● DMPA contains 2.9% PEG 3350 and PS80 	DMPA: 2 of 12 patients tested (+) on SPT DMPA diluted 1:100 or 1:10: 4 of 9 patients tested (+) on ID 10% PEG 3350: 5 of 12 tested (+) on SPT 0.1 or 1% PEG 3350: 2 of 2 tested (+) on ID	2 of 2 patients developed systemic allergic symptoms during ID skin test

DMPA, Depo-medroxyprogesterone acetate; ID, intradermal; MPA, methylprednisolone acetate; PS80, polysorbate 80; SPT, skin prick test.