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Commentary

Anaphylactic reactions to mRNA COVID-19 vaccines: A call for further study



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ARTICLE INFO

Article history:

Received 3 February 2021

Accepted 20 March 2021

Available online 6 April 2021

Keywords:

SARS-CoV-2

COVID-19

Spike

Allergy

Anaphylaxis

Hypersensitivity

Polyethylene glycol (PEG)

BNT162b2

Excipients

Lipid nanoparticles (LNPs)

1. Introduction

Several highly effective vaccines are available just over a year since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19). COVID-19 has claimed more than two million lives globally and over 450,000 in the United States. Two of the leading vaccines, which have provided hope that an end to the devastating effects of the pandemic may be in sight, are messenger RNA (mRNA)-based vaccines, a novel vaccine platform that has received authorization for emergency use by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

To overcome the inherent instability of the mRNA molecule under physiological conditions, mRNA is first modified and then encapsulated in lipid nanoparticles (LNPs) that effectively deliver the mRNA into cells at the injection site and the draining lymph nodes for translation into viral protein by the host protein synthe-

sis machinery [1–3]. Placing the mRNA, which contains N¹-methylpseudouridine instead of uridine, between two untranslated regions (UTRs) protects it from degradation, and its polyadenylation, or the addition of adenosine monophosphates, at the 3' end, further stabilizes the molecule; at the 5' end, the addition of a trinucleotide cap 1 analog ((m₂^{7,3'-O})Gppp(m^{2'-O}) ApG) serves as a recognition signal for the efficient binding of host cellular ribosomes that translate the mRNA [4]. The transiently expressed, mainly by dendritic cells and subcapsular sinus macrophages, membrane-bound spike protein of SARS-CoV-2 is then recognized by immune cells as a foreign antigen, eliciting both T-cell and B-cell responses to generate neutralizing antibodies, which are thought to contribute to protection against COVID-19 [4].

2. Composition and role of the ingredients of the two authorized mRNA vaccines

The composition and functions of the ingredients of the two mRNA vaccines authorized for emergency use include the Moderna mRNA-1273 and Pfizer/BioNTech's BNT162b2 vaccines, and are summarized in Table 1. The mRNA contained in both vaccines

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Table 1
Comparison of COVID-19 mRNA vaccines properties and composition.

		mRNA-1273 (Moderna)	BNT162b2 (Pfizer/BioNTech)
Pharmaceutical properties			
Formulation	Multidose vials	Dispersion for injection	Concentrate for solution for injection
Storage	Shelf life	7 months at –25 °C to –15 °C	Freezer storage only at –70 °C
Administration	Two IM* doses Interval between doses (days)	100 µg, 0.5 ml 28	30 µg, 0.3 ml each 21
Composition			
Active ingredient	Nucleoside-modified mRNA encoding the prefusion viral spike (S) glycoprotein of SARS-CoV-2	5'-capped mRNA produced by cell-free <i>in vitro</i> transcription from the corresponding DNA templates	
Excipients			
Lipids			
	Ionizable lipids	SM-102**: heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate	ALC-0315: (4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis(2-hexyldecanoate)
	Auxiliary lipids	1,2-Dimyristoyl- <i>rac</i> -glycero-3-methoxypolyethylene glycol-2000 [DSPC]	
	Pegylated lipids	Polyethylene glycol dimyristoyl glycerol [PEG 2000 - DMG]	ALC-0159: 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
	Membrane fluidity modulator/stabilizer pH adjustment	Cholesterol	
Buffer components			
		Acetic acid Sodium acetate trihydrate	Disodium hydrogen phosphate dihydrate
		Tromethamine	Potassium dihydrogen phosphate
		Tromethamine hydrochloride	Sodium chloride Potassium chloride
Sugars	Cryoprotectant	Sucrose	
Continuous phase	Dispersant	Water for injection	

* IM, intramuscularly.

** Proprietary to Moderna.

encodes for the full-length SARS-CoV-2 spike (S) protein modified with two proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the spike protein into the more immunogenic pre-fusion conformation [3,4]. The modified mRNA molecule is encased in LNPs, consisting of ionizable, auxiliary and pegylated lipids, as well as cholesterol, which is thought to contribute to the fluidity of the nanoparticle, thus increasing its ability to fuse with the plasma membranes of host cells to deliver the mRNA cargo.

Ionizable lipids are used to enhance the conjugation of the mRNA with the lipids as well as for its release into the cytosol. During the preparation of the conjugates, the pH is maintained at acidic values, rendering the lipids positively charged, thereby facilitating the mRNA/LNPs conjugation. During vaccine administration, because the normal arterial blood pH is ~ 7.5 (pH > pKa), the lipids are not charged, reducing cationic charge-mediated toxicity. Once in the acidic environment of the endosome, the lipids are positively charged again, regulating the release of RNA into the cytosol [2]. Two different ionizable lipids are used in these vaccines, namely SM-102 that is proprietary to Moderna, but most likely heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy)hexyl)amino) octanoate, and ALC-0315: (4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis(2-hexyldecanoate) (Pfizer/BioNTech) [4]. DSPC, or 1,2-Dimyristoyl-*rac*-glycero-3-methoxypolyethylene glycol-2000, is a neutral auxiliary (helper) lipid used as a bulking agent to the formation of LNPs.

The pegylated lipids are conjugated with polyethylene glycol (PEG), a nonionic macromolecule. They are used to decorate the LNPs surface with PEG chains, offering steric stabilization and extended physicochemical stability to the LNPs' dispersion. Additionally, LNP pegylation is a well-known technique for shielding against the mononuclear phagocyte system, contributing to improved safety and reduced clearance of the nanoparticles [2].

The pegylated lipid of Moderna's vaccine is polyethylene glycol dimyristoyl glycerol [PEG 2000 - DMG], while Pfizer/BioNTech uses ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide).

Apart from the mRNA/LNPs conjugates, several other ingredients are included in the formulation of the vaccines, such as buffer ingredients, sucrose and dispersant. It appears that Moderna uses two different buffer solutions: during mRNA/lipid conjugation, a buffer consisting of acetic acid and sodium acetate is probably used to maintain the pH at low values (4.75), while Tromethamine and Tromethamine hydrochloride buffers are probably used to regulate the pH at 7.4. According to their list of ingredients, Pfizer/BioNTech, on the other hand, uses only PBS buffer to regulate the pH of the final formula to 7.4.

The sugar that is used as cryoprotectant for both vaccines is sucrose. This ingredient is necessary during the lyophilization stage to maintain the LNPs intact, preventing their deformation or disruption. Both vaccines are concentrated dispersions, comprising water for injection (WFI) as the dispersant. The concentration of active ingredient (mRNA) is 200 µg/ml for Moderna and 100 µg/ml for the Pfizer/BioNTech vaccine, while the cold-chain requirements (at –25 °C to –15 °C and –70 °C respectively), render logistics and handling more challenging for the Pfizer/BioNTech vaccine.

3. Associations of mRNA vaccine ingredients with anaphylactic reactions

After implementation of vaccination programs, reports of anaphylactic incidents began to immerge [5,6]. Anaphylaxis is a serious adverse effect that is triggered by allergen-specific IgE antibodies that may develop after exposure to a previously encoun-

tered allergen. Although the two authorized COVID-19 mRNA vaccines are new, most of their ingredients have been used extensively in a wide variety of medical, cosmetic or food products, a fact that increases the likelihood of sensitization in the genetically predisposed population to some of these components [5]. Thus far, PEG is suspected to be the primary culprit of the cases of severe allergic reaction to the vaccines [6]. This hypothesis is based on the fact that few clinical cases of hypersensitivity to this molecule had been reported prior to the current pandemic era [7,8]. Previous studies showed that increasing PEG density not only reduces clearance of LNPs by the mononuclear phagocyte system via shielding their surface, but it also enhances their safety, reducing immunostimulatory potential [2]. In fact, Deak *et al.* reported the design and application of a nanoplatform for the test of nanoallergens [9]. The allergens that were decorated on the surface of LNPs contained the exact same basic ingredients as the LNPs of the new vaccines, namely a PEG-coated lipid, 1,2-distearoyl-*sn*-glycero-3-phosphocholine [DSPC], and cholesterol.

Although PEG allergy cannot be ruled out, the impact of the other ingredients should not be overlooked. Tromethamine (hydroxymethyl)aminomethane, or TRIS, is a buffer ingredient that has an extensive laboratory use particularly for regulating the pH of nucleic acids solutions. Apart from this use, it is also administered as a drug for the prevention of blood acid-base disorders with warnings to patients with known allergies to this substance [10]. PBS used in Pfizer/BioNTech vaccine, is well tolerated and does not cause any allergic reaction.

Glycerophospholipids like DSPC are substrates for phospholipases A2 (PLA2s) that play a pivotal role in proinflammatory mediator (eicosanoids) release, through the arachidonic acid (AA) that is produced from the hydrolysis of the ester bond at the *sn*-2 position of the glycerophospholipids. It is also reported that the cytosolic PLA2s may cause anaphylaxis and lysosomal PLA2s, complement activation. PLA2s may participate in intracellular signaling, leading to allergen induced production of inflammatory cytokines especially in asthmatic patients [11]. The ionizable lipids that are used in both vaccines are novel amino-lipids and their allergenic potential is not known.

Very recently published results on allergic reactions, including anaphylaxis, after receipt of the first dose of the two mRNA vaccines in the United States, report estimated rates of 4.7 vs. 2.5 anaphylaxis cases, with a strong female predominance, per million COVID-19 vaccine doses administered for the Pfizer/BioNTech vs. Moderna's vaccine, respectively [12–14]. Similarly, the overall rate of reported hypersensitivity reactions was 10.7 per million 2009 pandemic influenza A H1N1 vaccine doses [15], while after routine pediatric and adult vaccinations it is estimated at a rate of 1.31 per million vaccine doses in the U.S. [16]. Could the increased rate of anaphylactic reactions for Pfizer/BioNTech's BNT162b2 be related to differences in the vaccine excipients compared to Moderna? Further studies are warranted to find the true culprit for these rare, but serious allergic reactions.

In this regard we would recommend the following research agenda: (i) assessment of the potential IgE- and non-IgE-mediated allergy risk of each ingredient separately, but also of their combinations in each vaccine formulation; (ii) exploration of the prospect of developing rapid, non-invasive methods for the determination of anaphylactic risk to mRNA vaccine ingredients for sensitive population groups, prior to vaccination; or (iii) identification of biomarkers (blood or skin-based for example) indicating an elevated risk of IgE-mediated reactions to a vaccine component.

Given the considerable morbidity and mortality associated with COVID-19 infection, vaccination should continue in order to curtail the pandemic. The highly efficient and novel mRNA vaccines, which serves as a rapid platform for producing immunogens against emerging SARS-CoV-2 variants, ushers in a new era in vac-

cinology and provides hope that the end of the pandemic is near. Individuals at risk of severe allergic reactions should be identified through a history of anaphylactic reactions, and hopefully in the near future, biomarkers that could identify those at risk. In the meantime, the cause(s) for such reactions to these mRNA vaccines needs to be identified and research toward this end funded.

Declaration of Competing Interest

Dr. Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co., Medicago, GlaxoSmithKline, Sanofi Pasteur, Emergent Biosolutions, Dynavax, Genentech, Eli Lilly and Company, Janssen Global Services LLC, Kentucky Bioprocessing, AstraZeneca, and Genavant Sciences, Inc. Dr. Poland holds patents related to vaccinia and measles peptide vaccines. Dr. Poland has received grant funding from ICW Ventures for preclinical studies on a peptide-based COVID-19 vaccine. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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