Molecular Therapy
Editorial

# Pro-inflammatory concerns with lipid nanoparticles

The development of ionizable cationic lipids has been a pivotal factor in the clinical success of lipid nanoparticle (LNP)-RNAi (Onpattro) and the two LNP-mRNA vaccines against SARS-CoV-2 (Pfizer-BioNTech and Moderna).[1,2] The ionizable cationic lipids demonstrate improved efficacy and safety compared with the permanently charged cationic lipids.[1,2] However, acute side effects such as pain, swelling, fever, and systemic inflammatory responses have been reported in many human subjects receiving LNP-mRNA vaccines. Although these vaccines use N1-methyl-pseudouridine-modified RNA (which is poorly recognizable by the Toll-like receptors 7/8), it is not clear how these vaccines elicit reactogenicity, but this presumably indicates that LNPs have intrinsic adjuvant activity.[3] Infusion-related adverse effects have been noted in up to 19% of patients receiving Onpattro.

Now, Ndeupen et al.[4] and Tahtinen et al.[5] report a pro-inflammatory role for ionizable cationic lipids. Ndeupen et al.[3] show that intramuscular, intradermal, or intranasal administration of ionizable cationic lipid-containing LNPs in mice triggers rapid and robust neutrophil infiltration, activation of many different inflammatory pathways (e.g., retinoic acid-inducible gene I, nucleotide oligomerization domain-like and Toll-like receptor signaling) and secretion of inflammatory cytokines and chemokines (e.g., interleukin [IL]-1β, IL-6, and macrophage inflammatory proteins CCL3 and CCL4). Intranasal inoculation with LNPs led to massive inflammation in the lungs and a high mortality rate, but in a dose-dependent manner.[4]

Tahtinen et al.[5] go a step further and show ionizable lipid-dependent differences in NLRP3 inflammasome activation in human peripheral blood mononuclear cells. Empty and modified mRNA-loaded LNPs containing the ionizable cationic lipid SM-102 (which is used in the Moderna COVID-19 vaccine) potently activated the inflammasome pathway, showing robust IL-1β release, but low levels of IL-1 and the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra).[5] On the other hand, LNPs with the ionizable cationic lipid MC3 (commonly used in LNP-RNAi formulations) proved far less potent at stimulating IL-1β release, and the addition of R848 (a strong TLR7/8 agonist) did not fully rescue IL-1β release. Therefore, it is likely that SM-102 directly activates some intracellular pattern-recognition receptors, while both ionizable cationic lipids also trigger signals for the NLRP3 inflammasome, presumably, following transient disruption of plasma and/or endosomal membranes (e.g., through mitochondrial reactive oxygen species-mediated calcium flux and some caspases). Intriguingly, incorporation of the modified RNA in a liposome composed of the cationic lipid DOTMA neither induced IL-1β release nor any of its downstream cytokines.[5] Therefore, the reactogenicity of the modified RNA is tuneable depending on the choice of the cationic/ionizable lipid. On the other hand, systemic inflammation in response to unmodified RNA-cationic liposomes is species specific and regulated by the IL-1–IL-1ra axis.[5] While IL-1ra attenuates the effect of IL-1β in human cells only at low to moderate doses of LNP-RNA, in wild type mice (but not in IL-1ra-deficient mice), LNPs are well tolerated, where IL-1ra is dramatically induced relative to IL-1β. Robust upregulation of IL-1ra on LNP-RNA challenge is also reproducible in the peripheral blood mononuclear cells from two nonhuman primates. These species differences were partially explained by the lower frequency of monocytes in mice and the two nonhuman primate models compared with humans.[5]

The results of Ndeupen et al.[4] and Tahtinen et al.[5] are consistent with reactogenicity and immunogenicity observed in LNP-mRNA vaccination. Considering the rare episodes of human anaphylactic reactions following LNP-mRNA vaccine administration,[6] we cannot disregard a role for a subset of monocytes and dendritic cells in orchestrating these responses. Extensive studies are now needed to map the interactions between ionizable cationic lipids and intracellular pattern-recognition receptors and unravel integrated and multifaceted mechanisms by which these lipids induce inflammasome activation. Such information would be valuable for identification and tailoring of lipid-based adjuvant and delivery systems and for generating diversified portfolios of vaccine components and platforms to provide immunity in specific target tissues. Pertinent to realizing these goals are needs for the development, validation, and adaptation of humanized explant models that addresses intrinsic characteristics of different tissues (e.g., human spleen, tonsil, and lymph node tissues; human mucosal-associated lymphoid tissues from different regions of respiratory, gastrointestinal, and genital tracts). This pan-integrated approach further addresses cross-species translational variability seen in innate immune responses and should help identify appropriate pre-clinical models that resemble human responses, thereby de-risking vaccine development at early stages.

The success of LNP-based vaccines has prompted a surge in overenthusiastic research focused on the broader application of LNP-based nanomedicines.[2] Considering the pro-inflammatory nature of the currently available ionizable cationic lipids,[4,5] notably their undesirable immune cascade initiated through the IL-1β release, and of other cationic lipids,[7] the potential application of LNPs for systemic administration must be viewed cautiously. This is important, particularly when targeting biological barriers such as the blood-brain barrier with intravenously administered LNPs for the intended delivery of nucleic acid medicine to brain parenchymal cells to combat neurological diseases and disorders, which could initiate severe

inflammatory reactions in the brain either directly or through monocyte recruitment.

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