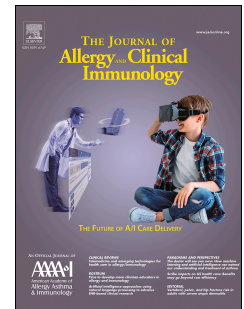


Journal Pre-proof

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PII: S0091-6749(21)00565-0

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

Reference: YMAI 15064

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 12 February 2021

Revised Date: 24 March 2021

Accepted Date: 1 April 2021

Please cite this article as: Risma KA, Edwards KM, Hummell DS, Little FF, Norton AE, Stallings A, Wood RA, Milner J, Potential Mechanisms of Anaphylaxis to COVID-19 mRNA Vaccines, *Journal of Allergy and Clinical Immunology* (2021), doi: <https://doi.org/10.1016/j.jaci.2021.04.002>.

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Potential Mechanisms of Anaphylaxis to COVID-19 mRNA Vaccines

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Disclosure of potential conflict of interest: All authors have served as consultants to the CDC sponsored Clinical Immunization Safety Assessment (CISA) Project; This work was not supported by CISA. K.M.E has grant funding from the Center for Disease Control (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.S. has support from Regeneron Pharmaceuticals. R.A.W., F.F.L., K.E., and J.M. have grant funding from the NIH. J.M. is providing expert witness testimony on trauma and hypersensitivity. R.A.W. receives research support from Aimmune, Astellas, DBV, HAL-Allergy, Genentech, Regeneron, and Sanofi, and royalties from Up To Date. A.E.N. and K.A.R have no external funding sources to declare.

38 **3551 words (limit is 3500)**

39 **Abstract:**

40 Anaphylaxis to vaccines is historically a rare event. The Coronavirus Disease 2019 (COVID-
41 19) pandemic drove the need for rapid vaccine production applying a novel antigen delivery
42 system: mRNA vaccines packaged in lipid nanoparticles (LNP). Unexpectedly, public
43 vaccine administration led to a small number of severe allergic reactions with resultant
44 substantial public concern, especially within atopic individuals. We reviewed the
45 constituents of the mRNA LNP vaccine and considered several contributors to these
46 reactions: 1) contact system activation by nucleic acid, 2) complement recognition of the
47 vaccine activating allergic effector cells, 3) pre-existing antibody recognition of polyethylene
48 glycol (PEG), a LNP surface hydrophilic polymer, and 4) direct mast cell activation, coupled
49 with potential genetic or environmental predispositions to hypersensitivity. Unfortunately,
50 measurement of anti-PEG antibodies in vitro is not clinically available, and the predictive
51 value of skin testing to PEG components as a COVID-19 mRNA vaccine-specific
52 anaphylaxis marker is unknown. Even less is known regarding the applicability of vaccine
53 use for testing (in vitro/vivo) to ascertain pathogenesis or predict reactivity risk. Expedient
54 and thorough research-based evaluation of patients who have suffered anaphylactic
55 vaccine reactions and prospective clinical trials in putative at-risk individuals are needed to
56 address these concerns during a public health crisis.

Key Words

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

Abbreviations

Ab	antibody
BAT	basophil activation test
BST	basal serum tryptase
C3a	complement component 3a
C5a	complement component 5a
CARPA	complement activation related pseudo-allergic reactions
CDC	Center for Disease Control and Prevention
COVID-19	Coronavirus disease 2019
CRH	Corticotropin releasing hormone
DC	dendritic cell
DMG	dimyristoyl glycerol
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
Fc	fragment crystallizable region
FDA	US Food and Drug Administration
HATS	Hereditary alpha tryptasemia
HK	high molecular weight kininogen
HMW	high molecular weight
ID	intradermal
Ig	immunoglobulin
LNP	lipid nanoparticles
MAT	mast cell activation test
MHC	major histocompatibility complex
MRGPRX2	Mas-related G protein coupled receptor X2
mRNA	messenger ribonucleic acid
PAF	platelet activating factor
PEG	polyethylene glycol
PEGylated	polyethylene glycol conjugated
PS80	polysorbate 80
RBD	receptor-binding domain
SPT	skin prick test
UK	United Kingdom
US	United States of America

On December 8, 2020, the world watched as the first dose of COVID-19 mRNA vaccine was given in the United Kingdom. . The subsequent United States (US) Food and Drug Administration (FDA) emergency use authorization of both Pfizer-BioNTech and Moderna mRNA vaccines was historic, as the use of the mRNA platform had never progressed beyond phase 1-2 trials (1). Encouraging preclinical data for the COVID-19 mRNA vaccines and phase 1-3 trial data (2) (3) (4) (5) (6) (7) (8) demonstrating 95% efficacy against COVID 19 disease had been published. Given the public health emergency and the worldwide death count of nearly 1.8 million, a vaccine to prevent COVID-19 disease was critically needed.

However, within twenty-four hours of the first vaccination, media reported that 2 individuals had developed anaphylaxis minutes of 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 US and 21 cases of anaphylaxis had been reported (9). One month later the CDC 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 2-5 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 (11)).

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 utilized surfactant, polysorbate 80 (PS80), present in numerous vaccines (12). However, since none of these excipients were included in the Pfizer BioNTech COVID-19 mRNA vaccine (Table 1, Figure 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 upon the allergy community to respond to these concerns. Recommendations for reasonable clinical management have been published (13) at a time when we have very limited understanding of the nature of these reactions; there also emerge a series of research-based questions which are critical to answer.

Immediate Reactions to COVID-19 mRNA LNP vaccines—pseudo-allergic or allergic?

The occurrence of anaphylaxis upon first exposure to the COVID-19 vaccine implies either pre-existing, antibody-mediated immunity (allergic) or a pseudo-allergic response independent of previous exposure. Although anaphylaxis related to known allergens is best understood via the classic paradigm of crosslinking IgE bound to Fcε receptors on mast cells and basophils, non-classical pathways such as antibody-dependent activation of complement or IgG mediated mast cell/granulocyte/platelet/basophil activation via Fcγ receptors have been described in animal models and in allergic responses to medications in humans (14) (15) (16) (17) (18). Additionally, a variety of pseudo-allergic 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 Figure 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 pro-inflammatory 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 (LNP) (1), Figure 1. Since 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 prior to 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 upon injection, should be examined. Measuring intact and cleaved high molecular weight 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 a variety of receptors including opioid receptors, Mas-related G protein coupled receptor X2 (MRGPRX2) receptors, and other yet to be defined receptors for contrast agents (14, 15). As 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 or the Moderna mRNA vaccines have been tested in vitro for their 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 (Figure 1, Table 1), reviewed in (1), (24). Additionally, the LNP includes neutral lipids and cholesterol which 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 hydrophilicity of the LNP surface and to provide stability to the mRNA carrier. Historically, PEG has been utilized 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 FDA approved 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). Pseudo-allergic reactions to Doxil were also subsequently demonstrated in porcine models, and were labeled as Complement Activation Related Pseudo-allergic Reactions (CARPA) (28). Doxil infusions led to the production of anaphylatoxins C3a and C5a that 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 pre-existing 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 post-vaccine 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 ethylenediaminetetraacetic acid (EDTA) tubes which prevents ongoing activation of complement. While these assays may certainly be useful as a research tool, due to the inherent instability of the complex, they require flash freezing of plasma on dry ice and storage at -60 to -80 C for shipment, thus limiting clinical utility.

Non-classical Allergic Reactions to the LNP

Allergic reactions to LNP 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 (31)). 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 (Figure 3).

The first documentation that antibody could form against PEG in humans came from the observation in 2005 that 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, pre-existing antibodies have been associated with adverse reactions upon first infusions in children with leukemia (34, 35). The proposed mechanism is a non-classical 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 due to an unacceptably high rate of anaphylaxis occurring upon first exposure, associated with pre-existing anti-PEG antibody (37, 38). Both IgM and IgG anti-PEG antibodies were documented in these patients; tryptase was elevated in 6/11 patients with severe

reactions; and complement C3a was also elevated at 90 minutes. Unfortunately, the authors did not report if 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 gold standard enzyme-linked immunosorbent assay (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 upon the assay and the cutoffs utilized by individual research labs (34, 35, 37, 40, 41).

As a side note, although the existence of pre-existing IgM and/or IgG antibodies against the LNP may adversely lead to non-classical allergic reactions, they may also lead to enhanced efficacy of the vaccine. Pre-existing IgG and IgM may enhance dendritic cell (DC) uptake of LNP through Fc receptors or complement receptors on DC (Figure 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 94-95% of infections (7) (8). If pre-existing, 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 tests (SPT) to HMW PEG or structurally related polysorbates (42) (43, 44) (45) (46) (47). Additionally, both SPT and intradermal (ID) testing have led to systemic allergic symptoms (Supplemental table), strongly pointing to an IgE mediated mechanism. The estimated prevalence of IgE mediated reactions to PEG is unknown. One recent study by Cosby et al, reviewed 25,905 reports of anaphylactic events to the FDA and found 53 reports with unique case identifiers for anaphylaxis and PEG containing products with an estimate of 4 per year during the range 2005-2017, (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 SPT to both HMW PEG and the vaccines themselves. Skin prick testing with PEG with molecular weight 3350 has been suggested by Banerji et al as a starting point for evaluation of anaphylactic reactions to the mRNA COVID-19 vaccines as PEG 3350 is readily available in the United States (13). 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 since class switching to IgE implies T cell engagement. Due to 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 IgG (31) 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 pseudo-allergic or non-classical allergic reactions may be anticipated to occur more frequently.

Most recently IgE against PEG was detected in the blood of a patient suffered immediate reactions to each of three different medications containing PEG -- Definity liposomes, oral bowel prep, and steroid injection (47). Anti-PEG IgE was measured by two independent immunoassays- chemiluminescent-based and dual cytometric bead assays (40, 47). Zhou et al showed that 6 additional patients with a history of reactions to HMW PEG had detectable IgE (40, 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 expected (40); 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 PS80 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 non-reactive basophils in up to 20% of individuals. These limitations can be overcome using a mast cell activation test (MAT), 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 MAT is that blood samples can be frozen and shipped to a research lab and the cultured mast cells may be confirmed to degranulate prior to experimentation.

A key set of experiments for evaluating COVID-19 mRNA vaccine reactions is the use of the BAT and/or MAT 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 males, with this difference emerging after puberty (reviewed by (53)). Few studies have examined these differences in drug allergy. The skewing of the allergic response to the COVID mRNA vaccine towards the female sex may be secondary to estrogen effects in promoting a TH2 response, or conversely, testosterone and progesterone's known role in diminishing TH2 responses (54) (55). Additionally 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 over-represented 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 (CRH) 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 non-steroidal 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 prior to 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 hypersensitivity (60) but could be relevant for the vaccine reactions as well. While 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. While 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). Those 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 (HATS)- is present in 5% of certain populations (64). HATS is significantly enriched amongst those with idiopathic anaphylaxis, severe hymenoptera reactions, mastocytosis, and even anaphylaxis within the context of mastocytosis (65, 66); Genotyping can be performed to identify if the vaccine anaphylaxis population is enriched for those with HATS, and a BST >8 ng/ml can be highly suggestive as well. Additionally, 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.

Conclusion:

The high efficacy of mRNA LNP vaccination against COVID-19 disease in Phase 2/3 clinical trials and the rapid successful mobilization of a useful vaccine suggests 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 to develop strategies to identify risk factors for immediate reactions, identify sensitive and specific mechanisms for diagnosis and risk stratification for future vaccination. Due to limited availability of clinical tools to assess for allergic responses to vaccines and the likelihood that non classical allergic responses and/or pseudo-allergic responses contribute to COVID-19 mRNA vaccine reactions, research studies are imperative.

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Figure 1. Components of COVID-19 mRNA LNP listed in Pfizer BioNTech and Moderna COVID-19 vaccine package inserts. PEG, polyethylene glycol; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; mRNA, messenger ribonucleic acid.

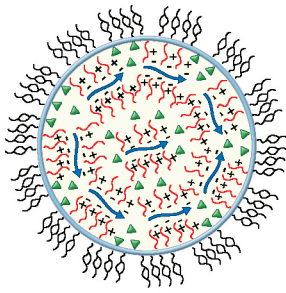
Figure 2. Potential allergic and pseudo-allergic triggers and modifiers of anaphylaxis. PMN, polymorphonuclear cells. MP, macrophage. HK, high molecular weight kininogen. PAF, platelet activating factor. TNF, tumor necrosis factor. CRH, corticotropin releasing hormone, LNP, lipid nanoparticle, MRGPRX2, Mas-related G protein coupled receptor X2.

Figure 3. Pre-existing antibodies against PEG- a threat for anaphylaxis and an immunologic advantage for vaccine efficacy. Yellow ovals represent complement (C) deposition. Ab, antibody. APC, antigen presenting cell, LNP, lipid nanoparticle, CARPA, complement activation related pseudo-allergic reactions, MHC, major histocompatibility complex

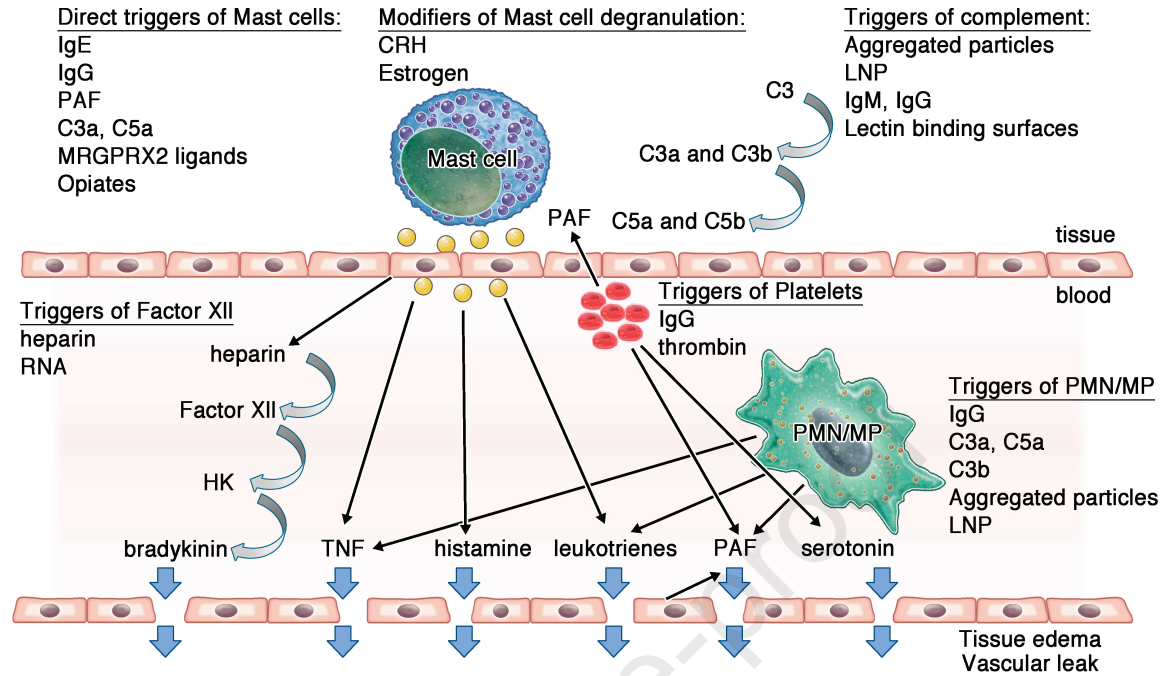
Table 1: Components of mRNA vaccines

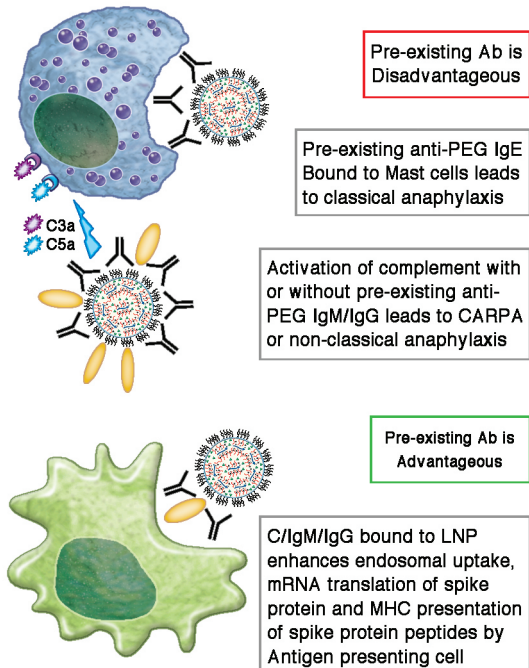
Description		Pfizer-BioNTech COVID-19 vaccine	Moderna COVID-19 vaccine
mRNA		Nucleoside-modified mRNA encoding the receptor binding domain (RBD) of viral spike (S1) glycoprotein and encoding T4 fibrin to achieve trimerization	Nucleoside-modified mRNA encoding the viral spike (S2) glycoprotein
Lipids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

* Neither vaccine contain eggs, gelatin, latex, or preservatives



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





Author, date, country	Patients	Implicated drug(s)	Skin test Results:	Systemic symptoms on skin testing
Wylon et al (42) 2016, Germany	1 woman	- Depo-Medroxyprogesterone acetate, ((DMPA) contains 2.9% PEG 3350 and PS80)) - Joint Injection w, lidocaine, bupivacaine, triamcinolone, (PEG 4000 and PS80) - Oral medication, PEG 6000	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 et al (43) 2016, Denmark	14 women 23 men	A variety of HMW PEG products (3350-20,000), Oral, vaginal and injection	Variety of HMW PEG products (3350-20,000): 19/22 patients tested (+) on SPT 0.0001%-10% HMW PEG: 4/5 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 (44) 2018, United States	2 men	- PEG 3350 bowel preparations - Methylprednisolone acetate injection ((MPA) contains 2.8% PEG 3350)	1 st patient: 0.17-17% PEG 3350: (+) on SPT PS80 in a variety of preparations: (+) on ID 2nd patient: 0.17-17% PEG 3350: (-) SPT MPA: (-) SPT/ID triamcinolone acetone preparation containing PS80: (+) on ID	1st patient developed systemic allergic symptoms during ID skin test 2nd patient had systemic allergic symptoms to oral challenge PEG 3350 despite negative skin tests
Sellaturay et al (45) 2020, United Kingdom	4 women 1 man	A variety of HMW PEG products (3350-20,000), Oral and injection	Variety of HMW PEG products (3350-20,000): 3/5 patients tested (+) on SPT 1% PEG 20,000: 1/2 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 (46) 2020, United Kingdom	15 women	Depo-Medroxyprogesterone acetate ((DMPA) contains 2.9% PEG 3350 and PS80))	DMPA: 2/12 patients tested (+) on SPT DMPA Diluted 1:100 or 1:10: 4/9 patients tested (+) on ID 10% PEG 3350: 5/12 tested (+) on SPT 0.1 or 1% PEG 3350: 2/2 tested (+) on ID	2/2 patients developed systemic allergic symptoms during ID skin test

PS80 polysorbate 80; PEG, polyethylene glycol; HMW, high molecular weight; ID, Intradermal; SPT, skin prick test; MPA methylprednisolone acetate; DMPA Depo-medroxyprogesterone acetate