REVIEW JASS@STEM

Analyzing the Function and Structure of Components within the mRNA COVID-19 Vaccination for Determining the Cause of Anaphylaxis Reactions within Vaccine Recipients

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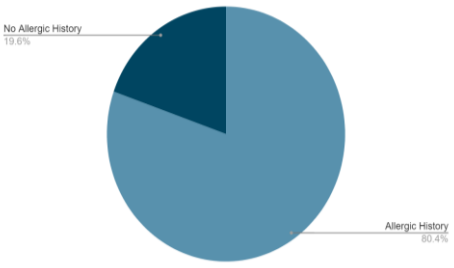
1. Introduction

As of January 3, 2021, over 20,000,000 cases of COVID-19 have been reported in the United States, and on top of this, 350,000+ deaths. 77.4% of vaccine recipients reported at least one systemic reaction during the 7 days after their vaccination. The frequency of these reactions was even higher in the younger population than the older age group (82.8% vs 70.6%). In each age group, patients had a higher frequency and severity of adverse reactions after receipt of Dose 2 than Dose 1. (1) Long-term results over the course of a lifetime from contracting COVID-19 are currently unknown; however, several symptoms and serious complications are being reported among COVID-19 survivors - including those who upon initial dosage, experience a mild acute reaction. (2) As of December 2020, more than 1,900,000 first doses of the Pfizer vaccine had been administered in the United States, and 0.2% (around 4,500) of recipients were experiencing adverse reactions. Among these individuals, approximately 200 case reports were identified for further review as potential cases of anaphylaxis.

Anaphylaxis is a life-threatening allergic reaction that can occur after vaccination, with the event presenting itself anywhere from a few minutes to several hours. Twenty-one cases were determined to be anaphylaxis from the Pfizer vaccination, out of which 17 possessed a documented history of allergies or allergic reactions, and 7 with a history of anaphylaxis. The interval from receiving the vaccine to onset in the system was 13 minutes, with the range expanding from 2 to 150 minutes. (3) Below, we can see the characteristics of allergic reaction case reports after receipt of the first dose of the Pfizer COVID-19 vaccination, including anaphylaxis and non-anaphylaxis allergic reactions, during the time frame of December 14–23, 2020, in the United States.

Scheme 1a. History of those who experienced Anaphylaxis Reactions (n=21) after receipt of Pfizer COVID-19 vaccination:



Scheme 1b. *History of those who experienced Non-Anaphylaxis Reactions (n=83) after receipt of Pfizer COVID-19 vaccination:*

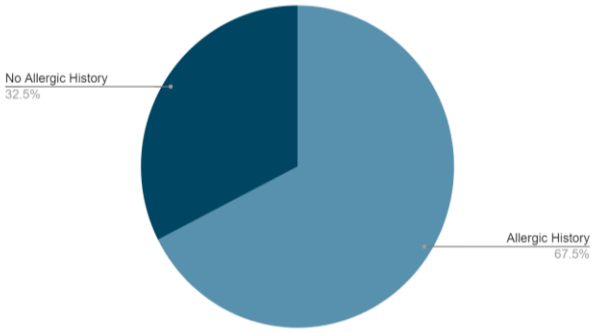


Figure 1: A comparison of patients with anaphylaxis and non - anaphylaxis allergic reactions who who received the Pfizer COVID-19 Vaccination (Vaccine Adverse Events Reporting System [VAERS, December 14–23, 2020) (3)

	Type of reported reaction, no. (%)	
Characteristic	**Anaphylaxis (n = 21)**	**Non-Anaphylaxis allergic reactions (n = 83)**
Median Age Years (range)	40 (27-60)	43 (18-65)
Female	19 (90)	75 (90)
Mins to symptom onset, median (range)	13 (2-150)	12 (<1–1,200 [20 hrs])
Symptom onset ≤ 15 mins	15 (71)	44 (61)
Symptom onset ≤ 30 mins	18 (86)	61 (85)
Document d history of allergies or allergic reactions	17 (81)	56 (67)

TABLE 1: *Characteristics of patients with anaphylaxis and non - anaphylaxis allergic reactions after receiving the Pfizer COVID-19 vaccine — (Vaccine Adverse Events Reporting System [VAERS, December 14–23, 2020)* (3)

Monitoring of the vaccine has detected 21 cases of anaphylaxis after an administration of approximately 1,900,900 first doses of the Pfizer COVID-19 vaccine (11.1 cases per million vaccine doses administered), as well as cases of less severe non-anaphylaxic allergic reactions, based on U.S. data for December 2020. 86% of anaphylaxis cases had symptom onset within 30 minutes of vaccination, and 81% had a history of allergies or allergic reactions, including some with previous anaphylaxis events. Up to 30% of persons in the general population might have some type of allergy or history of allergic reactions. (3) Anaphylaxis is potentially life-threatening and does require immediate treatment (4).

2. Structural Components of the Vaccine

In order to treat anaphylaxis, however, we must first understand the structural components of the vaccine itself. The Pfizer COVID-19 vaccine is made of the following ingredients:

Potassium Chloride, monobasic potassium phosphate, sodium chloride, and dibasic sodium phosphate dihydrate are the salts included in the vaccine, serving to help balance the acidity in your body. Basic table sugar, also known as sucrose, can also be found in the new COVID vaccine. This ingredient helps the molecules maintain their shape during the freezing process.

The main role of lipids within the COVID-19 vaccine is to protect the mRNA and provide somewhat of an oily exterior that helps the mRNA slide inside the cells. Lipids present are ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis, (2-hexyldecanoic), 2 [(polyethylene glycol)-2000]-N,N-di tetradecyl acetamide, 1,2-Distearoyl-sn glycero-3- phosphocholine, and cholesterol.

mRNA, also known as messenger ribonucleic acid, is the only active ingredient in the vaccine. The mRNA molecules contain the genetic material that provide instructions for our body on how to make a viral protein that triggers an immune response within our bodies. The immune response causes our bodies to make the antibodies needed to protect us from getting infected if exposed to COVID-19. (5)

Description	Pfizer BioNTech
mRNA	Nucleoside-modified mRNA encoding the viral spike (S) glycoprotein of SARS-Cov-2
Lipids	2[(polyethylene glycol)-200]-N,N-di tetradecyl acetamide
	1,2-distearoyl-sn-glycero-3-phosphocholine
	Cholesterol
	(4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
Salts, Sugars, Buffers	Potassium Chloride
	Monobasic Potassium Phosphate
	Sodium Chloride
	Dibasic Sodium Phosphate Dihydrate
	Sucrose

TABLE 2: *List of all ingredients of the Pfizer COVID-19 Vaccine (Centers for Disease Control and Prevention [CDC, February 11, 2021)* (6)

Production of mRNA-based vaccines is easier due to well-developed and validated laboratory techniques used to obtain mRNA (with predefined characteristics). Additionally, delivering mRNA to the cells avoids many complicated steps required to produce, express, and purify recombinant proteins in laboratories. mRNA vaccine production is also a cell-free process and entirely in-vitro with chemical processing - without the need for advanced biological methods and cell culture techniques, unlike most protein-based vaccines. Although there are rumors that mRNA vaccines will alter our DNA since the RNA molecule can convert information stored in DNA into proteins, it is critical to note that the mRNA vaccine never enters the nucleus of the cell itself, where our DNA is stored. Instead, after the injection, the mRNA from the vaccine is released into the cytoplasm of the cells, as opposed to DNA vaccines, which need external stimuli such as electroporation, to facilitate DNA penetration into the nucleus to integrate with the host's cells. Once the antibody is produced and on the surface of the cell, mRNA is broken down and the body permanently disposes of it, therefore making it impossible to change our DNA. Tied along with mRNA's short half-life; this facilitates their elimination from the body after doing their work; thus, preventing any interaction with the packed human genetic materials.

Despite the mentioned advantages of mRNA-based vaccines, delivering mRNA is a challenging process, and cannot be attained without encasing them into vehicles known as nanocarriers designated to carry the mRNA to the cell. These nanocarriers are especially crucial to the success of the vaccine due to mRNA's short half-life. Therefore, in the Pfizer COVID-19 vaccination, lipid nanoparticles are used to carry and protect mRNA during the manufacturing and transportation processes. Lipid nanoparticles are 50-100 nanometers of miniaturized drug delivery systems, such as liposomes.

Scheme 2: *Cationic lipids used within mRNA-based vaccines enable the encapsulation of mRNA, serving as immunogenic agents. Helper lipids (e.g. PEGylated lipids and cholesterol) are usually included in mRNA lipid nanoparticle formulations to enhance the stability of nanoparticles. They promote the cellular uptake and destabilization of the lipid bilayer, and thereby, improve the efficiency of nucleic acid delivery. PEGylated lipids extend the circulation time of lipid nanoparticles due to their steric barrier effect - thus assisting in the accumulation of the nanoparticles at disease sites. The steric barrier properties of PEGylated lipids may prevent fusion or aggregation of nanoparticles during manufacturing, and this would result in a homogenous mRNA vaccine formulation with a small particle size (50-100 nm).*

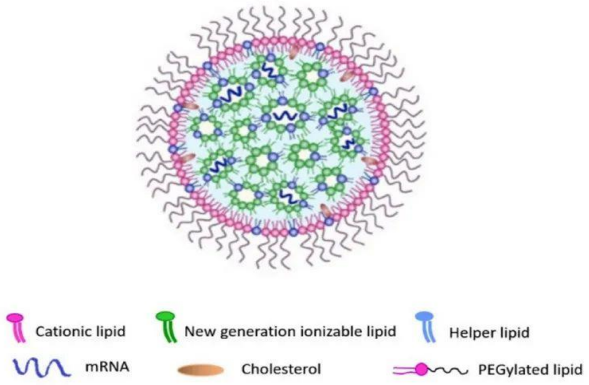


Figure 2: A diagram entailing the structural components of Lipid Nanoparticles, as well each component's role in helping to carry the mRNA from the Pfizer COVID-19 Vaccination to the cell. (7)

PEGylated liposomes - both covalent and non-covalent polyethylene glycol polymer chains attached or amalgamated onto molecules and macrostructures, such as a drug, therapeutic protein or vesicle - are the used nanocarriers in both mRNA vaccines. Liposomes are made of a bilayer of phospholipids and cholesterol, which mimics a cell's membrane and thus enables them to release their loaded mRNA and penetrate it into the cell. Although many other materials have been used to deliver macromolecules (such as exosomes, polymers, and carbon derivatives), liposomes are advantageous over other nanocarriers due to their biodegradability, biocompatibility, cell membrane-like nature, ability to confine both hydrophilic and hydrophobic drugs, and their positive charge which maintains their hold on negatively charged mRNA and allows for their penetration into cells. Once mRNA is released into a cell's cytoplasmic space, the mRNA will translate into a SARS-CoV2 spike protein, thus enabling the body to produce neutralizing antibodies against the produced protein. (8)

Scheme 3: *Both mRNA vaccine doses are given through intramuscular means. Lipid nanoparticles approach the cells following the injection, and release mRNA inside the cytoplasmic space of the cell to encode a full-length mutated SARS-CoV-2 spike glycoprotein onto the surface of cells. The human immune system recognizes the spike protein, marking it as a foreign body. Antibodies are then manufactured by the human body to fight the protein. Copies of the generated antibodies are then stored in the body to fight any potential COVID-19 infection in the future. The vaccine is administered in two doses, three to four weeks apart.*

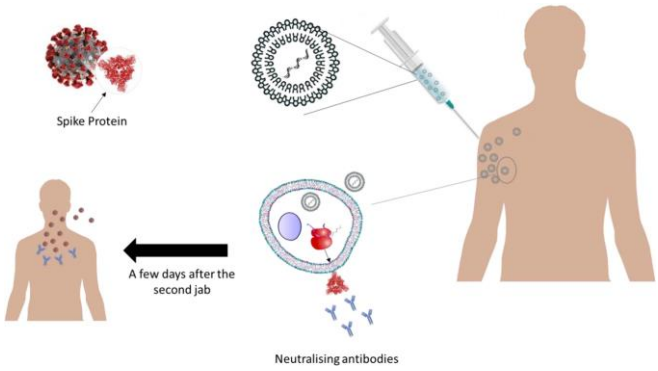


Figure 3: An illustration of the action taken by mRNA vaccines, and the response given in return by the immune system once the spike protein enters the body and rests on the surface of cells (8)

Protein purification is a series of processes conducted in order to isolate one or a few proteins from a complex mixture (usually being cells). It allows for the specification of the function and structure of the protein in question. When it comes to mRNA vaccines, the unwanted base pairs within the mRNA must be purified via these processes before it is loaded into the PEGylated liposomes, so that the vaccine can achieve optimal performance. The isolation and purification process for RNA can be conducted via a variety of approaches - including phenol-chloroform extraction, spin column purification, and the use of magnetic beads. (9)

1) Cell Lysis and Dissolution - can be achieved using buffers or reagents with chaotropic agents (e.g. guanidinium isothiocyanate, sodium dodecyl sulphate, phenol, chloroform). TRIzol or Qiazol or RNAlater can be used to maintain RNA integrity during lysis.

2) Denaturation of DNA, RNase, and Proteins - DNase is used to degrade DNA, and proteinase K is added to digest proteins. The repeated organic extraction, or the dissolving of samples in buffers containing guanidine salts, can also be used to isolate and purify the proteins. The RNase can be denatured and inactivated by using any of the chaotropic agents (like chloroform).

3) Removal/Separation of Cellular Components - RNA can be isolated from other cellular parts by adding chloroform to the solution and thus centrifuging the solution. This would result in the solution split into 2 phases - organic and aqueous, the aqueous of which contains RNA.

4) Precipitation - RNA is often recovered from the aqueous phase of using isopropyl alcohol. The RNA can also be selectively precipitated from DNA itself, via the usage of Ammonia Acetate. Similarly, lithium chloride is often used to selectively isolate RNA as well as DNA. (10)

Now that we understand the different components within the vaccine, as well as how these vaccines are made in the first place - we must now ask ourselves: What is it about the pFizer COVID-19 mRNA Vaccination that has caused so many anaphylaxis reactions in the first place?

Scheme 4: In order to produce Messenger RNA Vaccines, it is essential that the mRNA within the vaccination is purified and all unwanted base pairs have been disposed of. The steps in mRNA purification include: 1) The cloning of the cDNA construct is purified and amplified; 2) The linearized DNA constructs are transcribed into mRNA strands; 3) The transcripts are capped during transcription or post-transcription; 4) The capped mRNA is purified (many processes are available for such purification to occur, including precipitation or high-pressure chromatography), and ready to be put into a mRNA vaccine and a PEGylated liposome for it to go through an individual's body.

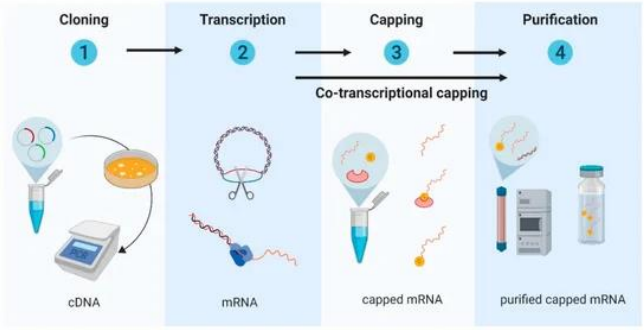


Figure 4: An illustration of the steps involved in RNA purification, and how it turns into the completed mRNA product that is eventually put into vaccinations and sent across the body via PEGylated liposome carriers. (11)

3. COVID-19 Vaccine and Anaphylaxis

A severe allergic reaction is often caused by a type of antibody made by the immune system known as an IgE antibody. When the immune system initially encounters something foreign, it learns how to fight it by using different types of antibodies. But if it creates too many IgE antibodies, it can result in an immune system overreaction.

IgE antibodies can have powerful effects, triggering other types of immune cells that retaliate harshly against a foreign substance. One example is a mast cell, which is full of cytokines, or proteins that can trigger inflammation. Allergic, inflammatory responses - also known as anaphylaxis - can cause rashes, swelling, hives and discomfort.

While this may seem mild, in more severe cases, such symptoms can result in blood pressure dropping, systemic inflammation, throat swelling and other life-threatening symptoms.

Many suspicions have been made regarding what component within the vaccine is causing such reactions. One such guess is that Polyethylene Glycol, a lipid found in both the Pfizer and Moderna COVID-19 Vaccinations, is a fairly large molecule, which can result in the immune system recognizing it and creating antibodies against it. PEG is often referred to as a "hidden antigen", often found in medications, toothpaste, shampoo, skincare, and laxatives - which have a history of causing allergic reactions within certain individuals. The COVID-19 vaccine is the first time PEG has been used as an additive in immunization. It is also the first time where an immunization platform has faced such a high rate of anaphylaxis reactions, leading scientists to believe that there is a link between the two.

The same rule applies to molecules that have a similar structure to PEG, such as polysorbates, which are oily liquids often found in pharmaceutical products, cosmetics and foods such as ice cream. Polysorbates are found in many vaccines, including the seasonal influenza vaccination. So if they have had an anaphylactic reaction to a vaccine that contains polysorbates in the past, their body is already making antibodies against similar structures, including Polyethylene Glycol - and therefore, could be at higher risk of their body resisting the COVID-19 vaccine, thus resulting in an anaphylaxis reaction. Records have shown that those with a history of allergic reactions are more likely to experience anaphylaxis reaction, and seeing as how PEG has been known to cause severe allergic reactions in the past, it is likely that PEG is the contributing factor behind anaphylaxis in vaccination recipients. (12)

Although allergy to PEG is rare, reactions can be severe or even fatal. The active ingredient of a vaccine is rarely the cause of a reaction, and more often, is due to the vaccine's excipients - an inactive substance serving as a vehicle for many drugs - such as PEG. However, a patient's hypersensitivity to PEG itself depends on its molecular weight.

Skin prick tests taken by a patient at the Addenbrookes Hospital of the United Kingdom on April 6th, 2021, were negative to all PEGs at 0.1% concentration, and to the Pfizer/BioNTech vaccine - however, at 1% of concentration, the patient in question tested positive to PEG 4000. Twelve minutes after skin testing, and 2 minutes after the skin test was established to be positive, the individual developed a systemic reaction with widespread pruritus and urticaria, a dropping systolic blood pressure, and coughing with throat constriction - key signs of an anaphylaxis reaction. The individual was treated with intramuscular adrenaline (0.5 mg), intravenous chlorpheniramine (10 mg), and hydrocortisone (200 mg). After these dosages, blood pressure improved, but coughing persisted within the patient, as well as a drop in oxygen saturation to 85%. A second dose of intramuscular adrenaline (0.5 mg) was then administered, and a rapid improvement was seen. PEG allergy was diagnosed as the cause of the patient's Pfizer/BioNTech vaccine anaphylaxis.

The case had a positive skin prick test to PEG 4000, and they experienced systemic reaction to skin prick testing, thus confirming a response to PEG. Their mast cell tryptase level did not increase in the index reaction, nor in the skin test–induced anaphylaxis, which could have occurred as the severe features of their skin test reaction were predominantly respiratory. The patient's history of multiple drug allergic reactions with sudden-onset anaphylaxis supported a diagnosis of PEG allergy. They also had reactions to products such as shower gels, shampoos, toothpaste and mouthwash - all of which are known to be PEG-heavy.

However, the patient's skin prick tests were positive only when tested with PEG 4000 - and not at higher molecular weights. PEG skin prick tests can become positive slightly later, anywhere from 10 to 30 minutes after administration. Thus, it is possible that the skin prick tests to the higher molecular weight PEGs could have become positive if read later, or if tested at a higher concentration, which would have been done had anaphylaxis not occurred already at the molecular weight of PEG 4000. (13)

TOPIC	FEATURES
History	Multiple drug allergies to unrelated drugs; May undergo anaphylaxis or severe systemic reactions.
Symptoms	Immediate onset; Symptoms may include pruritus, erythema, urticaria, angioedema, rhinitis, wheeze, dyspnoea and hypotension.
Common Drugs	Common drugs containing PEG: laxatives, Gaviscon double action, depot-corticosteroids, methylprednisolone, Depo Provera, penicillin.
PEG Molecular Weight	Higher PEG MWs appear more allergenic; Molecular weight and amount of PEG are a determining factor as to whether an allergic reaction occurs.
Topical Products	Mild cutaneous reactions (pruritus, rhino-conjunctivitis) to cosmetics, toothpaste, mouthwashes, shower gels, moisturizers, hand sanitizers and soaps; reported as mild due to the products' lower PEG molecular weights.
Vaccine Reaction	Immediate-onset (within minutes) of a severe systemic allergic reaction to the Pfizer mRNA COVID−19 vaccine.

Table 3: The clinical features of the aforementioned PEG allergic patient, which can help identify other patients at-risk for anaphylaxis reactions before vaccination, or if a COVID-19 vaccine reaction occurred due to PEG (Addenbrookes Hospital, United Kingdom, April 6th, 2021) (13)

PEG 2000 is one of the excipients of the Pfizer COVID-19 Vaccine. It is true that the aforementioned patient experienced no allergic reaction until the administration of the PEG 4000 skin prick test; however, we must keep in mind that only 1% of concentration was administered. In the vaccination itself, approximately 6% of a dosage consists solely of PEG 2000. Although the molecular weight of PEG 2000 is substantially lower than that of PEG 4000, the increased amount of concentration administered via the Pfizer vaccine results in a higher amount of PEG being released into the bloodstream than the skin prick test above. For PEG-allergic individuals, taking the vaccination can lead to life-threatening results.

Scheme 5: The amount of milligrams per dose for each ingredient present in both the lipid nanoparticle components and the overall structure of the Pfizer COVID-19 vaccination. For the PEG-lipid, approximately 50 micrograms are administered per dosage, and the Pfizer vaccination is given in an 800 microgram dose. It is shown that 6% of the mRNA vaccine is made up of PEG alone.

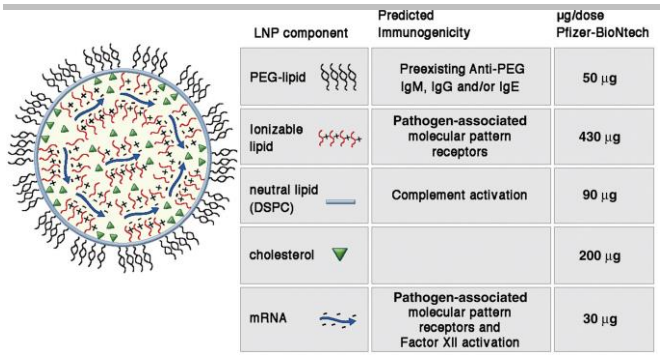


Figure 5: A graphical diagram of the mass of each lipid nanoparticle component within the Pfizer mRNA vaccine, and the total amount of PEG-lipid in comparison to other components (e.g. mRNA, cholesterol) of the vaccine. (14)

4. Conclusion and Outlook

PEG can be confirmed as a cause of anaphylaxis to the Pfizer/BioNTech vaccine. COVID-19 vaccine anaphylaxis and PEG allergy are both rare in itself, so proof of PEG as the cause in one case of vaccine anaphylaxis is extremely critical. Undiagnosed PEG-allergic patients are at risk of anaphylaxis to mRNA vaccines containing PEG, which must be identified before vaccination. In a patient with suspected or proven PEG allergy, mRNA vaccines containing PEG must be avoided.

Acknowledgment

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Keywords: COVID-19 • Vaccine Development • RNA • Patient • Nanotechnology

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