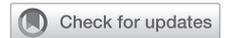


Potential mechanisms of anaphylaxis to COVID-19 mRNA vaccines



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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;147:2075-82.)

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

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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

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

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 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 lysteriolysin 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

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)azanediyl)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.

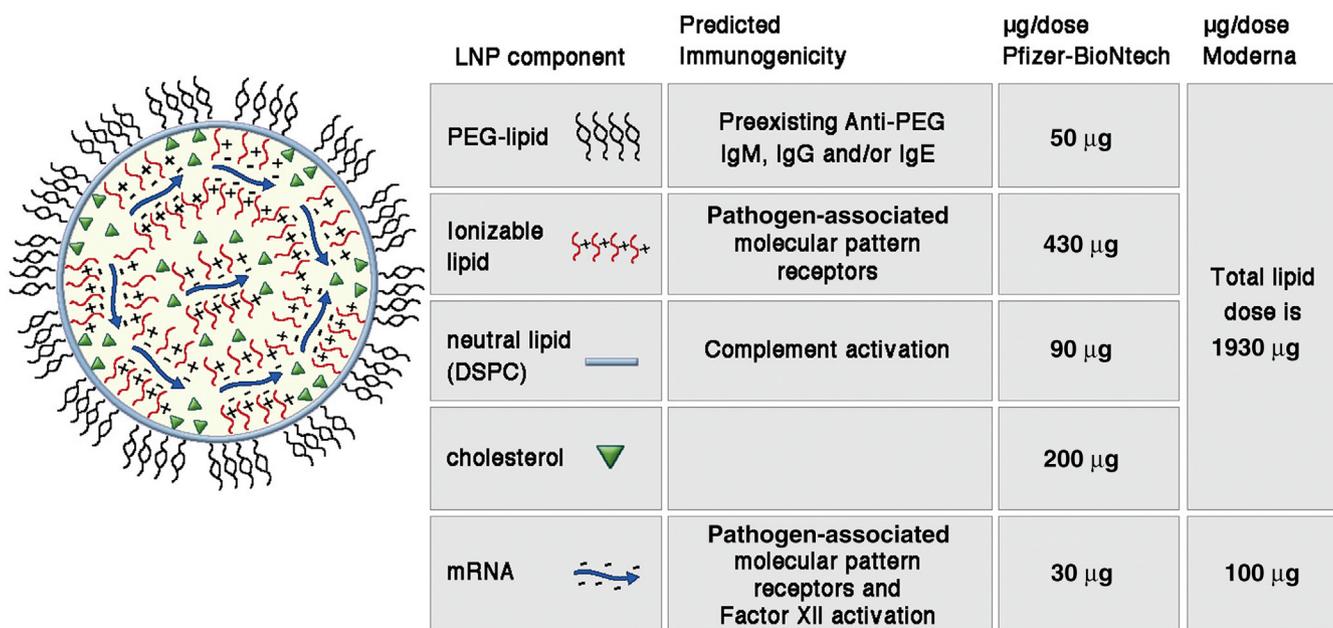


FIG 1. Components of COVID-19 mRNA LNP listed in Pfizer-BioNTech and Moderna COVID-19 vaccine package inserts. DSPC, 1,2-Distearoyl-sn-glycero-3-phosphocholine.

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 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).

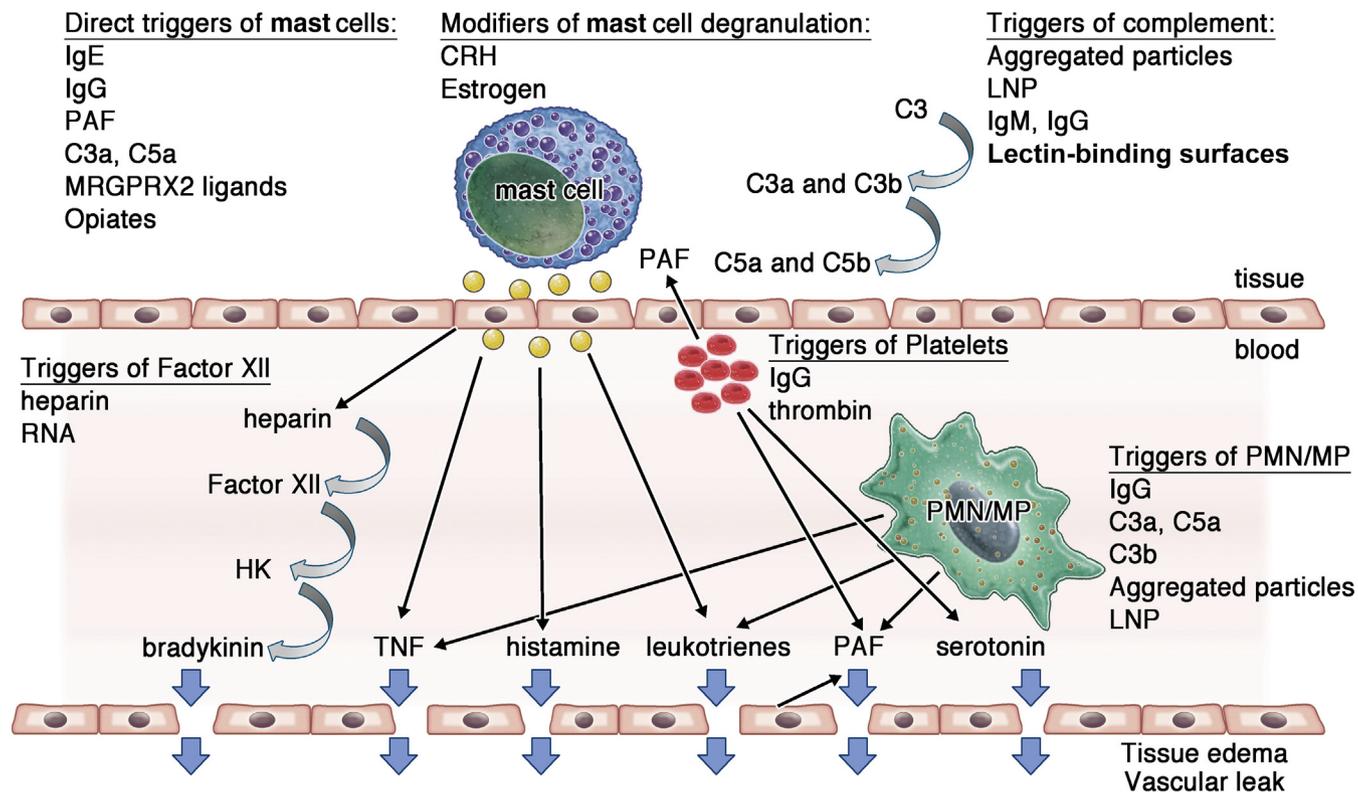


FIG 2. Potential allergic and pseudoallergic triggers and modifiers of anaphylaxis. CRH, Corticotropin-releasing hormone; HK, high-molecular-weight kininogen; MP, macrophage; MRGPRX2, Mas-related G protein-coupled receptor X2; PAF, platelet-activating factor; PMN, polymorphonuclear cell.

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 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

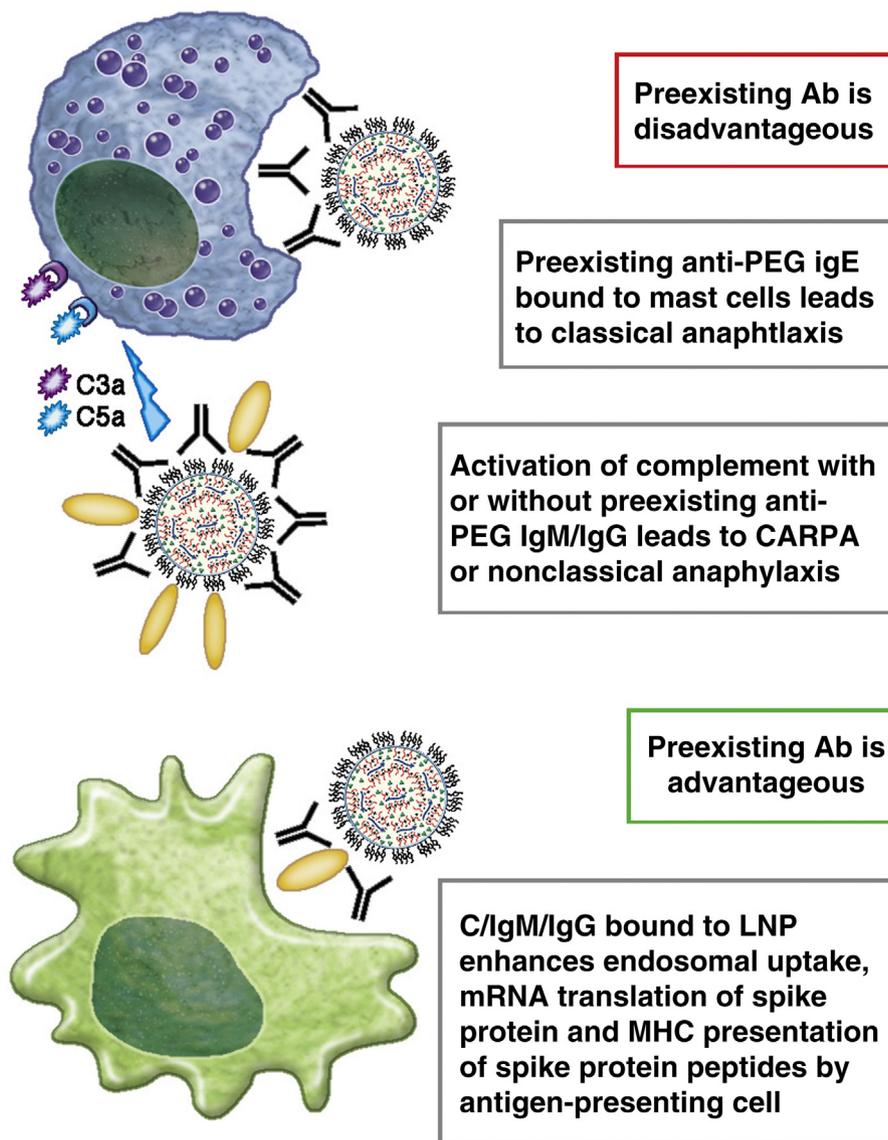


FIG 3. Preexisting antibodies against PEG—a threat for anaphylaxis and an immunologic advantage for vaccine efficacy. Yellow ovals represent complement (C) deposition. *Ab*, Antibody; *CARPA*, complement activation–related pseudoallergic reactions.

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 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.

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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 acetamide 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.