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Current Opinion in Virology

Future considerations for the mRNA-lipid nanoparticle vaccine platform

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Vaccines based on mRNA-containing lipid nanoparticles (LNPs) pioneered by Katalin Karikó and Drew Weissman at the University of Pennsylvania are a promising new vaccine platform used by two of the leading vaccines against coronavirus disease in 2019 (COVID-19). However, there are many questions regarding their mechanism of action in humans that remain unanswered. Here we consider the immunological features of LNP components and off-target effects of the mRNA, both of which could increase the risk of side effects. We suggest ways to mitigate these potential risks by harnessing dendritic cell (DC) biology.

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Introduction

LNPs have grown in popularity as a delivery and adjuvant system for mRNA vaccines. An abundance of preclinical animal studies have shown the promise of this platform [1] and human clinical trials by Moderna and Pfizer/BioNTech of mRNA-LNP based SARS-CoV-2 vaccines reported above 90% protection rates. The advantages of using LNPs for vaccines are numerous. In addition to being a safer alternative to viral vectors for the delivery of mRNA vaccines, LNPs are self-adjuvating and highly customizable. Furthermore, the LNP-mRNA platform can be manufactured on a large scale and adapted easily to emerging pathogens. Also, the recent development of thermostable variants [2•] will overcome the necessity of cold-chain storage, which is required to different degrees for the current mRNA-LNP based SARS-CoV-2 vaccines. However, because this is a new approach for human vaccination, with different levels of reported side-effects [3•,4•,5•,6•], there remain many unknowns and caveats that should be considered.

Immunological features of LNPs

LNPs are ~100 nm size carriers that consist of different ratios of phospholipids, cholesterol, PEGylated lipids and cationic/ionizable lipids. The LNPs' phospholipid and cholesterol components have structural and stabilizing roles, whereas the PEGylated lipids support prolonged circulation [7•]. Cationic/ionizable lipids are included to allow complexation of the negatively charged mRNA molecules and to enable the exit of the mRNA from the endosome to the cytosol for translation [7•].

Innate immune features of LNPs

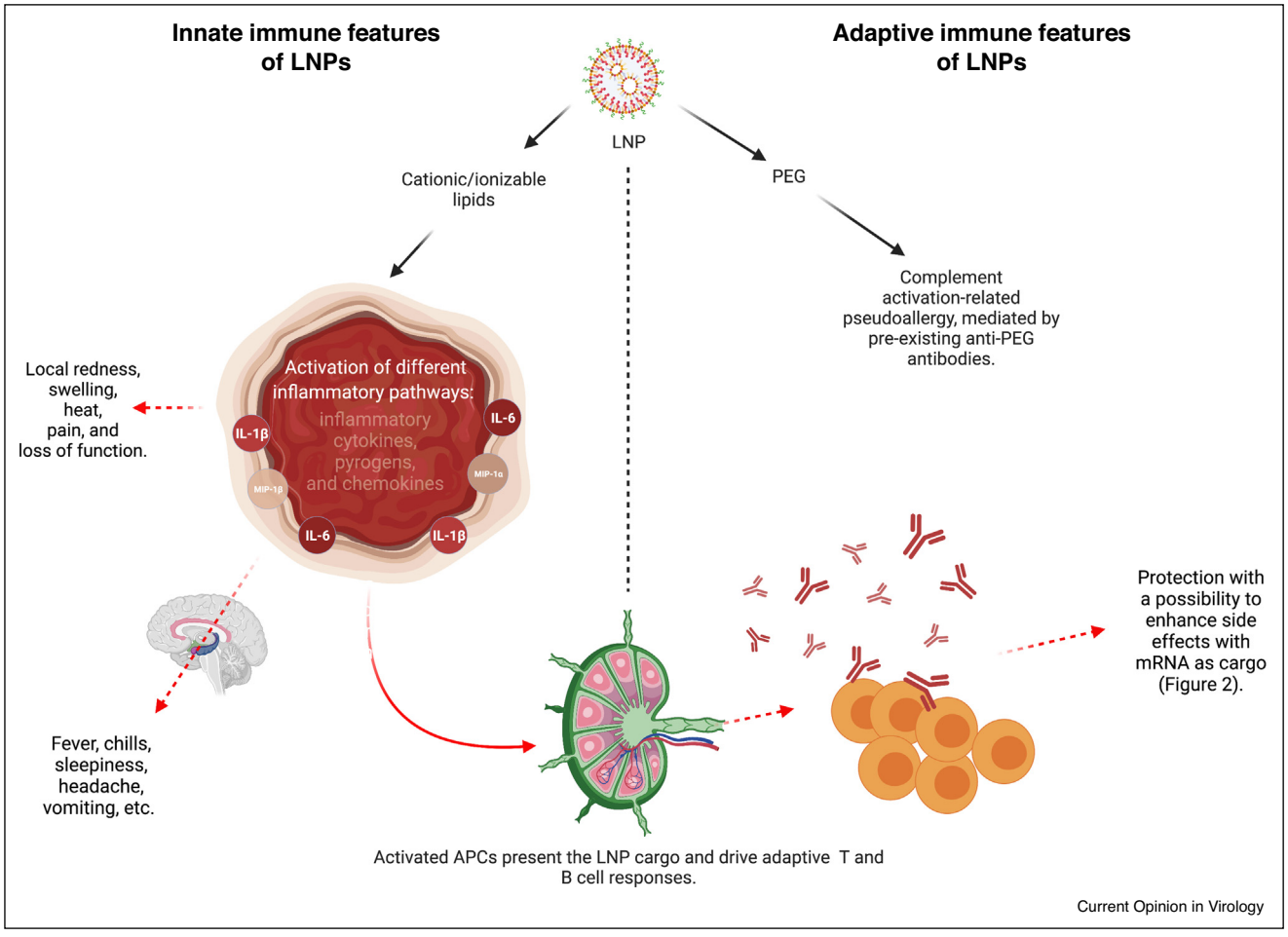
The phospholipid and cholesterol components of the LNPs also occur naturally in the mammalian cell membranes. Thus, they are unlikely to trigger any significant innate immune recognition and inflammatory responses. A less natural component of the LNPs is the cationic/ionizable lipid. Some cationic/ionizable lipids can induce inflammation by activating TLR pathways [8–10,11•] and cell toxicity [7•]. The LNPs that were widely used in preclinical vaccine studies and similar in composition to the ones used for the human SARS-CoV-2 vaccines were shown to have adjuvant effect when complexed with mRNA [12]. However, the potentially inflammatory nature of this mRNA-LNPs platform has not been assessed [1,12]. The LNP component in this platform contains proprietary ionizable lipid and supports the induction of robust humoral immune responses [12]. Humans receiving the mRNA-LNP based SARS-CoV-2 vaccines often presented with typical side effects of inflammation, such as pain, swelling, and fever [3•]. Since the mRNA of these platforms are nucleoside modified to decrease innate immune recognition and activation [13], we hypothesized that this mRNA-LNP platform's adjuvant activity could stem from the LNPs' inflammatory properties. Indeed, we recently reported that the LNP component of the mRNA-LNP platform used in preclinical studies is highly inflammatory [14]. Intradermal injection of these LNPs alone or in combination with non-coding poly-cytosine mRNA led to rapid and robust innate inflammatory responses, characterized by neutrophil infiltration, activation of diverse inflammatory pathways, and production of various inflammatory cytokines and chemokines. The same dose of LNP delivered intranasally led to similar inflammatory responses in the lung and resulted in a high mortality rate. As expected, based on previous literature [7•], the

inflammatory nature of these proprietary LNPs was dependent on the ionizable lipid component. Furthermore, LNPs lacking the ionizable lipid failed to support the generation of adaptive immune responses (manuscript under review). Thus, these LNPs' potent adjuvant activity and reported superiority comparing to other adjuvants in supporting the induction of adaptive immune responses could stem from the inflammatory nature of the ionizable lipid component. These preclinical LNPs assessed by us are similar to those used for human vaccines. Thus, the inflammatory milieu induced by the LNPs could be partially responsible for reported side effects of mRNA-LNP based SARS-CoV-2 vaccines in humans, and are possibly contributory to their reported high potency for eliciting protective immunity (Figure 1). Whether with repeated injections, innate memory responses [15] to the ionizable lipid component of the LNPs will form and contribute to the adaptive immune responses' and side effects' modulation remains to be determined.

Adaptive immune features of LNPs

A growing number of reports show that polyethylene glycol (PEG) can be immunogenic, and repeat administration of PEG can induce anaphylactoid, complement activation-related pseudoallergy (CARPA) reaction [16,17•,18]. Humans are likely developing PEG antibodies because of exposure to everyday products containing PEG. Therefore, some of the immediate allergic responses observed with the first shot of mRNA-LNP vaccines might be related to pre-existing PEG antibodies (Figure 1). Since these vaccines often require a booster shot, anti-PEG antibody

Figure 1



Immunological features of LNPs.
The cationic/ionizable lipid component of the LNP can trigger highly inflammatory responses through the activation of different innate immune sensing pathways. The resulting inflammatory milieu can support local and systemic side effects and help develop adaptive immune responses. The adaptive immune responses are dependent on the innate inflammatory environment induced by the cationic/ionizable lipids. The generated adaptive immune responses will protect from subsequent infections and might contribute to the development of side effects, as detailed in Figure 2. The PEG component of the LNP can support the development of complement activation-related pseudoallergy.

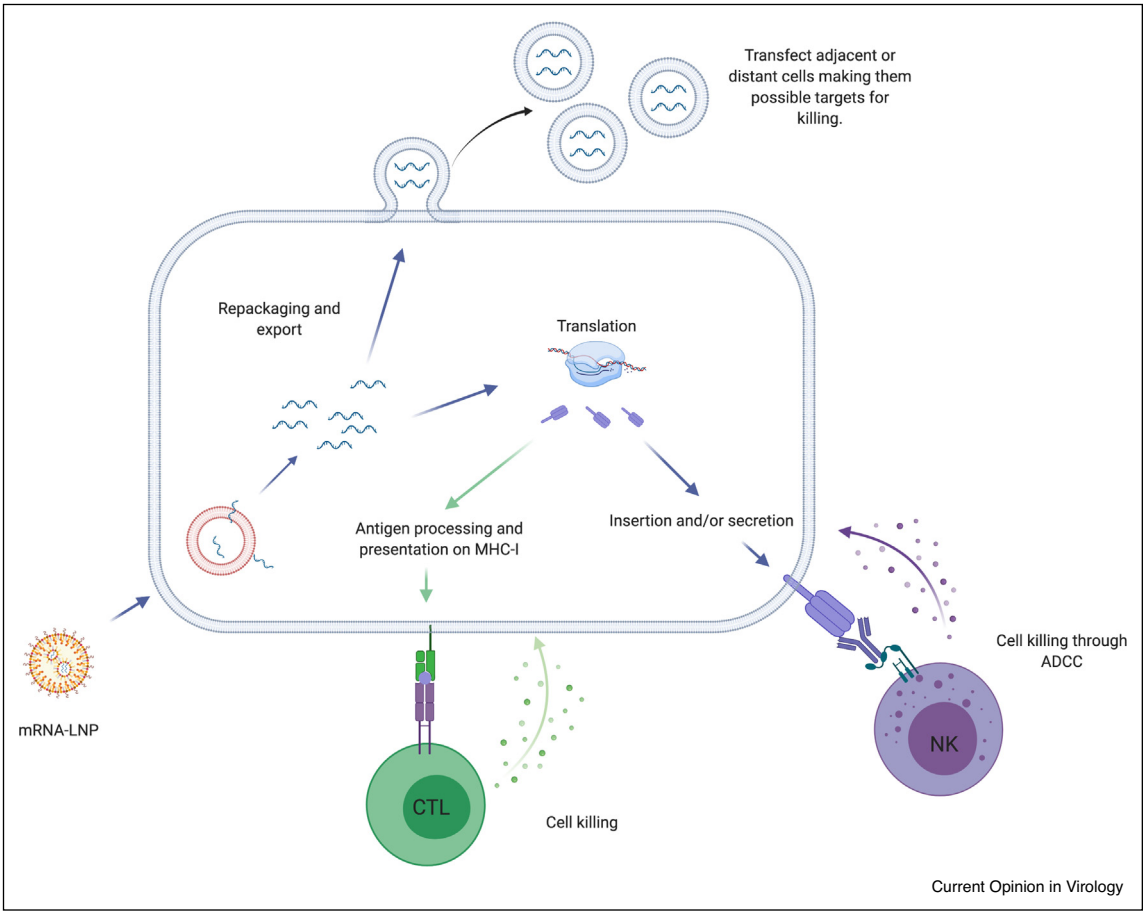
formation is expected after the first shot. Thus, the allergic events are likely to increase upon re-vaccination.

Off-target effects of vaccine mRNA

Based on the current mRNA-LNP vaccine design, LNPs can be taken up by almost any cell type, near or far from the site of injection, transfecting them with the antigen-encoding mRNA [19]. Moreover, the mRNA used in these vaccines are nucleoside-modified to decrease inflammatory responses [13] and increase its stability *in vivo*, allowing extended periods of mRNA translation [20,21]. Also, a significant portion of the mRNA can be re-packaged and expelled from transfected cells in extracellular vesicles (EVs) [22••]. These vesicles could reach cells far from the injection site, further increasing the number of cells translating the antigen and extending the duration of its expression.

Long-term mRNA translation in non-professional antigen-presenting cells (APCs) might lead to unanticipated cell killing. Similar to any other self-proteins, synthesized vaccine proteins have access to antigen presentation on major histocompatibility complex (MHC) class I molecules on any nucleated cells [23]. Thus, any cell presenting antigenic determinants from the vaccine could become a target of T cell-mediated killing. The so called 'Covid-arm', a delayed-type hypersensitivity reaction, that develops in some patients several days after vaccination [24•], could be indeed an indication of effector $CD8^+$ T cell responses targeting the cells expressing the vaccine-derived peptides. Furthermore, if vaccine-derived proteins become inserted into the plasma membrane or secreted and associated with the cell membrane, these cells could become targets of antibody-dependent cellular cytotoxicity (ADCC) [25]. Both $CD8^+$ T cell-mediated killing and ADCC should

Figure 2



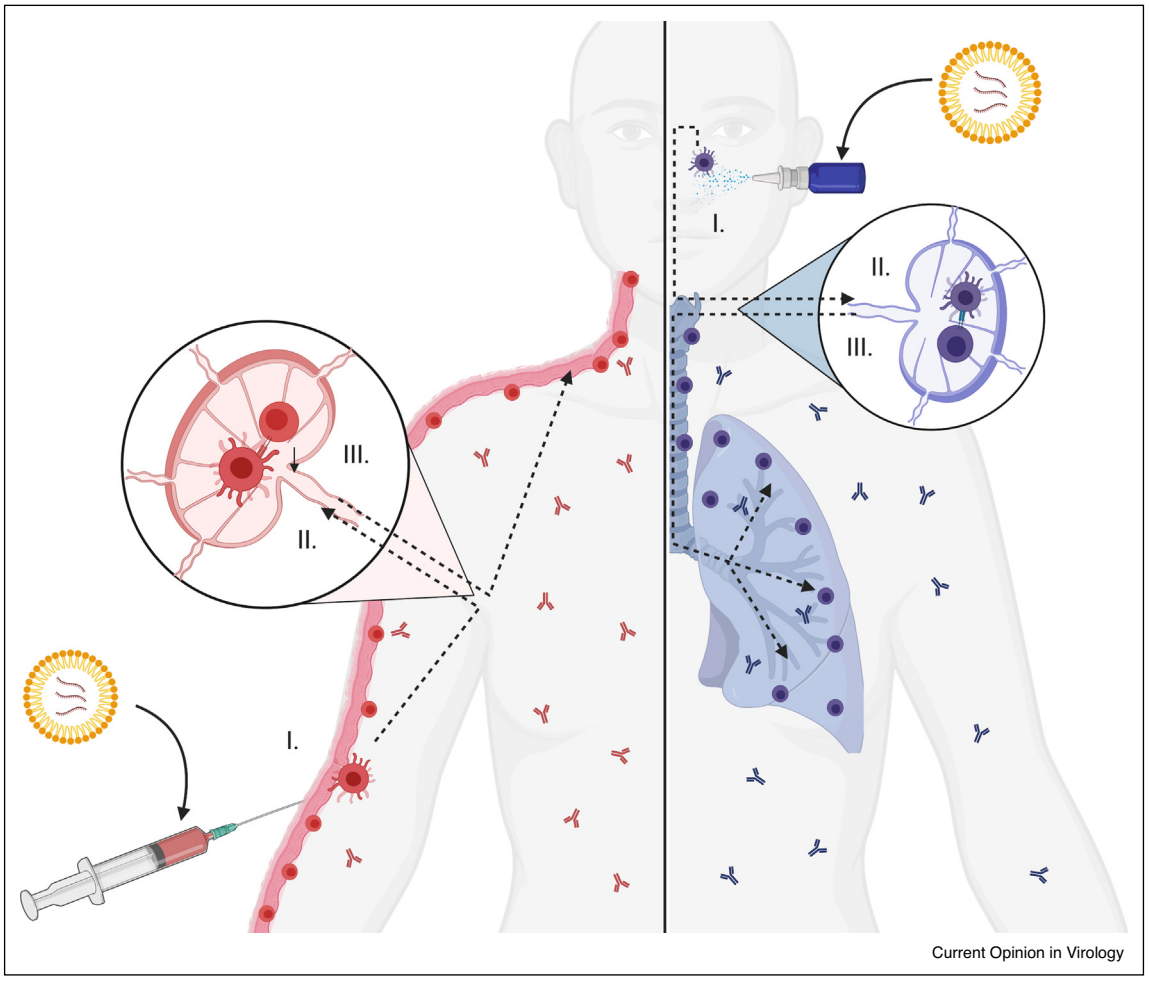
Possible off-target effects of the mRNA-LNP platform.
The mRNA reaching the cytosol can have different fates. They can be re-packaged in EVs that can transfect adjacent or distant cells, making them target of the immune response induced by the vaccine. The intact antigen coded by the mRNA can in theory reach the plasma membrane marking the cells for killing through ADCC. The ADCC should become evident after the antibodies specific to this antigen are formed. Since all the nucleated cells express MHC-I, the translated protein can be processed and presented, like any other self-proteins, on MHC-I to CD8 T cells. This can lead to cell killing after the effector T cells are formed. The killing should be accentuated upon booster shot when the tissue memory T cells are also present.

become evident after an adaptive immune response has been generated and may be accentuated upon secondary immunization in the presence of memory cells and preformed antibodies (Figure 2). In line with this, systemic adverse events from the mRNA-LNP based SARS-CoV-2 vaccines were indeed more common after the second vaccination, particularly with the highest dose [3•]. Strategies that allow delivery of the mRNA exclusively to DCs may limit the possible off-target effects. For this purpose LNPs should be conjugated to DC-targeting antibodies or ligands, such as anti-Langerin, anti-CLEC9A, anti-DEC205, mannose, and so on [26–33].

Considering DC biology

The success of mRNA-LNP vaccines depends not only on cellular internalization of the LNPs but on the release of mRNA from the endosomal compartment, to enable translation. It is thought that, in most cells, the ionizable lipid component becomes protonated in the progressively acidic environment of the endosome, leading to endosome destabilization and mRNA release [7•]. However, DCs have specific biology that may interfere with this process. Specifically, DCs have been reported to retain intact protein antigens for days [29,34] in mildly acidic endosomal compartments [34]. This likely allows DCs more time to display antigenic determinants to T cells and intact antigens to B cells [29,35–37]. However, the low acidity environment of the DC endo-lysosomal compartment may inhibit the endosomal escape of mRNA by failing to ionize the lipids in the LNPs. While it remains to be tested, lipid carriers that fuse with the plasma membrane and release their mRNA cargo into the cytosol

Figure 3



Route of immunization determines tissue protection by T cells.
DCs upon migration to the draining lymph nodes imprint the antigen-specific T cells to migrate and reside at their tissue of origin. Thus, vaccines administrated in the skin will imprint the T cells in the skin draining LNs to migrate to the skin and reside there as tissue-resident memory cells; while DCs, from the airway epithelia upon intranasal immunization, will instruct the T cells in the local LNs to populate the airways including the lung tissue. These cells would then confer protection at these sites upon exposure to the pathogen. The DCs will also initiate antibody responses that through lymph and bloodstream can provide systemic protection.

might be preferred when it comes to aiding mRNA translation and subsequent antigen presentation in DCs.

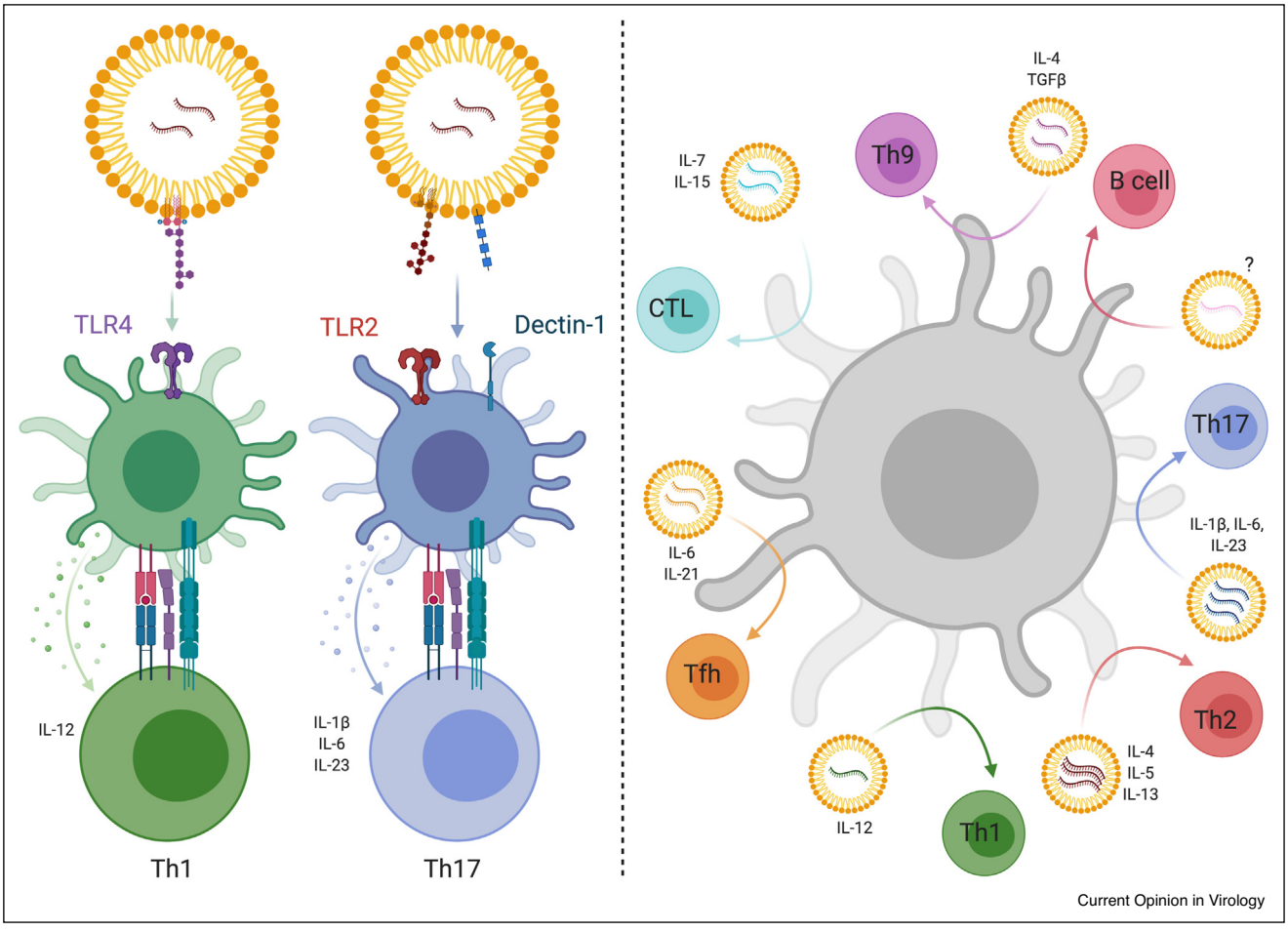
Considering pre-existing inflammation

It has been shown that mRNA-LNP vaccines have an altered tissue distribution, dynamics, and uptake in animals that have been pre-exposed to inflammatory agents [7•]. These findings suggest that people with pre-existing inflammatory conditions might show altered immune responses to these vaccines and might present with more severe side-effects.

Considering vaccine delivery route

The route of vaccine delivery determines which tissue will be protected by the cellular immunity. Peripheral DCs program antigen-specific T cells in the lymphoid organs to migrate to and reside in the DC's tissue of origin [38]. However, most current vaccines, including the mRNA-LNP based SARS-CoV-2 vaccines, are delivered into the muscle. This delivery route is expected to support the formation of antibodies that provide systemic protection and T cells that patrol these organs but not the site of natural exposure and infection, the airway epithelium. The presence of virus-specific T cells in the right tissue would be highly desirable because these cells can also provide cross-protection across different strains of viruses [39–43]. Therefore, we would propose tailoring the vaccine's route of administration to the pathogen's natural route of infection and developing intranasal vaccines for respiratory viruses such as influenza virus and SARS-CoV-2 (Figure 3).

Figure 4



Strategies to exploit DC biology with the mRNA-LNP platform.
Here we present two not mutually exclusive strategies to use the mRNA-LNP platform to support a variety of different adaptive immune responses by targeting DCs. On the left panel, we present a strategy that takes into consideration that not all DC subsets express the same PRR repertoire, thus they are more functionally specialized. In this case, we can have the LNPs containing different PRR ligand(s). By changing the ligands and targeting certain DC subsets we can achieve again a variety of different adaptive responses. In this case the engagement of different PRRs will lead to secretion of distinct polarizing cytokines by the DCs. On the right panel, we propose to have mRNAs coding for certain polarizing cytokines along with the antigen (protein or mRNA coding for the antigen) delivered to all the DCs. By changing the polarizing cytokines, we could make any DC subset to support different adaptive immune responses.

Broadening vaccine-induced T cell responses

Our knowledge of immune mechanisms of mRNA-LNP vaccines is still very limited. Vaccine-derived mRNAs are expected to be translated and presented by MHC class I but largely excluded from MHC class II [23]. Yet, the existing mRNA-LNP vaccination studies clearly show that both $CD8^+$ T cell and $CD4^+$ T cell responses are induced [1].

The type of Th cell response induced depends on the DC subsets and pattern-recognition receptor (PRR) pathways engaged [44]. So far, the mRNA-LNP platform has been reported to induce Th1 and T follicular helper cells, likely through the engagement of TLRs by cationic lipids [7•,11•]. To induce other Th cell subsets with the mRNA-LNP platform, we propose two strategies. First, PRR ligands could be included in the LNPs and, second, mRNAs encoding T cell-polarizing cytokines could be added to the LNPs. The first option is more restrictive as not all DC subsets express the same PRR repertoire, and thus not all DC subsets will be able to respond to the stimuli carried by the LNPs. Delivering mRNAs encoding polarizing cytokines would overcome this problem and would allow any DC subset, independent of its PRR profile, to polarize naive $CD4^+$ T cells towards the desired lineage (Figure 4).

Thus, with mRNA technology, there is almost no limit to modifying DC biology to match our needs.

Conclusion and future perspectives

The mRNA-LNP platform is very versatile, and as we have seen with the recent pandemic, it can provide us with a vaccine candidate in a matter of weeks. However, being a relatively new vaccine platform, as presented above, there are many unknowns and possible caveats that should be addressed before we label it safe for human use. Therefore, the discussed possible off-target effects of the mRNAs should be addressed. The LNPs can support very robust adaptive immune responses in animal models compared to other FDA-approved adjuvants. However, their higher efficacy probably relies on their highly inflammatory nature. The presentation of self-antigens in a highly inflammatory environment could lead to a break in tolerance [45]. Therefore, we believe more careful characterization of LNPs is needed to balance positive adjuvant and harmful inflammatory properties as LNP-associated vaccines move forward. Some DC subsets at optimized antigen dose can induce protective antibody responses in the absence of inflammatory agents [29,31,46]. These data suggest that LNP-based vaccine platforms that lack inflammatory cationic/ionizable lipids could be a viable option to induce protective antibody responses if targeted to specific DC subsets. The LNPs, unlike other adjuvants, can serve a dual purpose, as both delivery vehicles for different cargos and as an adjuvant. Therefore, the adjuvant properties of these LNPs should certainly be further exploited as a platform in combination with proteins, subunit vaccines, or even in combination with existing attenuated vaccines [47–52].

Conflict of interest statement

Nothing declared.

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