Molecular Therapy
Perspective

Allergic Reactions and Anaphylaxis to LNP-Based COVID-19 Vaccines

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Mortality from coronavirus disease 2019 (COVID-19) in populations at high risk, such as the elderly, certain ethnic groups (e.g., Black/African American, and Hispanic/Latino persons), obese individuals, and those with endothelial dysfunction is substantial. While treatment options are limited, vaccination against SARS-CoV-2, the virus that causes COVID-19, is the most important global strategy in controlling the pandemic. Thus, two lipid nanoparticle (LNP)-based mRNA vaccines (Pfizer-BioNTech and Moderna) against SARS-CoV-2 have already received emergency use authorization by the US Food and Drug Administration (FDA). During December 14–23 2020, after administration of 1,893,360 first doses of the Pfizer-BioNTech COVID-19 vaccine, 175 cases of severe allergic reaction were submitted to the Vaccine Adverse Event Reporting System (VAERS).[1] Of these, 21 cases were determined to be anaphylaxis (based on Brighton Collaboration definition criteria for anaphylaxis),[2] including 17 persons with a medical history of allergies or allergic reaction, and 7 of whom had a history of anaphylaxis. Furthermore, in the same period, 83 nonserious cases of nonanaphylaxis allergic reactions after Pfizer-BioNTech COVID-19 vaccination were submitted to VAERS. Vaccination with the first dose of the Moderna COVID-19 vaccine commenced on December 21, 2020, and in the period from December 21, 2020 to January 10, 2021, out of 4,041,396 recipients, 10 cases were determined to represent anaphylaxis.[3] Of these, 9 persons were confirmed to have a documented history of allergies or allergic reactions and 5 had a previous history of anaphylaxis. Most recently, the California Department of Public health has halted the distribution of one batch of Moderna COVID-19 vaccine (lot 41L20A) following reports of multiple episodes of severe allergic reactions at a single clinic in less than 24 h. Furthermore, 29 fatal cases of the first dose of Pfizer-BioNTech COVID-19 vaccine in elderly patients (>75 years of age) were reported in Norway, and some of these individuals apparently experienced anaphylaxis.[4,5] What is causing these allergic reactions, including anaphylaxis?

Anaphylaxis is a serious allergic reaction that is rapid in onset (over minutes to hours) and potentially life threatening.[6] Anaphylaxis can occur after exposure to different allergens (e.g., food ingredients, venom, and drugs), but anaphylaxis after vaccination is rare and, in some vaccine recipients, not even related to the vaccine components. Typically, natural rubber latex (e.g., in the syringe plunger and vial stoppers) could potentially cause anaphylaxis in sensitive individuals. However, the Pfizer-BioNTech's product is presented in a 2 mL type I glass vial with a synthetic bromobutyl rubber, which has no latex. On the other hand, allergic responses to a syringe containing latex have been reported in the literature.[7] With all confirmed cases of anaphylaxis after injection of either the first dose of the Pfizer-BioNTech or Moderna COVID-19 vaccine, however, none of the individuals had a documented history of latex allergy. While latex sensitivity might still account for the nonanaphylaxis allergic reaction in some of the nonserious cases after the Pfizer-BioNTech COVID-19 vaccination, using disposable glass syringes could resolve allergic reactions in individuals with a latex allergy.

Individual vaccine components, such as ovalbumin, gelatin, and milk proteins, have long been implicated in acute vaccine reactions,[8] but these components are absent in both the Pfizer-BioNTech and Moderna COVID-19 vaccines. The synthetic mRNA is also least likely to instigate allergic responses per se, since the disclosed mRNA in the Pfizer-BioNTech COVID-19 vaccine contain N1-methylpseudouridine instead of uridine[9] to dampen immune responses and cytotoxicity induced by introducing mRNA into cells.[10] However, truncated and modified RNA trace impurities are present in the Pfizer-BioNTech COVID-19 vaccine;[9] thus, there is a possibility that aberrant proteins could be expressed and initiate delayed immunological reactions in some subjects.

This leaves LNPs and other excipients as possible sources of allergens. The list of disclosed excipients in the Pfizer-BioNTech COVID-19 vaccine includes sucrose, sodium chloride, potassium chloride, disodium phosphate dihydrate, potassium dihydrogen phosphate, and water for injection.[9] In the Moderna COVID-19 vaccine, the excipients are tromethamine, tromethamine hydrochloride, acetic acid, sodium acetate, and sucrose.[11] Collectively these excipients are not classified as allergens. The LNPs in the Pfizer-BioNTech vaccine comprise four components: cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), ALC-0315 [(4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2- hexyldecanoate)], and ALC-0159 (2-[(polyethylene glycol)$_{2000}$]-N,N-ditetradecylacetamide). The first two components have been widely used in regulatory approved liposomal medicines (e.g., Doxil) and are also features in the Moderna COVID-19 vaccine. ALC-0315 is an ionisable aminolipid that is responsible for mRNA compaction and aids mRNA cellular delivery and its cytoplasmic release through suspected endosomal destabilization. The ionisable lipid in the Moderna COVID-19 vaccine is not disclosed, but it is most likely

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heptadecan-9-yl8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate. The LNPs in the Pfizer-BioNTech COVID-19 vaccine contain low levels (<2 mol %) of ALC-0159, which contributes to nanoparticle stabilization by a steric mechanism through its poly(ethylene glycol) (PEG) moiety. In the Moderna COVID-19 vaccine, ALC-0159 is replaced with another PEGylated lipid (1,2-dimyristoyl-rac-glycero-3-methoxyPEG$_{2000}$). There are speculations on a possible role for ALC-0159 (the PEGylated lipid) in triggering anaphylaxis, based on earlier reported anaphylactic reactions in some recipients of intravenously infused PEGylated nanomedicines.[12] For example, with PEGylated nanomedicines such as Doxil, complement activation was initially thought to account for anaphylactoid reactions (the so-called complement activation-related pseudoallergy [CARPA] hypothesis); however, the validity of CARPA has been recently questioned and, instead, a direct role for macrophages and other immune cells have been proposed.[13,14] Anaphylatoxins might play minor roles in potentiating anaphylactoid reactions; for instance, intradermal injection of low doses of anaphylatoxins (C3a, C4a, or C5a) in healthy volunteers was shown to induce immediate wheal-and-flare reactions.[6] If LNP-based vaccines can trigger immediate local complement activation, then complement activation is expected to proceed in almost all vaccine recipients, but anaphylaxis with the Pfizer-BioNTech and Moderna vaccines is very rare, and complement activation alone cannot account for anaphylaxis episodes. With PEGylated nanomedicines such as pegnivacogin, anaphylactic reactions have been most notable in individuals with high titers of anti-PEG immunoglobulin G (IgG), but, again, not all individuals with high levels of such antibodies experienced allergic reactions.[15] Thus, there are either inter-individual differences in susceptibility to antibody-triggered reactions or differences in the properties of anti-PEG antibodies. Nonetheless, the molecular basis of these reactions in humans remains unknown, but, in the murine model, antigen-induced anaphylaxis appears to proceed through the IgG, low-affinity FcγRIII, effector cells, and platelet-activating factor pathway.[16]

In responsive individuals, an intradermal titer of possible anti-PEG IgGs (if present) is expected to be extremely low to account for anaphylaxis in vaccine recipients. On the other hand, anaphylaxis to LNP-based vaccines could be related to the pre-existence of assumed anti-PEG IgEs,[17] but PEG is widely present in daily hygiene and cosmetic products, and these products are presumably used frequently by individuals who have shown allergic responses to either the Pfizer-BioNTech or Moderna COVID-19 vaccines. It is also plausible that other IgEs that cross-react with a heterogeneous group of allergenic determinants could recognize different epitopes on LNPs, LNP-aggregates, possible co-existing vesicles and PEG-lipid micelles, and traces of lipid/mRNA impurities. Still assuming that PEG is the main culprit, allergic responses will most likely persist with related coating stabilizers that share similar structures to PEG (e.g., polysorbates [also known as Tweens] and non-ionic block copolymers of the Pluronic series). Perhaps other neutral stabilizers, such as polyvinylpyrrolidone (and its derivatives), could be the materials of choice for future vaccine modification. Indeed, polyvinylpyrrolidone has been administered as a plasma expander to over half a million subjects and has shown an excellent safety record.[18] It is worth noting that both Oxford/AstraZeneca and Johnson & Johnson COVID-19 vaccines also contain Tweens (polysorbate 80). So far, there are no reports of anaphylactic reactions to the Oxford/AstraZeneca vaccine.

The PEGylation strategy, depending on nanoparticle size and adlayer thickness of the PEG, not only minimizes nanoparticle aggregation at the injection site (intramuscular and intradermal sites), but also improves nanoparticle spreading and drainage into the initial lymphatic vessels.[19] A weak steric barrier (a PEG adlayer thickness of 1–2 nm), while still minimizing nanoparticle aggregation, does not compromise nanoparticle recognition and uptake by macrophages and other immune cells (e.g., dendritic cells).[19] Considering their low ALC-0159 content, LNPs in the Pfizer-BioNTech COVID-19 vaccine most likely display a weak steric barrier of PEG. Accordingly, LNPs (and possible co-existing PEG-lipid micelles and PEGylated vesicles) spread easily from the injection site and encounter a large population of responsive effector cells locally (and in draining regional lymph nodes) within the first hour of injection.[19] Thus, such rapid kinetic events could be problematic in individuals with mastocytosis, prompting the release of vasoactive and other mediators of anaphylaxis either through IgE cross-linking by LNPs (and other possible co-existing particles) or LNP interaction with other signaling receptors. Additionally, LNP-mediated tissue damage/inflammation could prompt interleukin-33 (IL-33) release. IL-33 binding to the orphan receptor ST2 on IgE-sensitized mast cells can induce anaphylaxis.[20]

Limited information is available on LNP size distribution, polydispersity index, particle number, and presence of likely co-existing vesicles and micelles in the Pfizer-BioNTech and Moderna vaccines. Batch-to-batch variations in these parameters could further play a modulatory role in allergic reactions, and these possibilities were previously suggested for liposomes.[21] The Pfizer-BioNTech COVID-19 vaccine assessment report by the European Medicines Agency notes visual particulate matters in some finished product batches.[9] This might be indicative of LNP (and/or vesicular) aggregates. Whether these aggregates can instigate allergic responses remains to be evaluated. As a safety precaution, batches with visual particulate matter should preferably be disposed of or at least filtered prior to vaccination. From previous experience in nanomedicine, filtration of Doxil prior to infusion has dramatically reduced episodes of allergic responses.[14]

In summary, anaphylaxis to LNP-based COVID-19 vaccines remains a rare event, and vaccination against COVID-19 is the most promising strategy for successfully defeating this pandemic. These vaccines have proven the translational value of years of fundamental genomics and nanomedicine research and are beginning to save lives today.[22] In line with these developments, we must also promote further research in anaphylaxis to better understand the immunopathogenesis and pathophysiology of this life-threatening condition, particularly

within the remit of modern genetic vaccines and nanomedicine developments.

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