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Polyethylene Glycol (PEG) in mRNA-Based COVID-19 Vaccines: Impact, Immunogenicity, and its Potential Role in Post-Vaccination Induced Hypersensitivity

Mohamed Ibrahim[1*], Sherif Emam[2], Omar Helmy Elgarhy[1], Hatem A. Sarhan[1], Amal Kamal Hussein[1]

[1] Department of pharmaceutics and industrial pharmacy, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt.
[2] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Zagazig University; 44519 Zagazig, Egypt.

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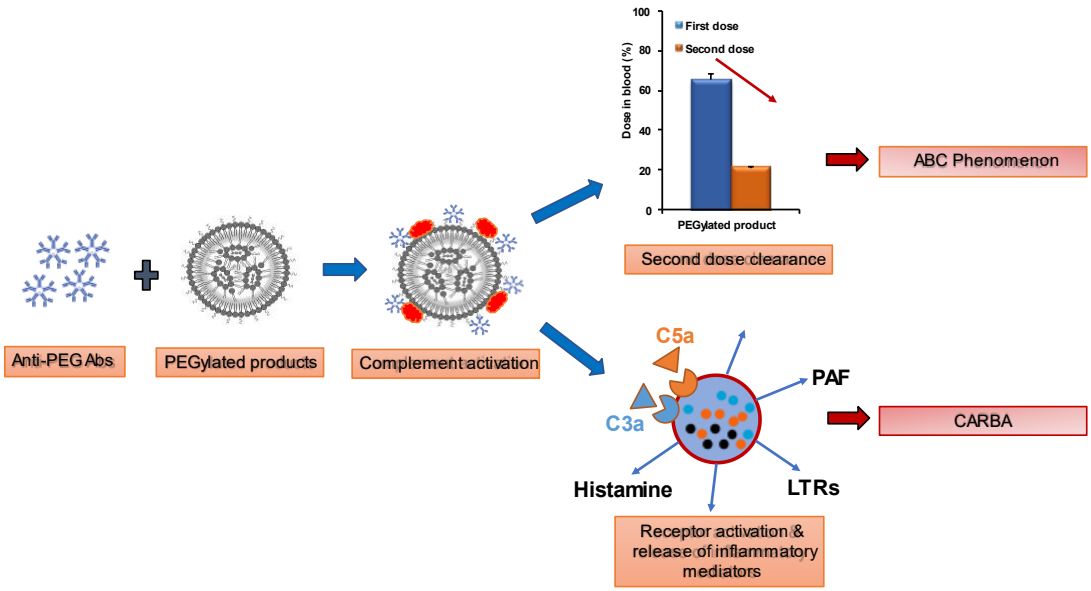
Abstract

Polyethylene glycol (PEG) is the most commonly used hydrophilic polymer in cosmetics and polymer-based drug delivery systems. PEGylation of nanocarriers has gained a lot of interest nowadays as it improves the circulation half-life and formulation stability. Recently, messenger ribonucleic acid (mRNA)-based coronavirus (COVID-19) vaccines depend on the delivery of mRNA to the cytosol and then being transcribed into the antigenic proteins that prime the immune system to produce specific antibodies (Abs) that can protect against the coronavirus infectious diseases. PEG is considered one of the main components used in the formulation of lipid nanoparticles (LNPs) encapsulating mRNA genomic material. Despite the previous reports that PEG is considered a stealth and non-immunogenic polymer, anti-PEG Abs were detected following the treatment with PEGylated products. Unfortunately, anti-PEG Abs were found not only in patients treated with PEGylated therapeutics, but also in healthy individuals who had never used a PEGylated product before. Several cases of hypersensitivity have been reported following mRNA-based COVID-19 vaccination. It is thought that PEG plays a crucial role in the development of anaphylactic reactions reported post-vaccination. So, in our study, we tried to highlight the mRNA-based COVID-19 vaccine products and the role of PEG in the formulation of mRNA-LNPs. Also, we focused on the immunogenicity of PEG and its effect on the clearance of PEGylated therapeutics. In addition, we tried to demonstrate the potential role of PEG in these reactions. Finally, we introduced the possible hypoth esis to overcome PEG-induced hypersensitivity and the recent recommendations that should be taken into consideration before administration of PEGylated products.

Keywords

PEG - mRNA vaccines, Accelerated blood clearance, Complement activation-related pseudoallergy (CARPA), Hypersensitivity, COVID-19



PEG, Polyethylene glycol; Abs, Antibodies; C3a and C5a, complement fragments; LTRs, Leukotrienes; PAF, Platelet activating factor; CARPA, complement activation-related pseudoallergy.

**** Correspondence:*** *Mohamed Ibrahim*
Tel.: *+ 01060904196*
Email Address: *mohamed_ibrahim@mu.edu.eg*

1. Introduction

The main aim of pharmaceutical research is to develop an effective, safe, and economic drug delivery system (DDS). The distribution of the drug molecules in the body represents the main area of interest in drug design. Ideal dosage forms should improve the drug's accumulation in the desired part of the body and decrease its unwanted distribution in other body parts, which are responsible for undesirable side effects [1]. Novel nanocarriers as liposomes, LNPs, dendrimers, exosomes, and polymeric micelles are effective drug delivery systems which success in achieving both criteria improves drug targeting and minimizes unwanted side effects [2]. They are widely used in many fields, as cancer therapy [3], gene therapy [4], delivery of proteins and peptides [5], and medical imaging [6].

Polymers have a crucial role in designing an immaculate DDS. They are used in DDS as stabilizers, solubilizers, penetration enhancers, drug targeting, release modifiers, and taste-masking agents [7]. A wide range of natural and synthetic polymers such as cellulose derivatives, starch, polyanhydrides, and polyesters are used in DDS. The Food and Drug Administration (FDA) approved the use of some biodegradable polymers in this DDS, such as poly (L-lactic acid) (PLA), poly (D, L-lactic-co-glycolic acid) (PLGA), PEG, and N-(2-hydroxypropyl) methacrylamide (HPMA) [8]. Despite the variety of polymers that can be used, PEG is considered the polymer of choice in design of DDSs. PEGs are hydrophilic polymers that consist of repeated monomer units called oxyethylene subunits **(Fig. 1)**. Each monomer consists of both non-polar $(CH_2)_2$ and polar oxygen atoms, thus it is soluble in a variety of both polar and non-polar solvents [9]. Over the past few decades, PEGs have been used as food additives, lubricants for devices, anti-freeze agents, and as a vehicle in tablets, parenterals, and dermatological formulations [10]. The use of PEG for the delivery of drugs and proteins was first introduced by Abuchowski and Davis in 1977, which is the so-called PEGylation technique [11]. PEGylation can be defined as the surface decoration of nanostructures or proteins with PEGs to improve the circulation half-life and improve the formulation stability [12, 13].

Despite the great importance of PEGylation, recent reports about the immunogenicity of PEGs may limit the use of this technique [14]. The immunogenicity of PEG may affect the therapeutic efficacy of subsequently administered PEGylated product, which is the so-called accelerated blood clearance (ABC) phenomenon [15]. In patient's serum, anti-PEG Abs were detected following administration of the first dose of PEGylated products [16]. surprisingly, Yang and Lai reported that anti-PEG Abs were detected at a significant level in 42 % of healthy individuals without a prior history of PEGylated products administration [17]. Pre-existing anti-PEG Abs can affect not only the therapeutic efficacy of PEGylated products but also may represent a possible cause of PEGylated products-induced hypersensitivity [18]. It is well established that the ABC phenomenon is accompanied by complement system activation, which may induce some allergic reactions due to the release of pro-inflammatory mediators upon PEGylated product administration [19].

Recently, upon the SARS-CoV-2 wide spread, several attempts have been made to introduce a safe and effective vaccination strategies to control the aggressive spread of this pandemic [20]. PEGylated LNPs were used as an effective delivery system for the coronavirus disease vaccination molecules, especially the mRNA-based coronaviruses-19 (COVID-19) vaccines [21]. Despite the reported efficacy of mRNA-COVID-19 vaccines, cases of COVID-19 post-vaccination hypersensitivity have been reported [22]. It is thought that the presence of PEG plays a vital role in post-vaccination induced allergic reactions [23]. So, in our study we will try to focus on the use of PEG in mRNA-COVID-19 vaccines and the immunogenicity of PEG molecules and their effect on the therapeutic efficacy of PEGylated products. In addition, we will try to explain the potential role of PEG in allergic reactions reported following COVID-19 vaccinations and try to introduce some strategies to overcome the undesirable allergic reactions associated with the use of PEG in nanoformulations. Also, we will explain some recent recommendations that should be taken into consideration during COVID-19 vaccination.

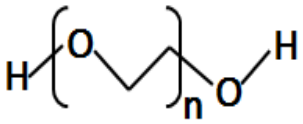


Figure 1. Structure of polyethylene glycol

The PEG polymer consists of repeated monomers of oxyethylene subunits. Each monomer consists of two (CH_2) groups attached to one oxygen atom. n represents the number of repeated molecules.

2. PEG properties

PEGs are also known as polyoxyethylene or polyethylene oxide, is a biocompatible, bio-inert polymer. they have amphiphilic nature which makes them soluble in a wide range of organic solvents, including ethanol, chloroform, acetone, acetonitrile, and in addition to a high solubility in water. they are thermally stable and electrically neutral at different pH levels. they can be synthesized with different molecular weights and a variety of properties. They are commercially available in wide range of molecular weights ranging from 200 to 35,000 Da and different degrees of branching [24]. According to the degree of polymerization during manufacturing and their final molecular weights, PEGs have specific states and melting points. PEGs with low molecular weights ranging from 100-700 Da are usually colorless, viscous liquids, while PEGs with molecular weights ranging from 1,000 to 2,000 Da are semisolids, in the same manner, those with higher molecular weights (more than 2,000 Da) are solid, waxy, and white in color, where their melting points proportional to their molecular weights [25].

3. COVID-19 vaccines

Vaccines are biological agents that provides an effective immune response against specific antigens or allergens and they are usually used to control or prevent infectious disease [26]. Upon the advances in vaccine technology, several pandemics have been reduced or completely eradicated, such as smallpox virus, measles, and polio [27]. Historically, vaccine approaches included, live-attenuated vaccines, inactivated pathogens, purified protein toxoids, purified inactivated viruses, purified polysaccharides, and virus-like particles [28]. Generally, vaccines can be classified into whole organism vaccines, combined antigens, purified macromolecules, synthetic peptides, recombinant vectors, DNA vaccines, and messenger RNA (mRNA) vaccines [29].

Since the coronavirus 2 (SARS-CoV-2) outbreaks in 2019 in China, many companies and researchers have focused on the development of effective, economic, and safe vaccines to control

the massive spread of this pandemic situation [30]. Coronaviruses are classified as envelope viruses with a single-strand RNA genome [31]. They can enter the host cells and develop an infection through fusion with the host cell membrane [32, 33]. It is well reported that coronaviruses encode one main surface protein called type I transmembrane spike glycoproteins (abbreviated as S-protein) **(Fig. 2)**, which is located on the virus envelop and is responsible for fusion of the viruses with the membrane of the host cell and receptor bindings [34]. In SARS-CoV-2, the spike protein first binds to angiotensin-converting enzyme 2 on the host cells and then endocytosed by the host cells [35]. Endocytosis is followed by fusion of viral and host cell endosomal membranes and subsequent release of the viral genome into the cytoplasm [36]. So, Abs that can bind to the S protein, mainly to its receptor-binding domain, can efficiently prevent the attachment of the virus to the host cells and protect them against viral infection. Based on previous knowledge, the S-protein is considered the main antigenic target for the development of vaccines against SARS-CoV-2 [37, 38].

Based on SARS-CoV-2 platforms, there are more than 180 vaccine approaches under development against SARS-CoV-2 [39]. The SARS-CoV-2 platforms are mainly classified into three main approaches, the first one is traditional or classical approaches such as live-virus or inactivated vaccines, the second is vectored vaccines, and recombinant protein vaccines, and the last one is DNA and RNA vaccines [40]. It is noteworthy that all the available vaccines that are currently in use were prepared for intramuscular administration. Despite the ability of this route to induce a strong IgG response, it can only protect the lower respiratory tracts. Unlike the natural antigen, it does not stimulate the secretory IgA responses which are responsible for the upper respiratory tract protection [41]. Regarding to the world health of organization (WHO) COVID-19 vaccine tracker, various vaccination platforms are under investigation including, protein-based vaccines (approximately represent about 34%), live attenuated virus (2%), inactivated virus (14%), replicating (2%) and non-replicating (15%) viral vector, virus like particle (4%), deoxyribonucleic acid (DNA) (10%), and ribonucleic acid (RNA)-based vaccines (16%) [42]. Despite the diversity of platforms against SARS-CoV-2, two mRNA-based vaccines (Pfizer/BioNTech BNT162b2 and Moderna mRNA-1273 vaccines) were approved for emergency use in August 2021 after ensuring their efficacy and safety.

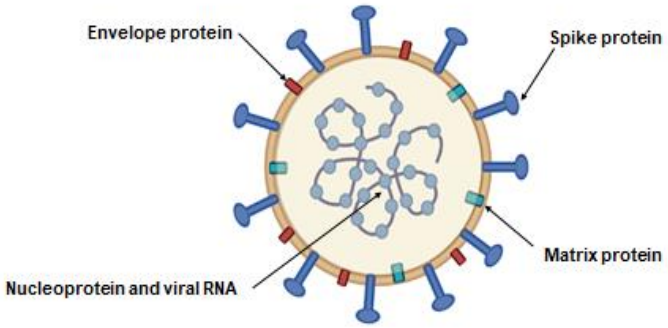


Figure 2. Structure of SARS-CoV-2 Virus

A schematic diagram of the structural proteins of the SARS-CoV-2 virus, including the lipid membrane, the envelope and matrix proteins within the membrane, the spike protein on the virus surface, and the genetic RNA material covered by the nucleoprotein inside the virus membrane.

4. mRNA-based COVID-19 vaccines

One of the recently approved strategies in the formulation of vaccines is messenger RNA (mRNA)-based vaccines. mRNA-based vaccines depend on the use of in vitro transcribed mRNA molecules that encode a specific antigen. It is well known that, the mRNA itself which is the so-called the naked mRNA is highly unstable and prone to undergo physical, chemical, or enzymatic degradation that decreases its stability and reduces its serum half-life to less than 5 min [43]. So, it is better to be formulated in a suitable delivery system to ensure the effective delivery of mRNA molecules into the cytoplasm of the host cells and prolong their circulation half-life [44]. Two types of RNA molecules are used in mRNA-based vaccines, including self-amplifying and non-replicating RNA molecules. The self-amplifying RNA molecules have a specific viral replicase gene together with the pathogenic antigen gene during the formulation of the delivery system that allows auto replication in the targeted host cells that increases the vaccine potency [44, 45]. It is well known that naked mRNA molecules have poor stability and are susceptible to being hydrolysed by extracellular RNases before they reach the target site for transcription (the cytosol). In addition to, their lower efficacy for transfection and cellular uptake. So, developing an efficient mRNA vaccine based on naked mRNA molecules faces great challenges [46]. Recently, several attempts have been made to improve mRNA stability and cellular uptake, including modifications to mRNA molecules' modifications and/or developing an efficient mRNA molecules' delivery system [47-50].

Upon SARS-CoV-2 infection widespread in December 2019 in Wuhan, China, many companies and researchers focused their efforts on developing effective vaccines against the SARS-CoV-2 virus. Several vaccine have been approved for protection against this pandemic [47, 51-55]. mRNA-based vaccines as Pfizer/BioNTech BNT162b2 and Moderna mRNA-1273 vaccines (developed by Pfizer/BioNTech and Moderna pharmaceuticals, respectively) were approved for the urgent use against SARS-CoV-2. Both vaccines are mRNA-based that encodes SARS-CoV-2 spike protein and formulated in Lipid Nanoparticles (LNPs) delivery system [56-58].

LNPs are lipid-based nanocarrier systems that are composed of cationic/ionizable lipids, polyetheylene glycol-containing lipids (PEG-lipids), helper lipids, and cholesterol **(Fig. 3)** [59, 60]. Cationic or ionizable lipids as D-Lin-MC3-DMA, DLin-KC2-DMA, Lipid A9, and 1,2-dioleyloxy-3-dimethylaminopropane (DODMA) are the major components of LNPs. Cationic lipids offered greater encapsulation efficiency of nucleic acids compared to neutral or anionic lipids due to electrostatic interaction between cationic lipids and the anionic nucleic acid molecules[61]. However, the *in vivo* administration of these positively charged lipids resulted in toxic effects on the cellular level. As a result, novel cationic lipids that are designed to be positively charged only in acidic conditions and become neutral at physiologic pH, have emerged and are called ionizable lipids [62, 63]. This astounding property of ionizable lipids could signifcantly improve the pharmacokinetics and toxicity profiles of LNPs. Other lipid components of LNPs have crucial roles as well. Helper lipids could assist in the formation of LNPs and maintain their stability. The helper lipids that are commonly used in LNPs are phosphatidylcholine lipids such as 1,2-Distearoyl-sn-glycero-3-phosphorylcholine (DSPC), 1,2-Dioleoyl-sn-glycero-3 phosphocholine (DOPC), or 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). Incorporation of cholesterol may also improve LNP stability, membrane regidity and

fusogenicity [64]. PEG-lipids could form a stealth layer that reduces LNP clearance and extends LNP circulation time by preventing LNP opsonization. Furthermore, the presence of PEG on the surface of LNPs prevents LNP aggregation during manufacturing and storage, and helps forming highly uniform and small-size LNPs[65, 66]. The full composition of Pfizer/BioNTech BNT162b2 and Moderna mRNA-1273 vaccines is summerized in **Table 1.**

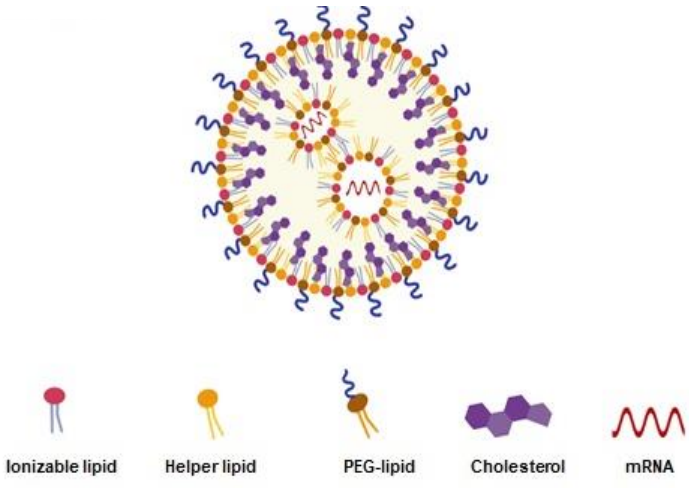


Figure 3. Structure of mRNA-based lipid nanoparticles (LNPs)

A schematic diagram of the structural arrangement of lipid components used in the formulation of LNPs. LNPs consist of four main lipids, including ionizable lipids (pink), phospholipids (yellow), PEG-lipids (brown), and cholesterol (violet), encapsulating single-stranded mRNA.

5. Impact of PEG-lipids in the formulation of mRNA-LNPs COVID-19 vaccines

PEG lipids represent one of the main constituents used in the formulations of LNPs. It is well established that PEG lipids are used for surface decoration of the LNPs and ultimately control the LNPs size. Witzigmann et al. studied the effect of PEG-lipid content (0.25-5.0%) on the LNP size and reported that there is an inverse relationship between the PEG-lipid molar percentage and LNP size. In addition, they reported that the formulation of LNPs with a molar percentage of 2.5% of the total lipid content can produce LNPs with a size of 78 nm [69]. The PEG-lipid structure is characterized by the presence of hydrophilic parts (PEG) and hydrophobic parts (lipid backbone), so it can form a hydrophilic shell surrounding the LNPs that sterically hinders the LNPs aggregation [70]. The main advantage behind the use of PEG-lipids in biomedical applications is their ability to increase the aqueous solubility of the PEGylated products due to their PEG chains, where their ethylene oxide subunits can interact with 2-3 water molecules. The polymer chains provide a conformational cloud around the LNPs that sterically hinders their interaction with other LNPs, and hence it prevents their aggregation and enhances their physical and chemical stability [71, 72]. In addition, PEGylation improves the circulation half-life of PEGylated therapeutics through increasing their hydrophilicity and decreasing the glomerular filtration rate, which subsequently decreases their blood clearance and improves their half-life [73]. As described in (Table 1), Pfizer/BioNTech uses 2-[(polyethylene glycol)-2000]-N, N ditetradecylacetamide (PEG2000-DMA) while Moderna decided to use 1,2-dimyristoyl-*rac*26 glycero-3-methoxy-poly (ethylene glycol)-2000 (PEG2000-DMG) in the formulation of mRNA-LNPs COVID-19 vaccines. Despite the importance of PEG-lipids in improving the LNP stability and extending their circulation half-life, some cases of allergic reactions have been reported following COVID-19 vaccination, which may be attributed to pre-existing anti-PEG Abs [74].

6. PEG immunogenicity

Several years ago, PEGs were considered biologically inert and non-immunogenic molecules, so they are widely used in pharmaceutical formulations and cosmetics. However, there are growing reports about the potential immunogenicity of PEG molecules [75]. These reports relied on the pre-existing anti-PEG Abs detected in healthy individuals without a previous history of PEGylated product administration. Armstrong et al. reported that anti-PEG Abs were detected in 25 % of blood donors without a previous history of PEGylated products administration [75]. In the same manner, Yang and Lai reported that anti-PEG Abs were detected in patients without a previous history of use of PEGylated therapeutics. They have proposed a tentative hypothesis for the source of these Abs in healthy individuals where they reported that frequent exposure to PEG-containing products such as soap, shampoos, and lotions may represent the main source of anti-PEG Abs in healthy donors because the use of these products can induce PEG skin penetration and stimulate the production of anti-PEG Abs, especially in cases of compromised skin or injury [17]. Although the PEGylation technique represents a great hypothesis to enhance the formulation stability and prolong their circulation half-life, it is well established that these PEGylated products can prime the immune system to produce anti-PEG Abs, which may affect the clearance and therapeutic efficacy of subsequent administered PEGylated products. Anti-PEG Abs were detected following administration of PEGylated liposomes in mice, rats, beagle dogs, and mini pigs [16, 76, 77]. Unfortunately, the presence of anti-PEG Abs may represent a great obstacle in usage of PEGylated therapeutics despite their reported benefits because it may attenuate their therapeutic response and play a potential role in PEGylated products-induced hypersensitivity reactions.

7. Accelerated blood clearance of PEGylated products

The concept of the ABC phenomenon was first introduced by Dams et al. in 2000, when they reported that the first dose of PEGylated liposomes enhanced the clearance of the second dose injected within one week in rats and rhesus monkeys [78]. In the same manner, Ichihara et al. reported that intravenous injection of PEGylated liposomes can prime the immune system to produce anti-PEG Abs in mice and rats [16]. Previous reports stated that the administration of PEGylated therapeutics as PEGylated liposomes, PEGylated LNPs, and PEGylated exosomes can prime the immune system for the production of anti-PEG antibodies. Pre-induced anti-PEG antibodies may enhance the clearance of subsequent administered PEGylated therapeutics [15]. Based on the previous findings, the mechanism of the ABC phenomenon was based on two phases: the induction phase and the effectuation phase. The induction phase includes differentiation and proliferation of the splenic B cells, which are responsible for Abs production. The effectuation phase occurs

following priming the biological system in the induction phase. The effectuation phase occurs 5 to 21 days following the initial dose of PEGylated product, and in this phase, the PEGylated products are opsonized by C3 fragments and engulfed by the Kupffer cells, which induce their rapid clearance from the systemic circulation [79, 80].

Several factors may influence the occurrence and the magnitude of the ABC phenomenon as; Time interval between the first dose and the subsequent doses where reports showed that prolonging the time interval between doses may diminish the effect of pre-induced anti-PEG antibodies. The route of administration is another factor may affect the magnitude of the ABC phenomenon. Li et al. stated that A slow intravenous infusion of PEGylated liposomes with a low lipid concentration was found to induce a more severe ABC phenomenon, compared to a bolus intravenous administration with the same dose of PEGylated liposomes [81]. Nature of the encapsulated drug may also affect the occurrence of ABC phenomenon. It is worth noting that the repeated administration of Doxil® (a PEGylated liposomal system encapsulating a cytotoxic drug doxorubicin did not induce the ABC phenomenon [82].

8. PEG-induced hypersensitivity reactions (HRs)

HRs are a group of undesirable immunological responses that occur following exposure to a specific antigen or allergen. HRs are classified into four types (types I, II, III, and IV) according to the type of immunologic response and the mechanism involved in tissue or cell injury [83]. Type I, II, and III are classified as immediate HRs as they occur within 24 hours following exposure to antigenic molecules, while type IV is classified as a delayed-HRs as its immune response usually appears within 24-48 hours following antigen exposure [84]. HRs symptoms range from mild to severe, depending on the antigen exposure and the immune response of the host cells. Symptoms include skin rash, fever, facial swelling, dry mouth, watery eyes, chest tightness, shortness in breath, and heart rate abnormalities [85].

Several years ago, PEGs were considered non-immunogenic molecules. However, HRs have been reported following administration of PEGylated products [86]. PEG-induced HRs were first introduced in the 1970s, as symptoms of hypersensitivity were reported in patients exposed to radiocontrast media containing PEG [87]. In 1995, the FDA approved the use of Doxil® as a PEGylated liposomal system to encapsulate doxorubicin for use in the treatment of various types of cancer. Some HRs or infusion reactions were reported in patients treated with Doxil® where symptoms of dyspnea, facial flushing, headache, hypotension or hypertension, and chest pain have been reported following Doxil® administration [88]. Nowadays, PEGylated LNPs are used as an effective drug delivery system for the delivery of therapeutics to the target site of action [89]. LNPs were used in Pfizer/BioNTech and Moderna COVID-19 vaccines. Despite the great benefits obtained from the use of these products, some cases of anaphylactic reactions have been reported following vaccine administration [90, 91]. The HRs reported for these vaccines were 5 and 2.8 cases per million for Pfizer/BioNTech and Moderna vaccines, respectively [92]. The rate of these reactions was high compared to similar vaccines (1.3 cases per million) [93]. Studying the history of these cases revealed that most of them used therapeutics or

Table 1: The composition of Pfizer/BioNTech BNT162b2 and Moderna mRNA-1273 vaccines .

Description	Pfizer/BioNTech BNT162b2	Moderna mRNA-1273
mRNA	Nucleoside-modified mRNA encoding SARS-CoV-2 spike (S) glycoprotein	Nucleoside-modified mRNA encoding SARS-CoV-2 spike (S) glycoprotein
Lipids	- ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) -1,2-Distearoyl-sn-glycero-3-phosphocholine - Cholesterol - 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide	- heptadecane-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate. - 1,2-Distearoyl-sn-glycero-3-phosphocholine - Cholesterol - PEG2000-DMG = 1,2-dimyristoyl-rac-glycerol, methoxypolyethylene glycol
Other additives	- Potassium chloride - Monobasic potassium phosphate - Sodium chloride, - Dibasic sodium phosphate dihydrate -Sucrose	- Tromethamine - Tromethamine hydrochloride - Acetic acid - Sodium acetate - Sucrose
Reference	[67]	[68]

cosmetic products containing PEG, such as azithromycin containing PEG 6000, Macrogol laxative (PEG 4000), and other PEG-containing cosmetics [23, 94]. McSweeney et al. reported a case of anaphylaxis following Pfizer/BioNTech COVID-19 vaccine administration. They reported that the patient experienced a positive PEG allergy test via basophil activation (BAT) test (with a positive signal detected upon stimulation of blood in vitro with PEG 4000 at a concentration of 0.2 mg/mL) [95]. In the same manner, Restivo et al. reported a case of a young Caucasian woman who had clear signs of anaphylactic reactions 5h following the first dose of the Pfizer-BioNTech vaccine. The allergic reactions include the appearance of erythematous spots on the neck and face, hoarseness, and a feeling of dry mouth. The BAT test was used to investigate a possible PEG allergy. Interestingly, the patient showed a positive BAT test where they reported a significant activation of the patient's basophils, which represents a major sign of PEG-allergy [96]. Also, Pickert et al. reported that a case of a24-year-old female experienced symptoms of allergic reactions both following the administration of PEG-containing medications such as Macrogol laxative (which contains PEG 4000) and the PEG-containing BNT162b2 vaccine (contains PEG 2000). She revealed a positive skin prick test (SPT) to these compounds which contain PEG, thus indicating clinically immediate-type sensitization to PEG molecules [97]. The reported immunogenicity of PEG may explain the potential role of PEG-induced HRs following administration of PEGylated therapeutics and vaccines such as the Pfizer/BioNTech and Moderna COVID-19 vaccines.

8.1. Mechanism of PEG-induced HRs

The exact mechanism of PEG-induced HRs is still under investigation and needs further studies to be fully understood. The early reports about PEG hypersensitivity were classified as type I reactions (IgE-mediated anaphylactic reactions), which require a prior sensitization (exposure) to the antigen or allergen. Upon first exposure, IgE Abs were produced by mature B cells and expressed on mast cells. After the second exposure, antigen binds to the surface bound receptors, which stimulates the activation and degranulation of the mast cell and the subsequently released inflammatory mediators responsible for allergy manifestations [98]. But recently, PEG-induced HRs have been reported upon first exposure to PEGylated products without prior exposure to PEG. These HRs are so-called complement activation-related pseudoallergy (CARPA), which is classified as a non-IgE-mediated allergy and very similar in symptoms to type I HRs [88, 99]. The proposed mechanism of PEG-induced hypersensitivity is that PEGylated products induce complement system activation via interaction with pre-existing anti-PEG Abs, which enhances anaphylatoxins' release as C3a and C5a. Upon their release, they activate immune cells as basophils, macrophages, and mast cells. Immune cell activation is accompanied by the release of vasoactive pro-inflammatory mediators such as histamine, tryptase, leukotrienes, and platelet activating factor [100]. Binding of the inflammatory mediators to their receptors leads to activation of smooth muscle cells and endothelial cells, which are responsible for CARBA symptoms such as systemic vasodilatation, pulmonary vasoconstriction, and bronchoconstriction (**Fig. 4)** [101, 102].

8.2. Approaches to overcome PEG-indued HRs

Hypersensitivity to PEGylated formulations and PEG-containing products is well documented in both animal and human studies [18]. Anaphylactic reactions are life-threatening reactions that occur minutes to hours after being exposed to an allergen [103]. Allergic symptoms range from modest erythema, headaches, and swelling of the face to more serious symptoms such as shortness of breath, hypothermia, hypotension, and finally uncontrollable hypersensitivity, which can result in death. [104]. To ensure the safe use of PEGylated drugs or PEG-containing items, current attempts have been made to overcome PEG-associated hypersensitivity. These strategies can be described as follows:

8.2.1. Premedication with anti-allergic medications such as antihistamines

Because histamine is one of the main allergic mediators in anaphylaxis, prophylactic antihistamines are important in controlling allergy symptoms [105]. is based on competitive inhibition of histamine binding to its cellular receptors, which are widely distributed throughout the body and include smooth muscles, nerve terminals, and glandular cells [106]. Histaminic receptors range from H1 to H4, with H1 and H2 being the most abundant in the body [107]. Antihistaminic medications are classified into three generations based on their selectivity to histaminic receptors and lipophilicity, and thus their ability to cross the blood brain barrier (BBB). The first generation includes Chlorpheniramine and Diphenhydramine, the second generation includes Loratadine and Cetirizine, and the third generation includes Desloratadine and Fexofenadine [108, 109]. So antihistaminic medication can be used as a prophylactic therapy in case of allergy to alleviate anaphylactic symptoms. As a result, antihistaminic medication can be used as a preventative measure in the event of an allergy to alleviate anaphylactic symptoms.

8.2.2. Use of complement inhibitor Factor H (FH):

As previously stated, complement activation is the primary noncellular component of the immune system and is a major contributor to the hypersensitivity reactions (HSRs) that occur upon administration of PEGylated products [110, 111]. Complement activation can occur via three different pathways: classical, lectin, and alternative. Complement activation via alternative is regulated by six plasma proteins [C3, and Factors B, D, Properdin (P), β1H and C3b inactivator (C3bINA)] [112]. FH is a soluble glycoprotein with a molecular weight of 155 KDa that is thought to be the main inhibitor of complement activation via the alternative pathway [113]. FH acts mainly by inhibiting factor B's binding of the third active component of complement (C3b), thereby preventing the formation of the C3bBb complex, which is responsible for the proteolytic cascade of the alternative pathway, as well as the ability of FH to accelerate the decay of the C3bBb complex if it has already formed [114]. It has been reported that FH has a high affinity for C3b, and that the level of this factor is inversely proportional to the severity of HSRs [115]. It has been reported that complement activation associated with the use of AmBisome (liposomal system loaded with Amphotericin B), Cremophore EL (solvent used with anticancer drugs), and Rituximab (anticancer monoclonal antibody) can be inhibited *in vitro* when exogenous FH is used, and that an artificial form of FH (mini FH) has a greater inhibitory effect on complement activation than exogenous FH [116]. According to the reports, using FH may be an effective strategy for inhibiting complement activation and, as a result, alleviating HSRs.

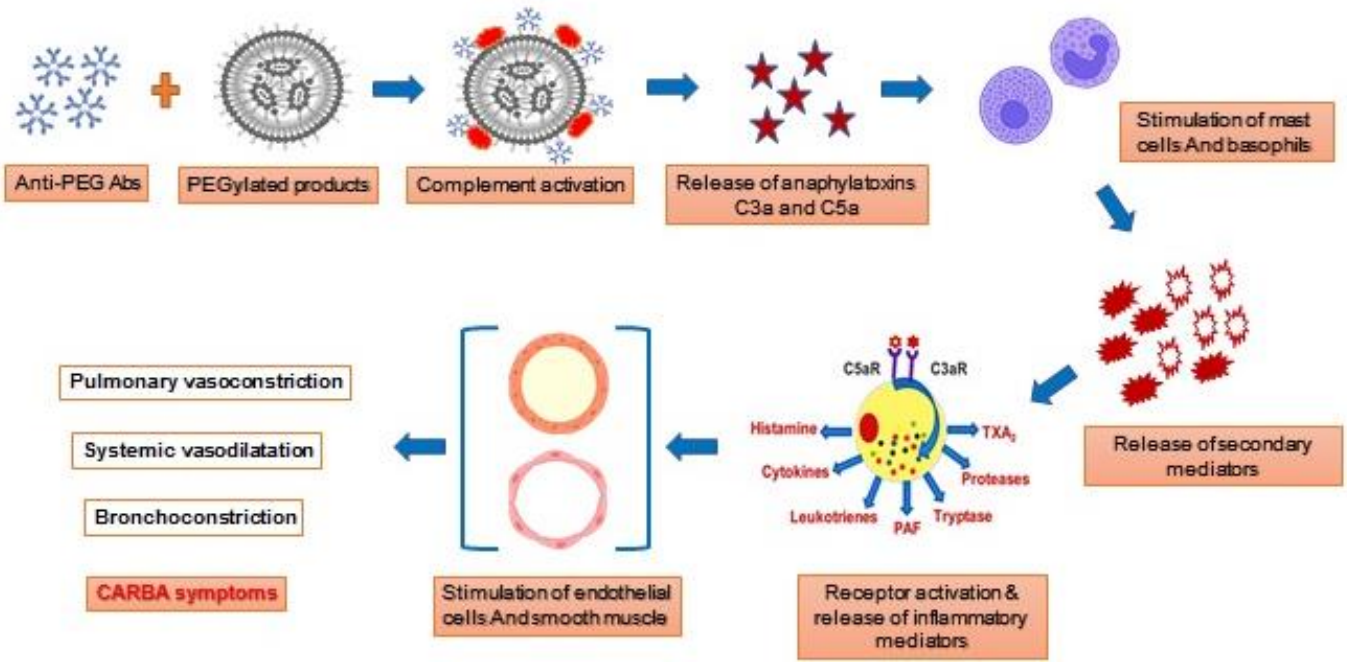


Figure 4. Mechanism of CARBA

PEGylated products interact with anti-PEG Abs and activate the complement system, which induces anaphylatoxins (C3a and C5a) release. Anaphylatoxins bind to their receptors on immune cells such as mast cells, macrophages, WBCs, platelets, and basophils. Activation of anaphylatoxins receptors is accompanied by the release of pro-inflammatory mediators such as histamine, leukotrienes, and platelet activating factor which result in CARPA symptoms. Modified from [95].

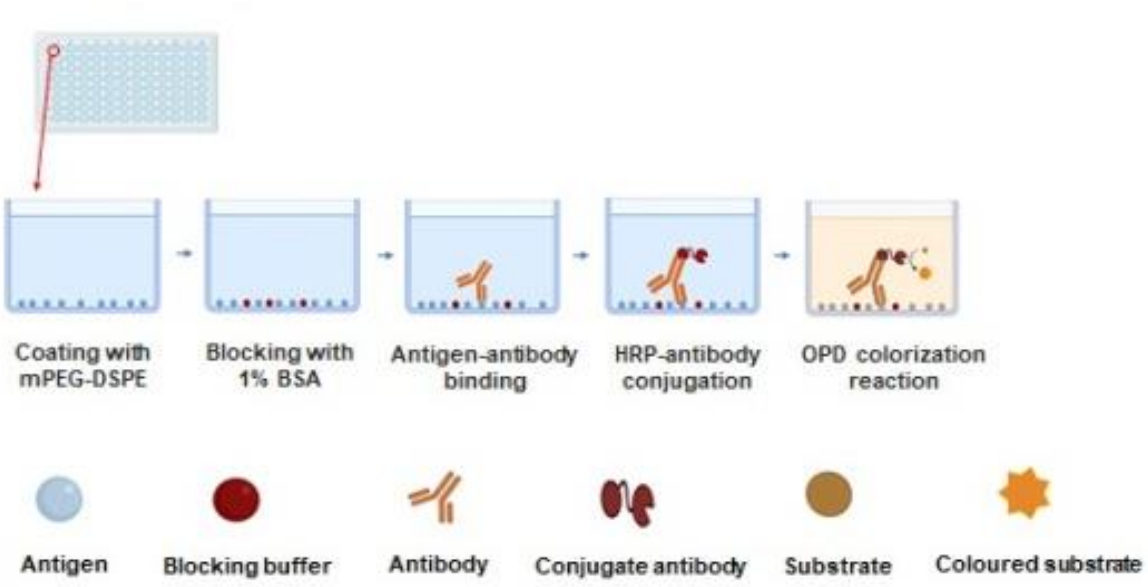


Figure 5. Principle of direct ELISA for anti-PEG antibodies detection

The corning® 96-well polystyrene microplate was coated with mPEG2000-DSPE, then the plates were blocked by 1% BSA. Serum samples containing anti-PEG Abs were added. And then, HRP gout anti mouse-IgM was added to each well. The OPD substrate for peroxidase was used to initiate the colorization reaction, which is measured spectrophotometrically using a microplate reader.

8.2.3. Tachyphylaxis induction with empty PEGylated products

Tachyphylaxis induction, also known as immune tolerance, is the self-induction of a decrease in the physiological and pathological response to a specific agent or drug molecule through pre-administration of the same agent or drug molecule. Immune tolerance with empty PEGylated products (Placebo) is regarded as a promising approach in anaphylaxis prevention [117]. The use of this strategy is based on the ability of empty PEGylated products to consume pre-existing mediators of hypersensitivity, such as anti-PEG Abs [118], and/or on the placebo's down-regulation effect on signalling processes that occur in cells that are primarily responsible for complement activation and the appearance of HSRs associated with the use of PEGylated products or PEG-containing products [119].

Szebeni et al. reported that infusion reactions caused by intravenous administration of Doxil® can be avoided by prior treatment with Doxebo® (empty PEGylated liposomal system), and that slow intravenous infusion of Doxebo® for a short time (15-30 min) in a pig model can effectively reduce or abolish HSRs caused by a subsequent dose of Doxil® [117]. Similarly, Duburque et al. reported that induction of tolerance can effectively reduce infusion reactions that occur after administration of Infliximab (monoclonal antibody to tumor necrosis factor) [120]. In summary, many publications have reported the success of desensitization strategies in inhibiting or reducing infusion reactions that can occur with some medications in patients who have a history of immunoglobulin-mediated anaphylactic reactions, such as Ciprofloxacin [121], Vancomycin [122], Insulin [123], and Allopurinol [124]. As a result, in the management of drug-induced anaphylactic reactions, a tachyphylaxis induction strategy should be considered.

8.2.4. Modification of PEG moiety

Modification of the PEG moiety is considered a promising approach for reducing PEG immunogenicity and minimizing PEG-associated HSRs. In the PEG moiety, there are two major modifications that can occur: The first is the replacement of the PEG moiety's terminal methoxy group. According to reports, substituting a hydroxy group for the methoxy group significantly reduces PEG immunogenicity as well as the accelerated blood clearance phenomenon that occurs when PEGylated products are used [125, 126]. Shimizu et al. reported that PEGylated liposomes prepared using PEG modified with a hydroxyl end rather than a methoxy terminal end effectively reduce anti-PEG antibody production, thereby attenuating the ABC phenomenon associated with second dose administration. Unfortunately, they also found that this modification increases complement activation in the presence of pre-existing anti-PEG Abs [127]. Increased complement activation may limit the use of these approaches because complement activation is believed to be the main contributor to PEG-associated HSRs [128, 129].

The second strategy associated with PEG moiety modification is changing the linker between PEG and the lipids used in PEGylated product formulation. When compared to un-cleavable PEG lipid derivatives, Xu et al. reported that using esterase-catalysed cleavable PEG lipid derivatives could reduce or eliminate the ABC phenomenon and complement activation associated with the second dose of PEGylated products. Their research is based on the use of a cleavable ester link between PEG and lipids, which can be easily cleaved by the esterase enzyme found in the body [130, 131]. Poppenporg et al. also reported that changing the succinic linker in PEGylated asparaginase to a more stable amide bond dramatically slowed the loss of asparaginase activity in pre-treated mice [132]. Chen et al. introduced another approach related to the previous strategy, basing their research on the usage of a pH-sensitive connection between PEG and the lipids utilized. They used a hydrazone bond to connect two ester moieties, which is a pH-sensitive bond that is quickly cleaved when the pH in the blood circulation changes. They demonstrated the strategy's efficacy in extending the circulation half-life of the produced formulation as well as its ability to prevent the ABC phenomenon that occurs when PEGylated goods are administered repeatedly [133].

8.2.5. Use of alternative polymers

Despite the numerous advantages of utilizing PEG in PEGylated products to extend plasma half-life and increase nanocarrier dosage form stability or in PEG-containing cosmetic products to act as an emulsifying agent and skin emollient, previous reports on PEG immunogenicity and its role in the formation of PEG-associated HSRs may limit the use of this polymer, prompting researchers to search for other alternatives that deliver the same benefits as PEG but have little or no immunogenicity. According to previous reports, using polymers such as polyvinyl alcohol (PVA) [134], polyvinyl pyrrolidine (PVP) [135, 136], polyglycerol (PG) [137] , or conjugation with biodegradable hydrophilic amin acids such as (proline, glycine, alanine or glutamic acid) [138-140] instead of PEG can extend the circulation half-life of the conjugated molecules without the development of HSRs. Whiteman et al. reported that surface modification of liposomal systems with Poly N-(2-hydroxypropyl) methacrylamide] (HPMA) increases the plasma half-life of produced liposomes, similar to that induced by PEG. [141]. In the same manner, Abu Lila et al. reported that surface fabrication of liposomes with PG instead of PEG significantly decreased the production of anti-PEG Abs, which are responsible for complement activation and, as a result, the occurrence of HSRs associated with the usage of PEGylated liposomes [142]. The PASylation technique is a process for fusing therapeutic proteins or drug molecules with (Proline, Alanine, and Serine) amino acids [143]. This approach has been shown to extend the plasma half-life and increase the stability of pharmaceutically active peptides and proteins [144]. Zvonova et al. reported that PASylation boosted the therapeutic efficiency of recombinant interferon-β1b (IFN-β1b), an effective medication for multiple sclerosis. In comparison to non-modified IFN-β1b, fusion with (proline, alanine, and serine) amino acids improves medication stability, solubility, and therapeutic action by a factor of two [145].

9. Recent recommendation on COVID-19 vaccination

Many incidences of hypersensitivity have been documented in the United States and the United Kingdom since the approval of the use of the mRNA COVID-19 vaccine in the prevention of viral infection and the improvement of post-viral infection symptoms [146, 147]. In fact, even if these HSRs are related to mRNA or other substances utilized in the formulation of mRNA-based nanoparticle vaccines, the major cause and mechanism of these HSRs has not been thoroughly explored. As previously stated, multiple findings suggested that these HSRs were linked to the presence of PEG in nanoparticle vaccines formulations [23, 146]. The role of PEG in COVID-19-induced hypersensitivity has been emerged from studying the previous history of the

reported cases, which revealed that these cases used PEGylated medicines or PEG-containing cosmetics regularly. Several recommendations have been made based on these findings in order to prevent or reduce the occurrence of HSRs. [148]. The following are some of these recommendations:

9.1. Patient history:

Consideration should be given to the previous history of anaphylaxis related to the use of PEG-containing medications before administration of PEGylated therapeutics or vaccines. Several products containing PEG are commonly used as Macrogol laxatives, Depo Methylprednisolone acetate, Depo Medroxyprogesterone or Clopidogrel [149]. In addition to PEGylated therapeutics such as Doxil®, Somavert®, Pegasys®, Jivi®, Oncaspar®, and Empaveli® [150]. In addition to PEG, that may be used in cosmetic formulations such as shampoos, lotions, soaps, and sunblocks [10, 151].

9.2. PEG hypersensitivity assessment

Although no specific test for PEG allergy has been approved, several reports suggest that SPT could be used as a diagnostic tool for PEG allergy. They reported, however, that negative results are not a sufficient marker for the avoidance of post-vaccine hypersensitivity [152]. If the skin test is negative and there is a contradiction between the patient's history and the skin test, a drug provocation test (DPT) with the appropriate PEG-containing medicine can be used as a confirmatory tool for assessment of vaccine hypersensitivity [153]. Since previous reports have shown that anti-PEG Abs play a vital role in complement activation, and subsequently in the development of HSRs, measuring anti-PEG Abs may provide insight into the possibility of an allergic reaction. [154, 155]. Anti-PEG Abs can be measured using the previously reported ELISA protocols (**Fig. 5)** [156]. Briefly, a 96-well plate was coated with mPEG2000-DSPE in 100% ethanol and left to dry overnight. Then, the plate was blocked by the addition of Tris-buffered saline (50 mM Tris, 0.14 M NaCl, pH 8.0) containing 1% BSA and left for 1 h. The plates were washed three times using a washing solution (50 mM Tris, 0.14 M NaCl, 0.05% CHAPS, pH 8.0). Serum samples with appropriate dilution were added to wells in a triplicate manner (100 µl/well) and incubated for 1 h. Then, the plate was washed five times as previously described. Horseradish peroxidase (HRP)-conjugated antibody (1 µg/ml, goat anti-mouse IgM-HRP conjugate) in the conjugate diluents (Tris-buffered saline (pH 8.0) containing 1% BSA) was added to each well (100 µl/well). After 1 h incubation, the wells were washed again five times with a wash solution. o-phenylene diamine in enzyme buffer (63 mM anhydrous citric acid, 67 mM Na2HPO4, and 0.05 % hydrogen peroxide) (1 mg/ml) was added to initiate the colorization. After an appropriate incubation time (5-15 min), the reaction was stopped by addition of 100 µl of 2 M H2SO4. Finally, the absorbance was measured at 492 nm using a Microplate reader. All procedures were performed at room temperature. In the same manner, anti-PEG Abs can recently be detected *in vitro* using the basophil activation test (BAT) [157]. BAT is primarily based on *in vitro* measurements of basophilic activation caused by a specific allergen; basophilic reactivity is assessed by flow cytometric test in response to the expression of activated molecule CD63 on basophiles [158, 159]. Unfortunately, there is insufficient published evidence to support the routine use of these techniques and their relevance in detecting possible anaphylactic reactions. [160, 161]. Assessment of anti-PEG Abs or PEG hypersensitivity in patients prior to the use of PEGylated therapeutics and vaccines should be further studied to avoid PEG-induced HRs and accelerated blood clearance of PEGylated products.

Conclusion

The FDA-approved mRNA-based vaccines against the SARS-CoV-2 virus have gained great attention due to their reported therapeutic efficacy. LNPs represent an effective delivery system to deliver mRNA to its site of transcription. PEG-Lipid is considered a main component of LNPs due to its role in improving the circulation half-life and formulation stability. Unfortunately, several cases of anaphylactic reactions have been reported following the mRNA-COVID-19 vaccination. There are growing reports about the impact of PEG on these HRs. Complement system activation following administration of PEGylated therapeutics has been previously described and it is thought that it may play a crucial role in mRNA LNPs induced hypersensitivity. So, the source of COVID-19 induced hypersensitivity and the potential role of PEG should be further investigated and efforts should be concentrated to overcome these HRs.

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