Allergenic components of the mRNA-1273 vaccine for COVID-19: possible involvement of polyethylene glycol and IgG-mediated complement activation

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ABSTRACT

Following the emergency use authorization of the vaccine mRNA-1273 on 18th December 2020 in the US and the vaccine BNT162b2 one week earlier, two mRNA vaccines are in currently used for the prevention of coronavirus disease 2019 (COVID-19). Phase 3 pivotal trials on both vaccines excluded individuals with a history of allergy to vaccine components. Immediately after the initiation of vaccination in the United Kingdom, Canada, and in the US, anaphylactic reactions have been reported. While the culprit trigger requires investigation, initial reports suggested the excipient polyethylene glycol 2000 (PEG-2000), which is contained in both vaccines as PEG-micellar carrier system as the potential culprit. Surface PEG chains form a hydrate shell to increase stability and prevent opsonization. Allergic reactions to such PEG-ylated lipids are rarely IgE-mediated, but may result from complement activation-related pseudoallergy (CARPA) that has been described to similar liposomes. In addition,

mRNA-1273 also contains tromethamine (trometamol), which has been reported to cause anaphylaxis to e.g. gadolinium-based or iodinated contrast media.

Skin prick-, intradermal-, epicutaneous- tests, in vitro sIgE assessment, evaluation of sIgG/IgM, as well as basophil activation test are in use to demonstrate allergic reactions to various components of the vaccines.

On 18th December 2020 emergency use authorization (EUA) provided by the US Food and Drug Administration (FDA) authorized the immediate use of the vaccine mRNA-1273 developed by Moderna Therapeutics for the prevention of coronavirus disease 2019 (COVID-19). TheEUA allows the immediate distribution and use of mRNA-1273 COVID-19 vaccine in the United States in subjects 18 years of age and older [1]. It is the second vaccine to be granted an EUA by US regulators after the authorization received by Pfizer-BioNTech on 11th December 2020 for the use of the vaccine BNT162b2 [2]. The authorization of mRNA-1273 was based on early phase trials [3, 4] and the revision of the results of an ongoing phase III trial that involves 33,000 adult subjects that were randomized 1:1 to receive the mRNA-1273 vaccine in a two-dose regimen or placebo. The assessment performed by the FDA demonstrated that the vaccine was 94.1% effective for the prevention of COVID-19 as determined 14 days after the administration of the second dose [1]. 196 cases were evaluated for the efficacy analysis of which 185 cases of COVID-19 were observed in the placebo group versus 11 cases observed in the mRNA-1273 group. The secondary endpoint involvd assessment of severe cases of COVID-19 and included 30 individuls. All of thesevere cases occurred in the placebo group and none of them in the mRNA-1273 vaccinated group [5].

The FDA stated that the potential benefits of mRNA-1273 outweigh the potential risks [1].

Serious allergic reactions to the active components of the vaccine itself or other components are one of the potential risks of every vaccination product. According to the New York Times [6] on the 25th December, soon after vaccination started in the US a physician in Boston developed an anaphylactic reaction to mRNA-1273. He used his own adrenaline autoinjector that he carried for his shellfish allergy and recovered well.

Anaphylactic reactions to BNT162b2 were also reported in the United Kingdom (UK), Canada and the US [7, 8].

Allergic reactions to vaccines including severe anaphylaxis may be IgE-mediated but canalso be IgG and complement-mediated. They usually occur within the first 30 minutes after vaccination. The symptoms include urticarial rashes, generalized pruritus, erythema, wheezing, coughing, dyspnea, throat, tongue or eye swelling (angioedema), hypotension, dizziness, and vomiting, and these reactions may even be fatal. Severe anaphylactic reactions to vaccines are rare and the rate has been estimated to be 1.31 (95% CI, 0.90-1.84) per million vaccine doses [9].

The frequency of allergic side reactions to BNT162b2 was nearly the same in the verum and in the placebo group (0,6% versus 0,5%) [10].

Allergic reactions to the mRNA 1273 vaccine have not been reported in detail. During the phase I trial of the mRNA-1273 vaccine, one case of transient urticaria in the verum group treated with a vaccine dose of 25µg was reported after the first injection. The total number of participants in this phase I trial was 45 patients [34].

Both, BioNTech/Pfizer and Moderna excluded individuals with a history of allergic reaction to vaccines or components thereof their vaccines from the phase 3 pivotal trials. The exclusion criteria for mRNA-1273 state: *"History of anaphylaxis, urticaria, or other significant adverse reaction requiring medical intervention after receipt of a vaccine"* [11].

Individuals with previous allergic reactions to food or medications were not excluded but may have been underrepresented.

The anaphylactic reactions during the routine vaccination prompted authorities such as the Medicines and Healthcare Products Regulatory Agency (MHRA) in the UK or the FDA to issue an alert stating that individuals with a history of severe allergic reactions to vaccines, medicines, food, or any component of these particular vaccines should be advised against their administration and that a second dose should not be given to anyone who has experienced anaphylaxis following administration of the first dose of this vaccine [9].

Although the culprit trigger has yet to be determined, initial reports pointed at the excipient polyethylene glycol 2000 (PEG-2000), contained in the vaccine as a PEG-micellar carrier system, to be the potential cause of the anaphylactic reactions [7]. PEG-ylated microsomes used as the carrier of the vaccine can cause anaphylactic reactions in individuals with pre-existing PEG allergies as it has been previously observed for PEG-ylated drugs used in the cancer therapy and treatment of chronic diseases [7] [35] [12].

PEGs are also used as excipients in everyday products, such as toothpaste, cosmetics, shampoos, and some biologicals.

Lipid nanoparticles are similar to liposomes, which have been in use pharmaceutically for many years as carriers for drugs. Some of the approved liposome/LNP-containing drugs also contain a PEG-ylated lipid (e.g. in Caelyx pegylated liposomal® or Onpattro®). The PEG chains on the surface form a hydrate shell around the liposome/LNP. This increases stability and prevents opsonization, i.e. the mechanism

by which the surface of foreign cells (e.g. bacteria, viruses) that have invaded the body is covered with antibodies and factors of the complement system. In addition, the stability and half-life of the lipid particles are increased.

Pathophysiology

Allergic reactions to such PEG-ylated lipids may be IgE-mediated, however non-IgE-mediated reactions have to be considered as well [13].

IgE activates mast cells and basophilic granulocytes via cross-links of high-affinity IgE receptors, which is indirectly measurable in an increased expression of surface markers (CD63, CD203c) on basophils [8, 14].

The symptoms of anaphylactic reactions are particularly caused by mediators released mainly from mast cells and basophilic granulocytes such as histamine, prostaglandins, leukotrienes (LTB4, LTC4, and LTD4), tryptase, platelet-activating factor (PAF), heparin, proteases, serotonin, and cytokines [8, 14]. Besides IgE, other antibody classes may trigger similar symptomatology or amplify an IgE-mediated reaction [8, 14]. Possible non-IgE-mediated reactions include complement activation-related pseudoallergy (CARPA) and have been described in the context of liposomes [15-17].

Updating the Gell and Coombs' scheme of Type I–IV hypersensitivity reactions (HSRs) [18], CARPA may be regarded as an independent category within Type I reactions, representing "receptor-mediated" mast cell activations [17].

CARPA is partly attributed to the binding of pre-existing anti-PEG IgM to the liposomes with subsequent complement activation. Clinical symptoms of this non-IgE-mediated hypersensitivity have been described as hypo- and hypertension, airway obstruction with dyspnea and other anaphylaxis symptoms shortly after intravenous administration of liposome-containing drugs. Independent of PEG-ylation, liposomes have the potential to activate complement non-specifically depending on their different surface structures and charge) [15]. Complement products C3a, C4a, and C5a (anaphylatoxins) are considered to be particularly important mediators and, in addition to basophils, neutrophils and macrophages are also considered to be relevant effector cells that can be activated via immune complex receptors (CD16, CD32, and CD64, respectively) [8, 14]. Anaphylatoxins are liberated uncontrolled in blood during complement activation and function as efficient small molecular weight regulators of cardiovascular and autonomic organ functions [17, 19].

Possible sensitization to PEG by previous use of cosmetics or drugs containing PEG is conceivable. Little is known about the prevalence of anti-PEG antibodies in the population. Some report that as much as 72% of the population have at least some IgG or IgM antibodies against PEGs [20], while others report high levels in certain groups of individuals [16]. Evidence for a possible role of IgE in triggering PEG-induced hypersensitivity is also discussed [21]. Allergic reactions following the use of PEG as an excipient in a variety of products have been described; it is also referred to as a "hidden" allergen [21, 22].

Similar to BNT162b2, the mRNA-1273 COVID-19 vaccine is a messenger ribonucleic acid (mRNA) vaccine encoding the viral spike (S) glycoprotein of SARS-CoV-2. The list of excipients of both vaccines share certain components but also differs in others. Interestingly, PEG-2000 can also be found as an excipient in the mRNA-1273 COVID-19 vaccine (Figure 1). It has to be noted, that PEG-2000 has never before been used in any vaccine and both Pfizer-BioNTech and Moderna are the first ones to apply this substance. PEGs are hydrophilic polyether compounds that are used as additives in medical products, cosmetics, and food. It is branded under different names, e.g. macrogol. The molecular weight of different PEGs varies from 300 g/mol to 10,000 g/mol and hypersensitivity reactions may occur to PEGs of all molecular weights with a higher rate of reactions to molecular weights from 3350-6000 g/mol [21]. However, it has been suggested that the molecular weight threshold for PEG immediate reactions is still undetermined[23, 24].

Cross-reactivity between PEGs and its derivatives, i.e., structurally related polymers such as polysorbates, exist due to shared moieties (=CH_2CH_2 and =CH_2CH_2OH) [21]. Severe allergic reactions to PEG, although rare, have been described after administration of medications that contain this excipient. PEG has even been described as the high-risk hidden allergen, since it is difficult to detect as a possible cause of allergic reactions [21, 23-26]. PEGs are ingredients of laxatives or liquid preparations for parenteral use, gels, tablet coatings, wound dressings, ointment bases, lotions, toothpaste, oral hygiene products, food additives and even some of the biologicals that are used in clinical studies and more [21] [7]. PEG-ylation is successfully used for drug delivery to protect the drug from any damage by the immune system and deliver it to the targeted location. In addition, PEGs are additives in cosmetics and shampoos. Both, a primary cutaneous sensitization pathway and sensitization after systemic administration are possible [24]. The U.S. National Institute of Allergy and Infectious Diseases

(NIAID) is initiating a study in collaboration with the FDA to analyze the response to the vaccine in people who have high levels of anti-PEG antibodies or have experienced severe allergic responses to drugs or vaccines before [25].

Additionally, and contrasting to the Pfizer-BioNTech vaccine, mRNA-1273 contains tromethamine, also named trometamol (molecular formula: $C_4H_{11}NO_3$), an organic amine that is widely used in several medications for topical, enteral, or parenteral administration. Tromethamine/trometamol is also used in cosmetic products as an emulsifier, and contact sensitization and allergy to this compound have been described [27]. Recently, the first case of anaphylaxis to trometamol as an excipient in a gadolinium-based contrast agent (GBCA) has been reported [28]. The reaction occurred immediately after GBCA injection in a 23-year-old woman and IgE-mediated trometamol allergy could be detected in this patient [28]. Trometamol can also be found in other contrast agents such as in iodinated contrast medium (IOM).

Diagnostic options

A thorough history taking is an important prerequisite to avoid severe anaphylaxis. Reactions to PEGs in e.g. laxatives, gels, wound dressings, lotions, toothpaste, mouthwash, cosmetics and shampoos may be indicative. The use of beta-adrenoreceptor antagonists, angiotensin-converting enzyme (ACE) inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) may lead to an increase in anaphylactic symptoms [14, 29].

In patients with elevated basal serum tryptase and/or mastocytosis, anaphylaxis may be particularly severe [8, 14, 29-31].

Allergy testing should be performed in specialized allergy centres. Skin prick tests should be performed very carefully with initial dilutions from 0.001% up to 10% with 30 minutes observation after every dose step. Since it is speculated that the individual threshold for positive reactions to PEG of different molecular weights varies [23], testing should be performed with PEGs of 2000g/mol molecular weight that are used in both vaccines; published algorithms should be followed [23]. Skin tests should be performed either before but not earlier than 2-4 weeks after the hypersensitivity reaction occurred. In addition, basophil activation test (BAT) and screening for specific IgE to PEG in blood serum may be performed in patients with suspected allergy to excipients of the vaccine. If PEG allergy can be confirmed, an emergency kit should be prescribed, and PEG-allergy information sheet provided. If not, intradermal testing with PEG of different molecular weights at a dilution of 0.01% can be carefully considered, but not

in high-risk patients since systemic reactions can occur [23]. In some settings, oral provocation test can be performed if needed [21].

Trometamol as a contact sensitizer is usually tested epicutaneously for allergic reactions of the delayed-type. Testing for suspected type 1 reactions can be done by skin prick testing (concentration 1:1) followed by intradermal testing with dilutions of trometamol from 1:1000-1:10 [28, 32].

Although allergic reactions to mRNA-1273 components such as PEG and trometamol have not been frequently reported, the fact that the vaccines for COVID-19 will be extensively administrated worldwide to a high proportion of the population should caution health care providers of the potential allergic reactions that may occur in individuals previously sensitized to the components of the vaccines, especially to PEG and PEG analogs as well as trometamol in the case of mRNA-1273 [33].

Therapeutic options

This allergy is of particular interest since some of the drugs used to treat anaphylactic reactions, such as antihistamines or injectable corticosteroids contain PEGs or polysorbates. Substances cross-reactive to PEG, i.e., polysorbates, are widely distributed and commonly used in bread, pastry, chewing gums, ice cream, and so on, but also in a high number of vaccines, biologics, and mediations to treat rheumatologic, cardiovascular, haematologic, gastrointestinal, or oncologic diseases, and during diagnostic procedures. It is very likely that hypersensitivity reactions to such agents have been underestimated in the past.

Further on, two doses of the vaccine have to be administered to achieve an effect so that sensitizations might even occur during the administration of the first dose or individuals may develop allergic reactions to the second dose. Whether the new route of delivery of PEG via intramuscular injections might play a role in its allergenicity has to be determined. Since both mRNA-1273 and BNT162b2 contain PEG-2000, PEG allergic patients or patients allergic to components cross-reactive to PEG do not have a current alternative for preventive vaccination against COVID-19 and should not be vaccinated with those substances. Further on, physicians should be aware of this potential risk and carefully interrogate for previous allergic reactions to PEG, PEG analogues, or tromethamine, and should be trained to respond to potential anaphylactic reactions during vaccination. In these patients, administration of emergency medications

containing PEG such as cetirizine, levocetirizine, fexofenadine, desloratadine, methylprednisolone acetate and triamcinolone acetonide should be avoided.. Alternatives should be considered, for example, clemastine solution for intravenous injection, cetirizine syrup for oral intake, soluble prednisolone or methylprednisolone for oral intake or injection, and of course as recommended for all patients with severe anaphylaxis most importantly adrenaline [23].

Figure legend.

Figure 1. Representation of the Moderna COVID-19 vaccine: The principle of the PEGylated-lipid nanoparticles as a delivery system for the mRNA is illustrated together with the full list of ingredients that contains the vaccine. PEG-2000 and tromethamine / trometamol as potential triggers of allergic reactions indicated in red colour are shown on the left side. Different ways of exposition to PEG and PEG-analogous are illustrated on the right side. Biorender software was used to create the figure under an academic license.

Table 1: Excipients listed in BNT162b2 and mRNA-1273 (according to [1, 2]). Ingredients of the BNT162b2 and mRNA-1273 vaccine are indicated in black colour, shared ingredients are underlined, ingredients with allergenic potential are indicated in red colour and ingredients shared by both vaccines with allergenic potential are indicated in red colour and underlined.

References

1. FDA. *United States Food and Drug Administration (FDA). Emergency use authorization for Moderna COVIC-19 vaccine.* . 2020 28.12.2020]; Available from: https://www.fda.gov/media/144636/download.
2. FDA. *United States Food and Drug Administration (FDA). Emergency use authorization for Pfizer-BioNTech COVID-19 Vaccine* 2020 28.12.2020]; Available from: https://www.fda.gov/media/144412/download
3. Anderson, E.J., et al., *Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults.* N Engl J Med, 2020. **383**(25): p. 2427-2438.
4. Widge, A.T., et al., *Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination.* N Engl J Med, 2020.
5. Moderna, I., *Moderna Announces Primary Efficacy Analysis in Phase 3 COVE Study for Its COVID-19 Vaccine Candidate and Filing Today with U.S. FDA for Emergency Use Authorization. 30 November 2020*. 2020, Moderna, Inc.
6. Wu, K.J., *Boston Doctor Reports Serious Allergic Reaction After Getting Moderna's Covid Vaccine*, in *The New York Times*. 2020: New York, USA.
7. Cabanillas, B.A., Akdis CA.; Novak, N.,, *Allergic reactions to the first COVID-19 vaccine: a potential role of Polyethylene glycol?* . Allergy 2020 In Press, 2020.
8. Klimek L, N.N., Hamelmann E. et al., *Severe allergic reactions after COVID-19-Vaccination with the Pfizer/BioNTech Vaccine in Great Britain and USA Position Statement of the German allergological Societies AeDA, DGAKI and GPA.* . Allergo Journal International 2020. https://doi.org/10.1007/s40629-020-00160-4.
9. McNeil, M.M., et al., *Risk of anaphylaxis after vaccination in children and adults.* J Allergy Clin Immunol, 2016. **137**(3): p. 868-78.
10. Polack, F.P., et al., *Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine.* The New England journal of medicine, 2020.
11. ModernaTX, I., *A Study to Evaluate Efficacy, Safety, and Immunogenicity of mRNA-1273 Vaccine in Adults Aged 18 Years and Older to Prevent COVID-19*. 2020, Clinical Trials.gov: ModernaTX, Inc.
12. Krantz, M.S., et al., *Anaphylaxis to PEGylated liposomal echocardiogram contrast in a patient with IgE-mediated macrogol allergy.* J Allergy Clin Immunol Pract, 2020. **8**(4): p. 1416-1419 e3.
13. Demoly, P., et al., *Predictive capacity of histamine release for the diagnosis of drug allergy.* Allergy, 1999. **54**(5): p. 500-6.
14. Ring, J., Beyer, K., Biedermann, T., Bircher, A., Fischer, M., Fuchs, T. et al., *Leitlinie (S2k) zu Akuttherapie und Management der Anaphylaxie - Update 2021.* . AllergoJournalInternational in press 2021, 2021.

15. Inglut, C.T., et al., *Immunological and Toxicological Considerations for the Design of Liposomes.* Nanomaterials (Basel, Switzerland), 2020. **10**(2).
16. Mohamed, M., et al., *PEGylated liposomes: immunological responses.* Sci Technol Adv Mater, 2019. **20**(1): p. 710-724.
17. Szebeni, J., *Complement activation-related pseudoallergy: a stress reaction in blood triggered by nanomedicines and biologicals.* Mol Immunol, 2014. **61**(2): p. 163-73.
18. Gell, P.G.H., Coombs, R.R.A.,, *Classification of Allergic Reactions Underlying Disease.* Blackwell, Oxford, 1963. Classification of Allergic Reactions Underlying Disease. .
19. Hugli, T.E., *Structure and function of the anaphylatoxins.* Springer Semin Immunopathol, 1984. **7**(2-3): p. 193-219.
20. Yang, Q., et al., *Analysis of Pre-existing IgG and IgM Antibodies against Polyethylene Glycol (PEG) in the General Population.* Anal Chem, 2016. **88**(23): p. 11804-11812.
21. Wenande, E. and L.H. Garvey, *Immediate-type hypersensitivity to polyethylene glycols: a review.* Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 2016. **46**(7): p. 907-22.
22. Wylon, K., S. Dölle, and M. Worm, *Polyethylene glycol as a cause of anaphylaxis.* Allergy, Asthma & Clinical Immunology, 2016. **12**(1): p. 67.
23. Sellaturay, P., S. Nasser, and P. Ewan, *Polyethylene Glycol-Induced Systemic Allergic Reactions (Anaphylaxis).* J Allergy Clin Immunol Pract, 2020.
24. Stone, C.A., et al., *Immediate Hypersensitivity to Polyethylene Glycols and Polysorbates: More Common Than We Have Recognized.* The journal of allergy and clinical immunology. In practice, 2018. **7**(5): p. 1533-1540.e8.
25. Calogiuri, G., et al., *Polyethylene glycols and polysorbates: Two still neglected ingredients causing true IgE-mediated reactions.* The journal of allergy and clinical immunology. In practice, 2019. **7**(7): p. 2509-2510.
26. Zhou, Z.-H., et al., *Anti-PEG IgE in anaphylaxis associated with polyethylene glycol.* The journal of allergy and clinical immunology. In practice, 2020.
27. Singh, M., S.M. Winhoven, and M.H. Beck, *Contact sensitivity to octyldodecanol and trometamol in an anti-itch cream.* Contact Dermatitis, 2007. **56**(5): p. 289-90.
28. Lukawska, J., et al., *Anaphylaxis to trometamol excipient in gadolinium-based contrast agents for clinical imaging.* J Allergy Clin Immunol Pract, 2019. **7**(3): p. 1086-1087.
29. Worm, M., et al., *Factors increasing the risk for a severe reaction in anaphylaxis: An analysis of data from The European Anaphylaxis Registry.* Allergy, 2018. **73**(6): p. 1322-1330.
30. Brockow, K., et al., *Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients.* Allergy, 2008. **63**(2): p. 226-32.
31. Schuch, A. and K. Brockow, *Mastocytosis and Anaphylaxis.* Immunology and allergy clinics of North America, 2017. **37**(1): p. 153-164.
32. Brockow, K., et al., *Skin test concentrations for systemically administered drugs -- an ENDA/EAACI Drug Allergy Interest Group position paper.* Allergy, 2013. **68**(6): p. 702-12.
33. Klimek L, Jutel.M., Akdis CA. et al., *ARIA-EAACI statement on severe allergic reactions to COVID-19 vaccines – an EAACI-ARIA Position Paper.* Allergy, 2020. (**in press)**.
34. Jackson LA, Anderson EJ, Rouphael NG et al. N Engl J Med 2020; 383:1920-1931 DOI: 10.1056/NEJMoa2022483
35. Krantz M, Liu Y, Phillips EJ, Stone CA. COVID-19 vaccine anaphylaxis: PEG or not? 2020. Allergy in press