Drug delivery

https://doi.org/10.1038/s41551-024-01265-9

Immunogenic amines on lipid nanoparticles

Preeti Sharma, Dor Breier & Dan Peer

Amine headgroups in the ionizable lipids of lipid nanoparticles contribute to their immunogenicity.

The success of lipid nanoparticles (LNPs) in delivering messenger RNA (mRNA)-based vaccines has transformed the biopharmaceutical industry. Indeed, the efficacy of the vaccines Comirnaty and Spikevax in curbing the spread of COVID-19 boosted research on LNPs, with their use expanding beyond viral vaccines to the treatment of cancers, bacterial infections, genetic disorders and other conditions[1–3]. The latest addition to the list of LNP-based drugs approved by the US Food and Drug Administration is mRESVIA, which is indicated for older adults who need protection from severe infection by the respiratory syncytial virus[4].

Nucleic acids within LNPs are shielded from degradation and delivered to the cytoplasm, where they are translated into the encoded proteins[5]. Incorporating targeting ligands into LNPs improves their organ and cellular specificity, reducing off-target effects[6]. The structural stability and delivery efficacy of LNPs are largely a result of cationic amino-ionizable lipids, cholesterol, conjugates of lipids with poly(ethylene glycol) (PEG), and helper lipids such as distearoylphosphatidylcholine. Among these lipids, the pH-sensitive amino-ionizable lipids ensure endosomal delivery yet also determine the in vivo behaviour of the LNPs. The ionizable lipids are key determinants of the expression of the delivered nucleic acids, as well as of the biodistribution, tolerance and immunogenicity of the LNPs. Because ionizable lipids are excellent adjuvants for vaccines, any ionizable lipid that is suitable for the delivery of LNPs to immune cells can elicit an immune response that may prove detrimental, particularly for applications involving protein replacement strategy[7]. Therefore, in the case of ionizable lipids, each application may require a tailored lipid.

LNP-induced inflammation presents a key challenge, as it prevents the clinical translation of LNPs for many indications other than vaccines, especially for conditions that involve pre-existing inflammation[8]. Immune responses therefore have a dominant role in determining the outcome of LNP-based therapeutic interventions. For example, the clinically approved SM-102 and DLin-MC3-DMA ionizable lipids have divergent immune profiles – SM-102 is immunostimulatory, whereas DLin-MC3-DMA is relatively inert[9].

In *Nature Biomedical Engineering*, Kathryn Whitehead and co-authors now report a study on the effect of the chemical structure of ionizable lipids on the immunostimulatory behaviour of LNPs (ref. 10). The authors designed a library of 15 ionizable lipids consisting of three alkylamine headgroups and five 10-to-13-carbon-long alkyl acrylate tails. Lipids with hydrophobic amine headgroups induced a greater inflammatory response after a single booster dose when compared to lipids containing a polar headgroup. The authors found that LNPs with amine headgroups mediated pro-inflammatory cytokine release

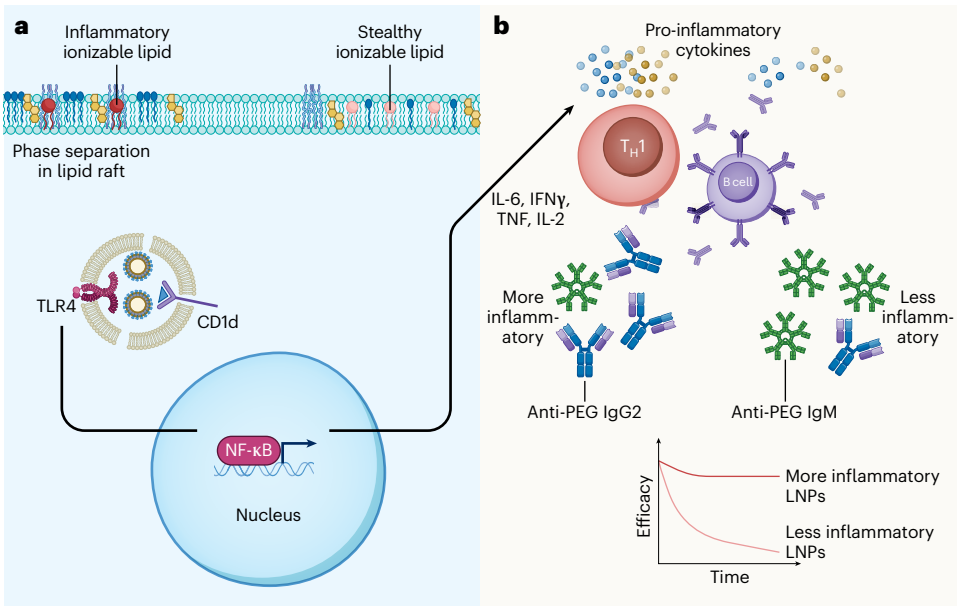

Fig. 1 | The effects of the structure of ionizable lipids on the innate and adaptive immune responses to LNPs. a, LNPs with ionizable lipids that contain amine headgroups are inflammatory, promote lipid-raft formation and induce stronger binding to TLR4 and CD1d receptors, activating the NF-κB transcription factor. **b**, This innate immune signalling results in the release of pro-inflammatory cytokines that trigger the activation of the adaptive immune system. Immunogenic LNPs trigger an immune response biased towards T_H1 cell responses and suppress the release of anti-PEG IgM, which helps the LNPs retain their delivery efficacy. TNF, tumour necrosis factor. Figure created with BioRender.com.

by activating Toll-like receptor 4 (TLR4) pattern recognition receptors on the cell membrane – a phenomenon that was previously described for LNPs incorporating fully charged cationic lipids[10]. In addition, the LNPs incorporating pro-inflammatory headgroups bind to CD1d, a receptor that recognizes and presents lipid antigens to cytotoxic cells (lymphocytes such as T cells and natural killer T cells). This led to the release of pro-inflammatory cytokines, particularly interleukin-6 (IL-6) and tumour necrosis factor. The immunostimulatory ionizable lipids also displayed higher lipid-raft-inducing properties, which is a prerequisite for TLR4 recruitment and assembly on the cell membrane. However, no consistent structural correlations could be deduced between changes in the tail structure of the ionizable lipids and the resulting immunostimulation.

To predict the potency and immunogenicity of LNPs, Whitehead and co-authors employed molecular docking simulations. They found increased binding affinity of the lipids with amine headgroups to a complex between TLR4 and myeloid differentiation factor 2, which also correlated with the activation of nuclear factor-κB (NF-κB) observed in immortalized murine macrophages. The less inflammatory lipids were buried in the binding pocket of the complex and thus interacted weakly with amino acid residues in TLR4. By using molecular dynamics simulations, the researchers observed the partitioning of ionizable lipids in a model plasma membrane. The pro-inflammatory lipids moved towards the centre of the bilayer and induced phase separation, which promoted the formation of lipid rafts and TLR4 recruitment, initiating an immune response; by contrast, when the headgroup was polar, the ionizable lipid was miscible with the other lipids of the bilayer, preventing phase separation (Fig. 1a).

Innate immune signalling is crucial for the activation of initial immune cascades that lead to an adaptive immune response. Hence, Whitehead and co-authors investigated how the polarity of the headgroup affected the adaptive immune system. After injecting mice with two doses of LNPs one month apart, the authors assessed the immune cell numbers and the levels of markers indicative of immune function in the blood and spleen on the second and seventh days following the second injection. They found that cytokines influencing T cell responses, such as interferon-γ, IL-2 and IL-6, were elevated in the serum of the animals; however, this increase did not result in T cell proliferation. Inspecting the humoral response revealed that the pro-inflammatory ionizable lipids polarize the immune systems towards a T helper 1 (T_H1) cell-type response, as evidenced by the upregulation of immunoglobulin G2 (IgG2) and the higher ratio of IgG2 to IgG1. Interestingly, the less inflammatory lipids formulated with the polar headgroup did not induce enhanced IgG levels; rather, an increase in the levels of IgM in the blood was observed in the mice. The enhanced IgM levels correlated with a drop in the efficacy of the LNPs on repeated dosing of the polar-lipid-containing LNPs. This IgM-driven efficacy loss was attributed to IgM antibodies produced in response to the PEG used in the LNP formulation. Contrary to this, the lower IgM levels in mice injected with the pro-inflammatory LNPs helped prevent their clearance on repeated dosing. Interestingly, blocking the inflammatory signal by injecting CD1d and TLR4 inhibitors led to an increase in PEG–IgM antibodies, indicating that inflammation correlates with sustained payload expression and with the prevention of LNP clearance (Fig. 1b).

In searching for potent drug-delivery vehicles, several research teams have rationally designed and synthesized libraries of ionizable lipids to investigate their structure–activity relationship. Changing the linker, head or tail components of the ionizable lipids modifies the physicochemical properties of the LNPs, hence altering their pharmacokinetics and ultimately leading to changes in organ and cellular tropism[11–14]. The study of Whitehead and co-authors provides a methodology that can be leveraged to test and improve the formulation of LNPs. The authors' computational pipeline appears promising when tested on lipids with known immunogenic properties, and future efforts may focus on predicting the immunogenicity of lipids with unknown immunogenicity. The computational pipeline would enable researchers to focus on refining designs of lipids for LNPs to tune their immunogenic profiles while minimizing adverse effects, thereby potentially broadening the clinical applicability of the LNPs. Naturally, one should bear in mind that the immune responses seen in the mice may not occur in humans, owing to differences in the specific mechanisms and functionalities of the immune system. Also, much remains unknown about how the route of administration (intranasally, intramuscularly or intravenously, in particular) will impact the immunogenicity of the LNPs.

Preeti Sharma[1,2,3,4], Dor Breier[1,2,3,4] & Dan Peer[1,2,3,4] ✉

[1]Laboratory of Precision NanoMedicine, Shmunis School for Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel. [2]Department of Materials Sciences and Engineering, Iby and Aladar Fleischman Faculty of Engineering, Tel Aviv University, Tel Aviv, Israel. [3]Center for Nanoscience and Nanotechnology, Tel Aviv University, Tel Aviv, Israel. [4]Cancer Biology Research Center, Tel Aviv University, Tel Aviv, Israel, Tel Aviv, Israel.

✉e-mail: peer@tauex.tau.ac.il

Published online: 23 October 2024

References

1. Kon, E. et al. *Sci. Adv.* **9**, eadg1036 (2023).
2. Kon, E. et al. *Nat. Rev. Clin. Oncol.* **20**, 739–754 (2023).
3. Zhang, G. et al. *Signal Transduct. Target. Ther.* **8**, 365 (2023).
4. Mullard, A. *Nat. Rev. Drug Discov.* https://doi.org/10.1038/d41573-024-00095-3 (2024).
5. Cullis, P. R. & Felgner, P. L. *Nat. Rev. Drug Discov.* **23**, 709–722 (2024).
6. Tarab-Ravski, D. et al. *Adv. Sci.* **10**, 2370141 (2023).
7. Connors, J. et al. *Commun. Biol.* **6**, 188 (2023).
8. Sharma, P. et al. *Adv. Drug Deliv. Rev.* **205**, 115175 (2024).
9. Tahtinen, S. et al. *Nat. Immunol.* **23**, 532–542 (2022).
10. Chaudhary, N. et al. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-024-01256-w (2024).
11. Kedmi, R., Ben-Arie, N. & Peer, D. *Biomaterials* **31**, 6867–6875 (2010).
12. Naidu, G. S. et al. *Adv. Sci.* **10**, 2301929 (2023).
13. Dilliard, S. A., Cheng, Q. & Siegwart, D. J. *Proc. Natl Acad. Sci. USA* **118**, e2109256118 (2021).
14. Ni, H. et al. *Nat. Commun.* **13**, 4766 (2022).

Competing interests

D.P. receives licensing fees (to patents on which he was an inventor) from the following entities: ART Biosciences, BioNtech SE, Earli Inc., Geneditor Biologics Inc., Kernal Biologics, Inc., Newphase Ltd., NeoVac Ltd., RiboX Therapeutics, Roche and Teva Pharmaceuticals Inc. D.P. has also invested in, consults or serves on the scientific advisory boards or boards of directors of some of these companies, and has lectured (and received a fee) or conducts sponsored research for the companies. All other authors declare no competing interests.