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Applying lessons learned from nanomedicines to understand rare hypersensitivity reactions to mRNA-based SARS-CoV-2 vaccines

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After over a billion of vaccinations with messenger RNA-lipid nanoparticle (mRNA-LNP) based SARS-CoV-2 vaccines, anaphylaxis and other manifestations of hypersensitivity can be considered as very rare adverse events. Although current recommendations include avoiding a second dose in those with first-dose anaphylaxis, the underlying mechanisms are unknown; therefore, the risk of a future reaction cannot be predicted. Given how important new mRNA constructs will be to address the emergence of new viral variants and viruses, there is an urgent need for clinical approaches that would allow a safe repeated immunization of high-risk individuals and for reliable predictive tools of adverse reactions to mRNA vaccines. In many aspects, anaphylaxis symptoms experienced by the affected vaccine recipients resemble those of infusion reactions to nanomedicines. Here we share lessons learned over a decade of nanomedicine research and discuss the current knowledge about several factors that individually or collectively contribute to infusion reactions to nanomedicines. We aim to use this knowledge to inform the SARS-CoV-2 lipid-nanoparticle-based mRNA vaccine field.

Respiratory infections caused by viral pathogens (for example, influenza, respiratory syncytial virus and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)) cause substantial mortality and morbidity worldwide.

Despite being among the most effective instruments against infectious diseases, vaccines can cause side effects. Anaphylaxis is a severe adverse reaction that requires hospitalization and, when untreated, can be fatal. A detailed analysis of anaphylaxis occurrence after traditional vaccination in the United States revealed that among >25 million people vaccinated between 2009 and 2011, 33 (1.31 cases per 1,000,000 vaccinations) experienced anaphylaxis[1], which in some cases was attributed to vaccine excipients such as gelatine and thimerosal[2–5].

The US Centers for Disease Control and Prevention (CDC) established the Vaccine Adverse Event Reporting System (VAERS), which collects reports from healthcare workers and the public, and the CDC Wide-ranging Online Data for Epidemiologic Research (WONDER) database, which disseminates public health data and information[6]. According to these data systems, some people developed hypersensitivity reactions (HSRs) and anaphylaxis after immunization with SARS-CoV-2 messenger RNA (mRNA) vaccines[7]. In the United States, 99 out of 1,725 reports entered into VAERS by 1 March 2021 mention anaphylaxis[6]; this is a higher frequency than that reported for all seasonal flu vaccines during the past decade (447 out of 346,575 VAERS entries)[1,3]. According to the Israel Ministry of Health report of March 2021, 4 of 4,755,585 recipients of the first dose and 3 of 3,408,825 recipients of the second dose of the Pfizer-BioNTech vaccine developed anaphylaxis[8]. Delayed immune-mediated reactions were also reported[9]. According to the Israel Ministry of Health report of 8 August 2021, among 11.6 million individuals vaccinated with Pfizer-BioNTech formulation, 421,000 safely received a third dose[8]. Earlier reports of efficacy and safety are also available from other countries[10–13]. Although public adverse-event-collecting databases such as VAERS are passive in that they rely on individuals to enter the data and, consequently, may underestimate the actual incidence of adverse effects, they allow the identification of toxicities and promote the investigation of underlying causes. Given over a billion of vaccinated individuals[14], the rate of anaphylaxis in mRNA SARS-CoV-2 vaccination is very low. However, recent reports suggest that the overall incidence of HSRs to SARS-CoV-2 vaccines is higher than that of traditional vaccines[15,16], which makes a thorough analysis to understand the underlying cause(s) important.

Vaccine manufacturers and regulatory authorities have issued warnings calling for special attention to high-risk individuals, mandated 30-minute post-vaccination monitoring to provide pharmacological intervention in case anaphylaxis occurs and recommended excluding individuals with a known history of allergy to vaccine components from immunization. Examples of the high-risk category individuals disclosed to the public on the CDC website include cosmetic dermal filler recipients and persons with a history of anaphylaxis, autoimmune diseases, Guillain–Barré syndrome or Bell's palsy[17].

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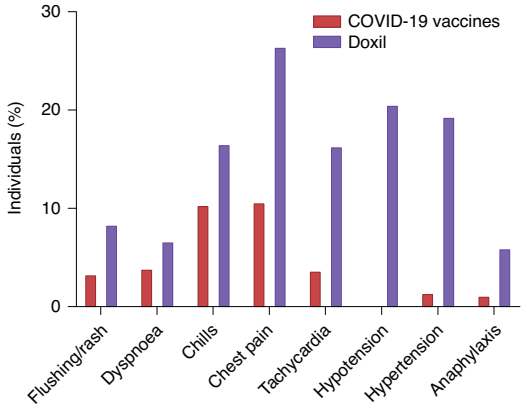

Fig. 1 | Some symptoms in recipients of SARS-CoV-2 mRNA vaccines are similar to that in nanomedicine-treated patients. Number of immunizations with SARS-CoV-2 vaccines in different countries is increasing; current data are available in ref. [14]. The rate of anaphylaxis at the time of proof-reading this paper is between 2.5-4.7 per million. However, it should be noted that information on the prevalence of vaccine-related anaphylaxis can vary depending on the inclusion criteria, method of evaluation, passive surveillance and type of data analysis. For this reason, the above rate substantially varies in the literature. Some symptoms experienced by SARS-CoV-2 vaccine recipients in the United States and reported to VAERS by 8 January 2021, resemble the symptoms described in response to the systemically administered nanomedicine Doxil. The per cent of individuals on this graph refers to the proportion of individuals with this adverse effect among 100 reports entered in VAERS for the Pfizer-BioNTech and Moderna SARS-CoV-2 vaccines by 8 January 2021, or registered for Doxil by the FDA adverse event reporting system[134] between 1995 (FDA approval of this formulation) and 2021.

A close examination of adverse events recorded for these SARS-CoV-2 mRNA intramuscular (i.m.) vaccines revealed that many reactions resemble infusion reactions, commonly experienced in response to intravenously (i.v.) administered established nanomedicines[18]. Some reactions to SARS-CoV-2 mRNA vaccines (for example, rash, dyspnoea, chills, chest pain, tachycardia, hypotension, hypertension and anaphylaxis) were also reported for the systemically administered nanomedicine Doxil; however, the frequency of these reactions to vaccines was much lower than that to Doxil (Fig. 1). The higher rate of infusion reactions to nanomedicines could be explained by their route of administration (that is, i.v. versus i.m. in vaccines), higher dose per kilogram of body weight (that is, mg kg^{-1} versus μg kg^{-1} in vaccines) and different distribution over the body. Nevertheless, these notions raise several questions about a potential contribution to HSRs by the lipid nanoparticles (LNPs) used as carriers in SARS-CoV-2 mRNA vaccines and suggest that lessons learned from managing infusion reactions to nanomedicines may help in understanding and overcoming HSRs to otherwise highly effective SARS-CoV-2 mRNA vaccines. Unlike infusion reactions to nanomedicines, anxiety was suggested among causes of HSR symptoms in vaccine recipients[19]. Understanding the mechanisms that underlie HSRs would help the medical community develop means to manage these reactions and prevent major effects and deaths, and so reduce people's anxiety and fears over these vaccines.

LNP–mRNA vaccines overview

Composition and physicochemical properties. The Pfizer-BioNTech and Moderna products, used for SARS-CoV-2 prevention in the United States, Europe and other parts of the world[20–22], use LNPs as mRNA delivery vehicles (Table 1). The vaccines' mRNA molecules differ in nucleoside type and sequence. Pfizer-BioNTech (BNT162b2) mRNA is a modified nucleoside mRNA chain that contains 1-methylpseudouridine instead of uridine. It expresses the SARS-CoV-2 full-length P2 mutant prefusion spike glycoprotein with two proline mutations that fix the S1S2 spike protein in a prefusion conformation. Moderna's vaccine mRNA is also modified with 1-methylpseudouridine and encodes the spike protein with two proline residues. The LNP carrier is made of an ionizable cationic lipid with apparent p*K*a values between 6 and 7, cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and a polyethylene glycol (PEG)-conjugated lipid intended to prevent LNP aggregation during storage as an aqueous dispersion. The ionizable cationic lipid serves two purposes: (1) to achieve an efficient encapsulation during the LNP preparation process and (2) after administration to provide a neutral particle surface at physiological pH but a positively charged particle surface once internalized by cells into more acidic intracellular compartments to facilitate intracellular mRNA trafficking. PEG terminal groups and the lipid conjugated to PEG differ between Pfizer-BioNTech and Moderna formulations. The mRNA dose is 30 μg and 100 μg in the Pfizer-BioNTech and Moderna vaccines, respectively. Although these LNP–mRNA vaccines' structural features are not published, approximately 100 nm spherical structures with an electron-dense core have been described for LNPs with a similar composition; inside those spheres, mRNA, ionizable cationic lipid and water molecules form an inverted hexagonal phase, whereas the external shell contains the PEG–lipid, part of the cholesterol and DSPC[23–25].

Pharmacology and mechanism of action. LNP–mRNA's fate in SARS-CoV-2 vaccines is not well understood, and detailed information about LNP–mRNA distribution and mRNA expression after i.m. injection in humans is not available. In mice, the route of administration is an important factor in determining the body sites in which the mRNA is translated, with the magnitude and duration of protein expression varying widely[26]. After i.m. injection, the produced protein was detectable at the injection site for 10 days and in the liver for 1–4 days[26]. A study in rhesus macaques on local immune events and protein expression after the i.m. administration of LNP loaded with mRNA-encoding influenza H10-haemagglutinin demonstrated a transient, local inflammation reaction that involved a rapid infiltration of immune cells, which included antigen-presenting cells (APCs), at the injection site and the draining lymph nodes. The injection of control LNP formulations showed that this immune cell influx effect occurs independently of the mRNA cargo. However, a marked activation of the adaptive immune system was observed only in the presence of LNP–mRNA. Monocytes and dendritic cells were the main cell types in which the protein was produced. The co-stimulatory receptors CD80 and CD86 were upregulated on the surface of the infiltrating APCs[27]. In a cynomolgus monkey model, no direct correlation was found between protein expression, immunogenicity and local tolerability for five LNP formulations with different ionizable cationic biodegradable lipids after i.m. injection. This suggests that inducing local irritation does not directly correlate with immunogenicity. Lipid H, which is identical to SM-102 in Moderna's vaccine (Table 1), stood out in terms of a high immunogenicity but low local oedema and erythema formation[28]. A more recent study in cynomolgus macaques investigated the fate of i.m.-injected LNP–mRNA in which the carrier contained a cationic lipid, based on an amino sugar, but no PEG–lipid[29]. The mRNA in this study was dual-labelled with a radionuclide positron emission tomography–computed tomography marker and a near-infrared probe. Muscle tissue and three draining lymph nodes were monitored for the presence of mRNA. The APCs were the major carriers of mRNA in these locations. In some lymph nodes, mRNA-enriched B cells were found. The authors did not mention mRNA uptake by the liver or spleen[29].

Table 1 | Composition of Pfizer-BioNTech and Moderna SARS-CoV-2 mRNA vaccines

Description	Pfizer-BioNTech BNT162h2 LNP–mRNA SARS-CoV-2 vaccine	Moderna 1273 LNP–mRNA SARS-CoV-2 vaccine
mRNA dose	30 μg in 0.3 ml	100 μg in 0.5 ml
LNPs	0.43 mg ALC-0315 (((4-hydroxybutyl) azanediyl) bis (hexane-6,1-diyl)bis(2-hexyldecanoate)) 0.05 mg ALC-0159 (2-((polyethylene glycol)-2000)-*N*,*N*-ditetradecylacetamide) 0.09 mg DSPC 0.2 mg cholesterol Total lipids: 2.57 mg ml^{-1} 0.77 mg per 0.3 ml dose	SM-102 (proprietary ionizable lipid) (heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy)hexyl)amino) octanoate) PEG2000-DMG (1-monomethoxypolyethyleneglycol 2000-2,3-dimyristylglycerol) DSPC Cholesterol Total lipids: 3.86 mg ml^{-1} 1.93 mg per 0.5 ml dose
Molar lipid ratios (%) (ionizable cationic lipid:PEGylated lipid:DSPC:cholesterol)	46.3:1.5:9.4:42.7[a]	50:1.5:10:38.5[b]
Molar N/P ratio[c]	6	5
Buffer[d]	0.01 mg phosphate (potassium dihydrogen phosphate, 0.07 mg disodium hydrogen phosphate dihydrate)	Tris(tromethamine) (0.31 mg tromethamine, 1.18 mg tromethamine hydrochloride)
Other excipients	0.01 mg potassium chloride 0.36 mg sodium chloride 6 mg sucrose Water for injection	0.043 mg acetic acid 0.12 mg sodium acetate 43.5 mg sucrose Water for injection

The table was prepared based on the FDA briefing documents presented to the public at the vaccine advisory board meetings in December 2020[20,21]. [a]Calculations are based on total lipids of 4.03 mM (4 μmol ml^{-1}) (ref. [129]); the molecular weight of ALC-0159 was estimated as 2,700 g mol^{-1}) for the purpose of calculations. [b]Calculations based on refs. [130,131]. [c]The mole ratio between cationic amines in the lipid excipient and the anionic phosphates of the RNA (based on ref. [132]). [d]To increase the temperature stability of the Pfizer-BioNTech vaccine, the pediatric (orange cap) 10 microgram, 0.2 ml IM formulation was Tris-buffered with 0.02 mg tromethamine, and 0.13 mg tromethamine hydrochloride; for the >12 years of age, the PBS buffered (purple cap) formulation has now been replaced with a tris buffered (gray cap) formulation (0.06 mg tromethamine, 0.4 mg tromethamine hydrochloride) in the US (ref. [133]).

No data on what happens after i.m. LNP–mRNA vaccination in humans has been published. One might speculate that, after i.m. injection, the PEG–lipid (ALC-0159 in the Pfizer-BioNTech vaccine and PEG2000-DMG in the Moderna vaccine; Table 1) desorbs from the LNP, as happens with short interfering RNA–LNP after i.v. administration to rodents and non-human primates (NHPs). In the case of i.v. administration, the PEG–lipid component of the LNP carrier rapidly desorbs from the nanoparticles after injection. Adsorption of apolipoprotein E to the surface of the LNP also occurs, and leads to the rapid endocytosis of the in vivo modified LNP by hepatocytes in the liver[24]. However, as discussed above, after i.m. administration in NHPs, LNP–mRNA complexes are rapidly endocytosed by cells that reside at or enter the site of injection (that is, APCs). The LNP complexes disintegrate in the endosomes, and the mRNA escapes into the cytosol via an endosomal membrane destabilization mediated by the ionizable lipid, from which it travels to the rough endoplasmic reticulum for translation; DSPC and cholesterol are seen as 'helper lipids', as they are critical to the formation and maintenance of stable LNP–mRNA complexes[30]. In humans, mechanistic details of cellular uptake and LNP–mRNA processing by APCs are still hypothetical. Nevertheless, a strong immune response that involves antigen-specific CD4$^+$ T_H1 (type 1 T helper) cells, CD8$^+$ cytotoxic T cells, B cells and plasma cells in vaccinated individuals is clearly documented[12,31,32].

Collectively, despite variation in the mRNA structure, LNP type and NHP species (rhesus versus cynomolgus), the limited studies available provide important information on the spatiotemporal trafficking of LNP–mRNA and antibody formation, and suggest that local tolerability and protein expression levels do not correlate with immunogenicity[27–29]. The relationships between the initial innate immune responses, spike protein production, resulting antigen presentation and specific antiviral immunogenicity are yet to be better understood. Although the incorporation of pseudouridine into mRNA reduces the Toll-like-receptor-mediated innate immune response against mRNA[33], additional studies demonstrated that other types of milder innate immune responses still occur and may reduce the mRNA translation[34,35]; these and other aspects regarding mRNA vaccines' pharmacology and mode of action are extensively discussed elsewhere[36].

Potential mechanisms that underlie HSR to LNP–mRNA vaccines

Although stimulation of innate and adaptive immune responses is a desirable outcome that underlies a vaccine's efficacy, it may lead to immune-mediated adverse effects (IMAEs) when overwhelming and left uncontrolled. Anaphylaxis is a life-threatening IMAE that belongs to the type I immediate-type hypersensitivity (ITH) category[37]. Anaphylaxis symptoms occur within minutes of exposure to the triggering agent (for example, an environmental allergen, Hymenoptera venom, a drug or an excipient). The classical ITH is triggered when an allergen binds to the allergen-specific immunoglobulin E (IgE) on mast cells of individuals who were previously sensitized, with subsequent degranulation of these cells, which leads to the release of preformed and newly synthesized mediators, such as histamine, tryptase, prostaglandins, leukotrienes and interleukins, among others. Mediator binding to tissue and cellular receptors induces local and systemic symptoms that affect the skin and respiratory, gastrointestinal and cardiovascular systems. Typical symptoms include swelling of the face, eyes, tongue and throat, skin eruptions, haemodynamic changes, respiratory failure followed by hypotension with circulatory collapse (that is, shock) and death within minutes to hours, unless medical intervention is applied. Anaphylaxis can occur without IgE, by direct activation of mast cells and/or basophils and through the newly described G-coupled protein receptors, such as the Mas-related G-protein coupled receptor member X2. Anaphylaxis treatment requires early recognition

Box 1 | Summary of early online reports that highlighted side effects to SARS-CoV-2 vaccination during the initial phase of immunization in 2020–2021. Reports from media are summarized; the incidence as reported is relatively low because millions of people received the vaccine. More recent research data about these side effects are currently available

Vaccine	Symptoms	Number of affected individuals and country	Reference
Moderna	Anaphylaxis	6, California, USA	https://www.healthline.com/health-news/why-california-put-a-pause-on-a-single-lot-of-the-moderna-covid-19-vaccine
Pfizer	HSR	6, France	https://www.archyde.com/six-cases-of-adverse-effects-caused-by-the-pfizer-vaccine-recorded-in-france/
Moderna	Anaphylaxis	6, USA	https://www.nbcsandiego.com/news/local/vaccination-super-station-near-petco-park-abruptly-closes/2495604/
Pfizer	Serious and fatal HSR	16 and 5, respectively, Switzerland	https://www.swissmedic.ch/swissmedic/en/home/news/coronavirus-covid-19/verdachtsmeldungen-impfstoff-covid19.html
Moderna	Anaphylaxis	1, USA	https://www.nytimes.com/2020/12/25/health/Covid-moderna-vaccine-allergies.html
Pfizer	Severe allergic reactions	175, USA	https://www.cdc.gov/mmwr/volumes/70/wr/mm7002e1.htm
Pfizer	Myocarditis	136, Israel	https://www.gov.il/BlobFolder/reports/vaccine-efficacy-safety-follow-up-committee/he/files_publications_corona_myocarditis-12.20-05.21.pdf
Pfizer	Anaphylaxis	10, Israel	https://www.gov.il/BlobFolder/reports/seav-25092021/he/files_publications_corona_side-effects-after-vaccination-25092021.pdf

of the symptoms and prompt use of i.m. epinephrine. Death varies depending on the trigger, and there is a 0.3% death rate from anaphylaxis of different origins[38]. The same symptoms and timeline could also occur due to the activation of the complement system[39]. This type of reaction is known as pseudoallergy, anaphylactoid reaction or complement activation-related pseudoallergy (CARPA)[39]. The enzymatic cleavage of complement proteins generates so-called anaphylatoxins (C3a, C4a and C5a) that act as cytokines and activate immune cells to produce secondary inflammation mediators[39]. Complement activation-generated anaphylatoxins can also activate mast cells, which results in classical anaphylaxis symptoms[40]. Allergen-specific IgM and IgG are known to activate the complement system; they also trigger type II and type III hypersensitivities, for which development takes minutes to hours and three to eight hours, respectively[37]. Complement activation also contributes to type III HSR[37]. Type IV HSR is delayed hypersensitivity mediated by T cells and can develop within 48–72 hours[37]. Although cytokines are produced by activated immune cells during ITH and other types of HSR and are considered part of the anaphylaxis phenotypes by some researchers[41,42], certain institutions separate anaphylaxis from cytokine release syndrome[43]. Wide discrepancies also exist in HSR definitions between health organizations, countries and fields of training[18]; the symptoms and underlying cellular and molecular players of HSRs frequently overlap, which complicates diagnosis and management[18]. Biomarkers recently became available for diagnosis, and provide a mechanistic approach to define the genotypes that underlie the different phenotypes. Significant elevations of tryptase above the baseline are typical for IgE and non-IgE mast-cell-driven reactions, and interleukin-6 (IL-6) elevations are diagnostic of cytokine release syndrome[44].

Despite recently described delayed reactions to SARS-CoV-2 vaccines[45], which, according to the timeline of occurrence and symptoms, suggest type III and type IV HSR, no mechanistic data or other reports are available. Therefore, here we focus on ITH, most frequently mentioned by the media at the time when vaccination began at the end of 2020 and early 2021 (Box 1), and discuss the mechanisms that underlie these reactions through the current knowledge about infusion reactions to nanomedicines[18]. We interrogate the potential role of LNP carriers, mRNA, excipients and antigens expressed from mRNA. We also touch on delayed responses as the same components that contribute to ITH, although via different mechanisms, are involved in delayed responses, all of which have been reported in the context of IMAEs to SARS-CoV-2 mRNA vaccines[7,10,46,47] (Box 1).

Systemic complement activation. There are three complement activation pathways—classical, mannose and alternative—that antibodies (IgM or some IgG isotypes), mannose-binding lectin and C3b binding trigger, respectively; additionally, autoactivation (via so-called C3 'tickover') can occur[39]. A prominent frequent consequence of immunization is transient local tissue swelling associated with redness and pain at the injection site. It occurs due to the accumulation of protein-rich inflammatory exudate that contains complement proteins, and it explains the availability of complement proteins at the injection site.

Pre-existing anti-PEG IgG and IgM that bind to the PEG backbone or terminal methoxy group have been found in healthy people[48,49]. Similarly, naturally occurring anti-cholesterol antibodies capable of reacting with cholesterol components of liposomes have been detected in healthy individuals and people with atherosclerosis[50,51]. Prior exposure to PEG, PEGylated nanomedicines and LNPs induces PEG-reactive IgM and IgG formation[52,53]. These pre-existing and induced antibodies trigger the classical complement activation pathway[51,52], which, when occurs in response to systemically administered PEGylated nanomedicines, leads to CARPA[52,54]. Nucleic acids also trigger the classical pathway[55]. Cationic lipids and LNPs activate the complement system in vitro and in vivo via the alternative pathway[56–59]. Anti-PEG antibody levels depend on the rate of PEG–lipid shedding off the LNP, with fast-shedding PEG–lipid being less immunogenic than its slow-shedding counterpart[53]. The LNP in both the Pfizer-BioNTech and Moderna vaccines contains a lipid conjugated to PEG2000, and local reactions in individuals with PEG-based cosmetic fillers have been reported (Box 1). Therefore, it is plausible that pre-existing anti-PEG antibodies contribute to complement-mediated anaphylactoid reactions to SARS-CoV-2 mRNA vaccines. However, as the lipid parts of the PEG2000-lipid and PEG terminal groups are different (2-((polyethylene glycol)-2000)-*N*,*N*-ditetradecylacetamide versus PEG-2000-DMG, and PEG versus methoxy-PEG (mPEG) in the Pfizer-BioNTech and Moderna formulations, respectively), these vaccines' reactogenicity, if indeed mediated by anti-PEG antibodies, may also differ. PEG's terminal group is a key contributor to its immunogenicity

in that mPEG is more immunogenic than hydroxy-PEG[60]; therefore, it is critical to clarify PEG's chemical identity in the Pfizer-BioNTech vaccine (2-((polyethylene glycol)-2000)-*N*,*N*-ditetradecylacetamide as stated in the package insert[20,61] versus mPEG-*N*,*N*-ditetradecylacetamide in the research catalogue[62] for ALC-0159). Some viruses (for example, mumps, parainfluenza, and SARS-CoV-2)[63], purified spike protein of SARS-CoV-2[64], and certain excipients (for example, acetic acid)[65] trigger the alternative pathway of complement activation. Spike protein, via direct binding to mannose-binding lectin, ficolin-2, and collectin-11, may also trigger the mannose pathway of complement activation[66]. Moreover, spike protein was detected in vaccinated individuals' blood as early as day zero after the first dose, peaked at day five, and remained until day 15[67]. This would explain spike-protein-triggered complement activation in the systemic circulation, along with the delayed anaphylaxis and repeated reactions reported in affected individuals. Although complement activation by therapeutic nucleic acids was reported in vitro and in vivo, NHPs overpredicted complement-mediated toxicities in humans[68], and no reliable data are available on nucleoside-modified mRNAs' ability to activate the complement system. Although the role of complement-related pattern-recognition molecules, pentraxins (pentraxin 3 and C-reactive protein), in response to viral infections, including that to SARS-CoV-2, has been established[69], it remains unknown for infusion reactions to nanomedicines and HSRs to LNP–mRNA-based vaccines.

Therefore, at least three components delivered by SARS-CoV-2 mRNA vaccines (PEGylated LNP carrier, mRNA payload and expressed spike protein antigen) can activate the complement system. Activation could occur after i.m. administration in the interstitial space and lymphatics, where complement is also present[70], as well as after particle distribution to the systemic circulation (Fig. 2a and Cytokine-mediated responses). It is unknown whether or to what extent the i.m.-injected SARS-CoV-2 vaccines distribute to the systemic circulation. Understanding such distribution is warranted, as the amount of LNP–mRNA injected i.m. to a human is minuscule. A recent murine study of the Pfizer-BioNTech mRNA vaccination demonstrated that accidental administration into the peripheral blood may inadvertently occur during the i.m. injection and is responsible for the systemic inflammation that leads to myopericarditis[71]. Such unintended i.v. administration could be another mechanism that leads to complement-mediated anaphylaxis. Recent reports about a reaction-free second dose after anaphylaxis to the first dose of SARS-CoV-2 mRNA vaccines[72,73] indirectly suggest complement is involved in the first reaction because a similar pattern of reduction in reactogenicity with repeated administration was reported for CARPA, but not for a true allergy to nanomedicines[74].

Intracellular complement. Many human cell types can produce complement proteins, collectively known as intracellular or local complement, which are different from the systemic complement present in the plasma and produced by the liver in that they are located inside the cell and can be cleaved via intracellular proteases. Pro-inflammatory cytokines produced during innate and adaptive responses to an antigen potently trigger intracellular complement activation and upregulation of anaphylatoxin receptors (C3aR and C5aR) on the surface of these cells[70]. The formed anaphylatoxins are released and bind to their cognate receptors to amplify the APC–T-cell interactions via autocrine and paracrine positive feedback loops[70]. Activated APC and T cells also produce complement factors B and D[70], which enables expansion of the chain reaction via the alternative pathway amplification loop. Although LNPs used in the Pfizer-BioNTech and Moderna vaccines have not been studied, cationic molecules and cationic nanoparticles are known to activate the intracellular complement system in a membrane-damage-related process similar to how cationic lipids enable mRNA escape from the lysosomes into the cytoplasm[75]. Thus, if the ionizable vaccine lipids indeed induce intracellular complement activation, it may coincide with the cytoplasmic translation of mRNA, just in time to stimulate APC–T-cell interactions. There are no data as to whether the excipients, mRNA or spike protein in vaccines can activate the intracellular complement; however, available knowledge about triggers of the intracellular complement[70,75] and the established facts about cytokine induction by SARS-CoV-2 mRNA vaccines[12,76] support this mechanism's plausibility and call for additional investigation. It is conceivable that the process is also important to the immunogenicity of SARS-CoV-2 mRNA vaccines.

Mast-cell activation through IgE and non-IgE mechanisms. Mast cells are myeloid-lineage-derived granulocytes that reside in connective tissue and are staffed with granules containing histamine, heparin, proteases and cytokines. Antigen-specific IgE molecules trigger mast-cell degranulation. PEG-specific IgE cross-reacting with polysorbate 80 has recently been described in two individuals with ITH to PEG-containing medications; moreover, a review of the US Food and Drug Administration (FDA) adverse effects database suggested 53 additional cases of IgE-mediated ITH to PEG[77]. High titres of anti-PEG IgE were detected in nine patients with a history of anaphylaxis to PEG3350-containing laxative and to PEG8000-containing ultrasound gel; the patients' blood also contained anti-PEG IgG[78]. Moreover, a recent computational study identified several spike protein epitopes as allergens[79], and a few cases of anaphylaxis were attributed to anti-PEG IgE[80,81]. Therefore, true anaphylaxis to SARS-CoV-2 mRNA vaccines due to anti-PEG IgE does exist. Antibody screening and skin-prick test appear as reliable, clinically available procedures to identify individuals at high risk; however, their utility to identify SARS-CoV-2 vaccine HSR is incompletely understood. Individuals with clonal and non-clonal mast-cell disorders, which include mastocytosis, asthma, myelodysplastic syndrome and acute myelocytic leukaemia, may present elevated tryptase levels that associate with an increased mast-cell activation. Thus, they could be at higher risk of developing HSR to SARS-CoV-2 mRNA vaccines, although recent vaccinations of two patients with systemic mastocytosis followed by 19 patients with multiple mast-cell-activation syndromes and elevated tryptase did not trigger symptoms of anaphylaxis[82,83]. A recently described syndrome, hereditary alpha tryptasemia, is associated with duplication and triplication of the tryptase gene *TPSAB1* on chromosome 16; elevated tryptase is linked to increased reactions to Hymenoptera venom, and patients with this genetic autosomal dominant trait may be more prone to reactions to vaccines and vaccine excipients[84]. Tryptase levels are a useful biomarker for diagnosing anaphylaxis[85,86].

A recent study demonstrated basophil and mast-cell activation in anaphylactic individuals injected with SARS-CoV-2 mRNA vaccines in the absence of IgEs specific to PEG or other vaccine components[87]. This points towards a complement-mediated reaction as anaphylatoxins C3a and C5a are known triggers of basophil and mast-cell degranulation[88]; other mechanisms are also possible.

Cytokine-mediated responses. Cytokines are the biomarkers of immunostimulation, which is an important prerequisite of immunogenicity. Similar to the complement system, innate immune cell activation is desirable to promote vaccine efficacy. However, excessive activation may lead to cytokine storm and cytokine-mediated host-tissue damage. The human population's genetic diversity leads to wide interindividual variability in cytokine responses[89,90]. A dose of the same immune adjuvant that results in the optimal cytokine response in one individual may be too strong or too weak for another individual because the innate and adaptive immune responses include multiple cellular and biochemical components and vary between individuals.

Lipids and lipid-based nanoparticles (for example, liposomes, LNPs and micelles) induce chemokines[91], whereas therapeutic

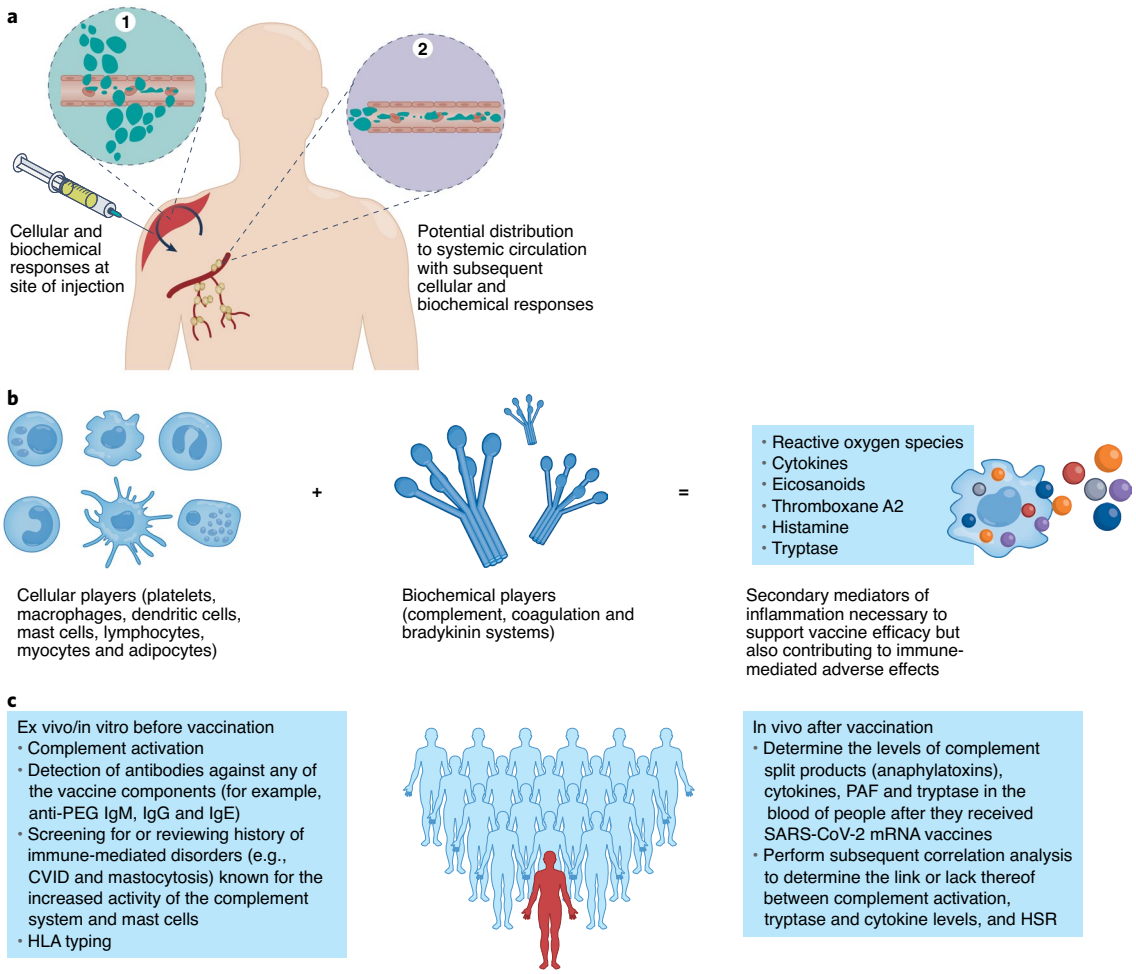

Fig. 2 | Adverse effects and strategy for overcoming them. a, Schematic of interaction between LNP–mRNA vaccine components and cells at the site of injection (1), along with a hypothetical mechanism of particle distribution to the systemic circulation (2). **b**, Cellular and molecular players responsible for the generation of an immune response to the LNP–mRNA vaccine that leads to both the desirable immunogenicity and, in some individuals, the adverse immune effects. **c**, Millions of people received vaccines, and only a very small proportion developed anaphylaxis. These individuals are currently either excluded from receiving the second dose of mRNA vaccine or the vaccination is done in hospitals under supervision. Studies outlined in this image would help to shed more light on the mechanisms of anaphylaxis and allow the immunization of more individuals.

nucleic acids (for example, mRNA) induce interferon responses[92]. Cationic moieties in LNPs, along with foreign antigens expressed after vaccine delivery, generate both danger- and pathogen-associated molecular patterns that collectively activate innate immune cell and B-cell pattern-recognition receptors. This triggers inflammatory signalling pathways, major histocompatibility complex class II upregulation and costimulatory molecule expression on APCs, and thereby promotes antigen presentation and immunogenicity. Cationic lipids, common nanocarriers for experimental vaccines, induce a broad spectrum of cytokines, chemokines and danger signals[93]. Endosomal Toll-like receptors (TLR7 and TLR8) and cytosolic receptors (RIG-I (retinoic acid-inducible gene I), LGP-2 (Laboratory of Genetics and Physiology 2), MDA-5 (melanoma differentiation-associated protein 5) and MAVS (mitochondrial antiviral-signalling protein) initiate an interferon response to the single-stranded RNA. Cationic molecules also trigger inflammasome activation, which specifically contributes to the generation of IL-1 family cytokines, a common feature of promising vaccine adjuvants[94,95]. Chemokine-mediated neutrophil recruitment is essential for allergic sensitization[96], whereas interferons provide antiviral immunity and orchestrate communication between innate and adaptive immunity[97,98]. Moreover, the SARS-CoV-2 spike protein contains the sequence and structural motif of a superantigen, which is responsible for the hyperinflammatory syndrome that involves direct T-cell stimulation and excessive cytokine production in some individuals[99].

Clinical trials of both the Pfizer-BioNTech and Moderna vaccines demonstrated a clear T_H1-specific cytokine response, including tumour necrosis factor, IL-1, IL-12 and interferon-gamma[21,31], which, besides driving inflammation and innate–adaptive immunity cross-talk, activate the coagulation system and increase the endothelial layer permeability in a time- and dose-dependent manner[100,101]. Therefore, along with the complement activation described above, these cytokines contribute to both the desired vaccine efficacy and the interindividual variability in IMAEs, such as fever and chills. These cytokines interact with complement and coagulation systems via a bidirectional loop, promoting particle distribution to the systemic circulation, which contributes to elevated inflammation. This warrants a more detailed investigation of these pathways in the context of SARS-CoV-2 vaccines.

Platelets, coagulation and bradykinin systems. Platelets contribute to CARPA and HSR by releasing biologically active molecules (for example, ATP, thromboxane and chemokines) and lipid

inflammatory molecules (for example, platelet-activating factor (PAF))[40]. Nanoparticles that contain cationic moieties activate platelets[91,102]. Both LNP–mRNA and spike protein also activate platelets[103,104]. PAF was recently proposed as a new biomarker of anaphylaxis[41]. Despite its short half-life, PAF has many potent biological effects on almost all tissues and organs; it is central in inflammation, triggers degranulation of perivascular mast cells, which leads to inflammatory responses and tissue injury, and induces the release of thromboxane and serotonins. Rupatadine and other anti-PAF drugs, alone or together with quercetin and luteolin, effectively manage PAF-mediated toxicities in SARS-CoV-2-infected individuals[43–45] and could be helpful in controlling anaphylaxis to SARS-CoV-2 vaccines.

Bradykinin production is triggered by an increase in heparin and the coagulation-factor-XII-mediated pathway, which cytokines, activated mast cells, complement and platelets trigger. Bradykinin activation leads to the increased vascular permeability responsible for hypotension in anaphylactic individuals[105] and therefore could contribute to particle distribution to the systemic circulation.

Oxidative stress. Oxidative stress, linked to HSR for some drugs (for example, sulfanilamides)[106] is a common cause of nanoparticle-mediated toxicities[107]. The induction of a chemokine response by common lipid-based excipients (for example, Cremophor EL) was attributed to oxidative stress[108] and is shared by lipid-based nanoparticles, which include LNPs[91,109]. Oxidative stress inhibits the negative regulation of complement, and so enhances complement-mediated toxicities[110].

Common variable immunodeficiency. Common variable immunodeficiency (CVID) is a disorder characterized by excessive activity of the complement system intended to compensate for B cells' deficient immunoglobulin production[111]. Persons with CVID, therefore, may be prone to complement-mediated toxicities triggered by LNP–mRNA vaccines, and reviewing the CVID history may help identify people with a high risk of HSR to SARS-CoV-2 mRNA vaccines.

Variability in human leukocyte antigens. Variability in the human leukocyte antigen (HLA) type is known to make some people more prone to developing HSR to certain types of drug products[112–116]. For example, individuals with HLA-B*57:01, HLA-B*15:02/A*31:01 and HLA-B*58:01 are at higher risk of T-cell-mediated reactions to abacavir, carbamazepine and allopurinol, respectively[117]. Anaphylaxis to PEG-asparaginase has been associated with HLA-DRB1*07:01[118]. Individuals with HLA-B*46:01 and HLA-B*15:03 have the lowest and the highest T-cell-mediated responses to SARS-CoV-2, respectively[119]. Similar variability in an individual's reactivity to the spike protein antigen encoded by the LNP–mRNA vaccines could exist and contribute to the development of HSRs to SARS-CoV-2 mRNA vaccines. A recent report linked HLA-A*03:01 and HLA-DPB1*11:01 with a decreased and HLA-B*08:01, HLA-C*07:01, HLA-DQA1*05:01 and HLA-DRB1*03:01 with an increased likelihood of reactions to SARS-CoV-2 mRNA vaccines[120].

Safety roadmap

In summary, all the components of LNP–mRNA vaccines (carrier, mRNA, excipients and expressed antigen) have various immunostimulatory effects on a broad spectrum of effector and target cells (myocytes, APCs, T and B lymphocytes, platelets and natural killer cells) and biochemical pathways (complement and coagulation) collectively required for vaccine efficacy (Fig. 2b). The same components, however, also contribute to HSR and other IMAEs due to the wide interindividual variability in both the quantity (for example, levels of cytokines, complement split products, tryptase and induced and pre-existing antibodies) and quality (for example, spectrum of inflammatory mediators) of immune responses.

Understanding the mechanisms behind HSR to SARS-CoV-2 mRNA vaccines and exchanging knowledge between the nanomedicine and vaccine fields, therefore, are beneficial not only for SARS-CoV-2 vaccines, but also for all mRNA-based vaccines and therapeutics for which nanoparticles are used as delivery vehicles. More basic research is needed to understand the Pfizer-BioNTech and Moderna vaccines' mechanisms of action and pharmacokinetics after i.m. injection. Clinical studies to evaluate plasma levels of anti-PEG and anti-cholesterol IgG and IgM in vaccine recipients, along with in vitro exposure of the plasma samples to SARS-CoV-2 mRNA vaccines with a subsequent analysis for the presence of complement split products, would help verify both the involvement of the complement system and the contribution of the pre-existing PEG- and/or cholesterol-specific antibodies. Similar analysis of plasma IgE specific to one or more vaccine components, along with basophil activation, is also needed. Clinical studies to assess the levels of complement split products (anaphylatoxins), cytokines, PAF and tryptase in the blood of people after SARS-CoV-2 vaccination, with subsequent correlation analysis to determine the link (or lack thereof) between complement activation, tryptase and cytokine levels, and HSR would offer further insight (Fig. 2c). The skin-prick test's applicability to identify individuals prone to developing HSR to SARS-CoV-2 vaccines requires verification. A clinical study to assess the safety of Pfizer-BioNTech and Moderna vaccine administrations to individuals with allergies recently began[121]. Supplementing it with basic research and clinical investigations that focus on the mechanisms underlying these reactions would further advance knowledge in this area.

The available information suggests that excluding anxiety-mediated responses[122], determining whether individuals have conditions known for their higher complement activity (for example, CVID) or hypersensitivity (for example, certain types of HLA and mastocytosis) and reviewing allergy history would further help to identify persons at high risk of HSR, and to develop strategies for safely vaccinating them. In the clinic, anaphylaxis is countered with antihistamines and adrenaline injections, such as the Mylan NV EpiPen. Premedicating patients with steroids, antihistamines and antipyretics similar to those used prior to nanomedicine infusion[18] may also prove helpful. However, the applicability of these treatments to vaccines requires investigation to ensure that they do not affect vaccine efficacy; once verified as not interfering, they may allow vaccination of a broader population. A specific HSR condition may call for a specific treatment to prevent a chronic immune reaction; in such cases, and depending on the underlying mechanisms, additional tools, such as PAF and complement inhibitors, may become helpful. Moreover, a better understanding of spike protein immunogenicity and cross-reactivity with the host's normal tissues, as recently described by two independent studies[123,124], will help address the problem by improving the overall immunological properties of the antigen used in mRNA and other SARS-CoV-2 vaccines. Basic research studies are underway to investigate safer PEG alternatives and the chemical modification of mRNA to reduce their undesirable immunoreactivity[125,126]. Collectively, these strategies would help to safely vaccinate individuals with known HSR to vaccine components. Recent reports about the safe delivery of a second dose to individuals who experienced anaphylaxis to the first dose are encouraging[72,73].

Note added in proof: Since this paper was accepted, a couple of studies were published that showed that the LNPs used in the mRNA vaccines cause a strong inflammatory response in mouse models[127] and that pigs injected with the Pfizer/BioNtech vaccine undergo hemodynamic changes reminiscent of those caused by the infusion reaction to nanomedicines[128].

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