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VACCINES

Making innate sense of mRNA vaccine adjuvanticity

mRNA vaccines such as those used to prevent COVID-19 owe part of their success to methylation that masks immunostimulatory properties of the mRNA, but the immunological mechanisms of adjuvanticity are unclear. Two new studies reveal distinct mechanisms for innate sensing of this hidden adjuvant.

Kouji Kobiyama and Ken J. Ishii

Successful vaccines contain two essential immunological components: a protective antigen and an adjuvant. Adjuvants are essential for optimal antigen-specific immune responses, the so-called 'immunogenicity', but are often a cause of reactogenicity (even toxicity) that results in local and systemic inflammation. Therefore, to ensure vaccine efficacy and safety, it is critical to understand the molecular and cellular mechanism(s) by which adjuvants provoke the immune system. Two papers in this issue of *Nature Immunology*, from Li et al.[1] and Tahtinen et al.[2], provide new insights into the understanding of the adjuvant mechanisms of mRNA vaccines (Fig. 1).

Adjuvants are often added as an exogenous material, but they are sometimes incorporated into vaccine formulations. Such 'built-in' adjuvants include nucleic acids, lipids, lectins, and proteins derived from pathogens, known as 'pathogen-associated molecular patterns' (PAMPs), which are able to activate pattern-recognition receptors expressed by antigen-presenting cells, resulting in the induction of antigen-specific T cell and B cell responses[3]. Nucleic acids such as DNA and RNA within virus- and non–virus-vectored vaccines are well-known built-in adjuvants; DNA can be recognized by TLR9 or cGAS, and RNA can be recognized by TLR3, TLR7, TLR8 (not valid in mice), TLR13 (not valid in humans), RIG-I and MDA-5, in a sequence- and structure-dependent manner[4]. The resultant activation of the innate immune system is cell specific and tissue specific.

However, many human vaccines do not contain PAMPs; instead, they utilize non-biological materials, such as organic oil, aluminum salt, squalene, liposomes or lipid nanoparticles (LNPs), all of which are known to function as adjuvants, to form micro-sized or nano-sized particles as delivery vehicles for antigens. Once injected into the body, these adjuvants not only carry the antigen but also can damage or kill host cells at the injection site. Such cell death is often a combination of apoptosis, necrosis, necroptosis, pyroptosis and/or NETosis, most of which in turn results in the induction or release of a variety of host adjuvant factors or damage-associated molecular patterns (DAMPs), such as nucleic acids and their metabolites (including cGAMP, uric acids, and nuclear cytokines such as IL-1, IL-18 and IL-33). For example, aluminum salt, a commonly used adjuvant, has been shown to induce cell death via necroptosis and NETosis in the injected tissue, resulting in the release of host DNA, uric acids and IL-1α, all of which can function as DAMPs[3].

Given the built-in or DAMP-inducing adjuvant effect of many vaccines, one might also wonder about the adjuvanticity of mRNA within LNP-mRNA vaccines. In early attempts to make LNP-mRNA particles, unmodified mRNA synthesized by in vitro transcription was a potent inducer of the production of type I interferons mediated by TLR3, TLR7, TLR8 and RIG-I, which hampered the translation efficiency of the encoded antigen protein and thereby resulted in low immunogenicity. This issue was overcome by the methylation of cytosine, adenine and uridine in the RNA, which reduces innate immune recognition by TLRs and RIG-I-like receptors (RLRs)[5,6]. Such methylated mRNAs have been used to make drugs with high efficacy and a tolerable safety profile in humans. Therefore, most researchers now believe that these modified mRNAs are invisible to the innate immune system.

However, one study showed that the ionizable lipid component, one of the two characteristic lipid components of LNPs, has adjuvant activity[7]. In this work, LNP itself was shown to induce IL-6 production, which resulted in potent antigen-specific CD4+ follicular helper T cell (T_FH cell) and germinal center (GC) B cell responses that were not dependent on RNA-sensing pathways controlled by the signaling adaptor IPS-1 (also known as MAVS). Although the LNP and mRNA used in this study[7] were not exactly the same as those in LNP-mRNA vaccines against the coronavirus SARS-CoV-2, the adjuvant effect of the LNP was observed with protein antigen and was more potent than that of a conventional squalene-based adjuvant, in an IL-6-dependent manner. Although TLRs and RLRs did not seem to be involved, these data suggest that a LNP alone can act as a built-in adjuvant, in addition to its primary function in forming a particle that mimics the size and behavior of a virus in vivo, thereby delivering mRNA into the cytosol of target cells and protecting it from nucleases.

The adjuvanticity of LNP-mRNA vaccines seems more complex than first thought. The mRNA in these vaccines is not immunogenic[5,6] and thus cannot function as a built-in adjuvant. Instead, it seems that the delivery of the LNP stimulates the innate immune system and functions as an adjuvant that is independent of TLRs and RLRs, although some reports have suggested some involvement of the pattern-recognition receptors TLR4, TLR7 and STING[8–10]. So what exactly in the LNP-mRNA acts as an in-built adjuvant, and which innate immune sensing pathways are involved?

Li et al. assessed the mechanism of the response to BNT162b2 LNP-mRNA, the officially approved Pfizer–BioNTech vaccine against COVID-19, using genetically modified mice that lack a series of innate immune sensors, including TLR2, TLR3, TLR4, TLR5, TLR7 and cGAS, and relevant genes encoding molecules involved in pyroptosis and necroptosis[1]. With one exception, none of these pattern-recognition receptors seemed to be needed for the immunogenicity of this vaccine. Only MDA-5, a receptor for long double-stranded RNA, was shown to be important for type I interferon responses and as a built-in adjuvant pathway for antigen-specific CD8+ T cell responses. These data indicate
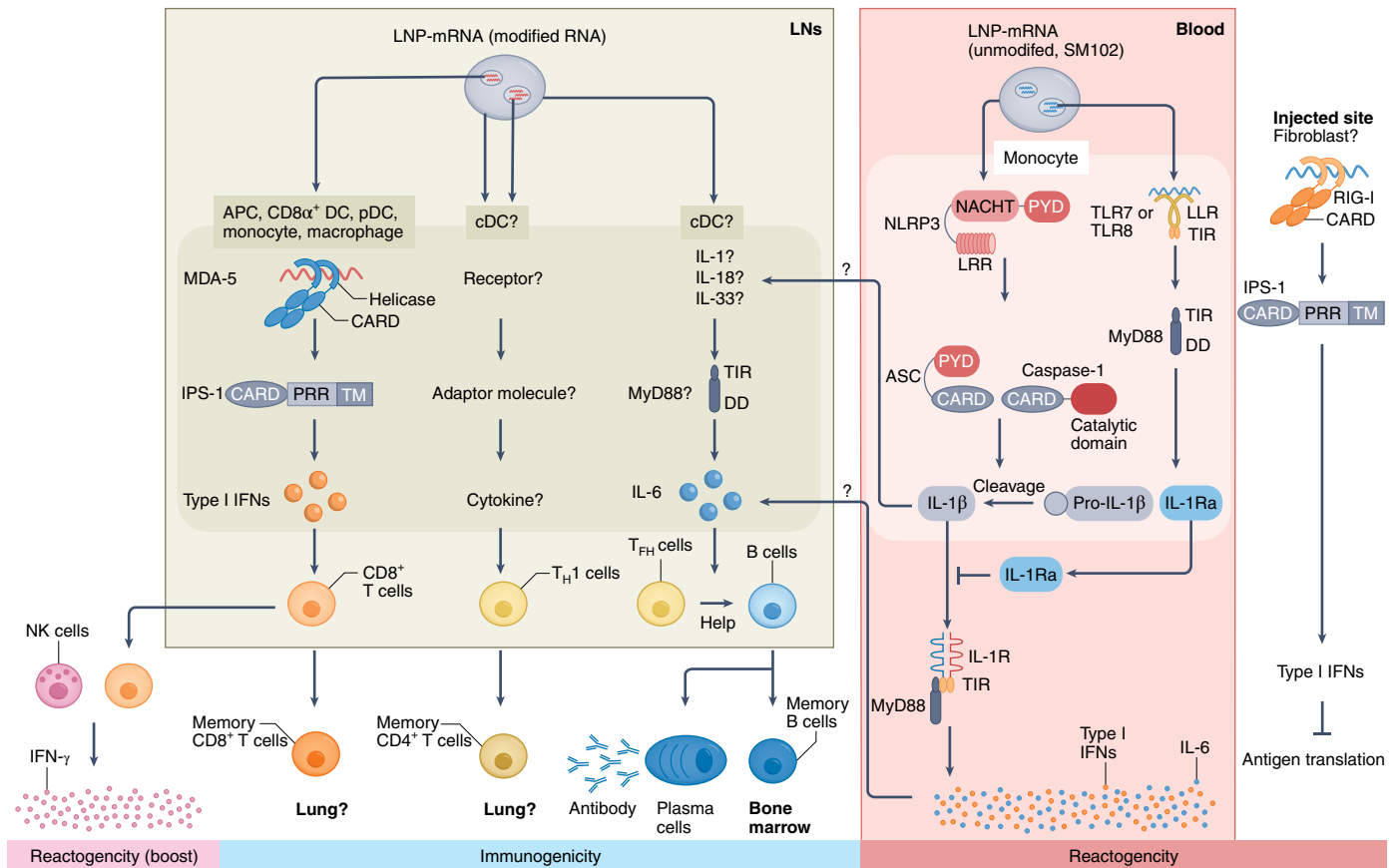
Fig. 1 | Mechanisms of immunogenicity of and reactogenicity to LNP-mRNA vaccines. In the lymph nodes (LNs), modified RNA sensed by MDA-5 results in the production of type I interferons (IFNs). Type I interferons induce antigen-specific CD8^{+} T cell responses. LNPs can activate innate immune responses in lymph nodes and elicit production of IL-6 that is essential for the induction of T_{FH} cells and GC B cells. These pathways are important for the immunogenicity of LNP-mRNA vaccines. When the mRNA is unmodified and a specific ionizable lipid (such as SM-102) is contained in the LNP, the mRNA is recognized by TLR7 and/or TLR8 and the ionizable lipid is recognized by the NLRP3 inflammasome in monocytes. Inflammasome-induced members of the IL-1 family trigger further inflammatory cytokine production. IL-1Ra is also produced as a negative feedback loop to inhibit IL-1 signaling and reduce inflammation. Unmodified mRNA is also detected by RIG-I, which results in the production of type I interferons and thus interferes with antigen translation. After booster vaccination, modified RNA–induced type I interferons also activate natural killer (NK) cells to produce IFN-γ. These signaling pathways might affect the reactogenicity of LNP-mRNA vaccines. APC, antigen-presenting cell; DC, dendritic cell; pDC, plasmacytoid dendritic cell; cDC, conventional dendritic cell; CARD, caspase-recruitment domain; PRR, proline-rich region; TM, transmembrane domain; TIR, Toll–IL-1R domain; DD, death domain; T_{H}1 cells, type 1 helper T cells; NACHT, nucleotide-binding domain; PYD, pyrin domain; LRR, leucine-rich repeat; ASC, apoptosis-associated speck-like protein containing a caspase-recruitment domain.

that mRNA might still be an important component of the built-in adjuvant and its MDA-5-mediated innate sensing pathway for antigen-specific CD8^{+} T cell responses, but they did not confirm that the true ligand for MDA-5 was from the vaccine or from the host RNA, although removing double-stranded RNA from LNP-mRNA preparations improves immunogenicity[11,12]. The molecular mechanisms of the innate sensing pathway of LNP-mRNA toward responses by antigen-specific CD4^{+} T cells, such as type 1 helper T cells and T_{FH} cells, remains unclear.

Interestingly, Li et al. showed that a single intramuscular immunization with the BNT162b2 vaccine can activate dendritic cells,

monocytes and macrophages in the draining lymph nodes, lungs and spleen to produce type I and type II interferons[1]. Notably, serum levels of interferon-γ (IFN-γ) and the expression of interferon-stimulated genes in monocytes were higher after a second immunization. These data might reflect the fact that the unique reactogenic incidence happened as an adverse effect after the second immunization with LNP-mRNA in humans, and might be connected to trained immunity (or innate immune memory), mediated by IFN-γ, and the consequent epigenetic modification of monocytes.

An aspect of immunogenicity that is controlled by the LNP-mRNA vaccine is reactogenicity, or systemic and local adverse

events, such as fever, fatigue, pain, swelling and redness after both primary vaccination and secondary vaccination. Are the same mechanisms or the same 'built-in' adjuvants involved in the induction of reactogenicity to the LNP-mRNA vaccination? Tahtinen et al. tried to address this issue by focusing on the varying systemic reactions to mRNA vaccines in humans, non-human primates (NHPs) and mice[2]. They analyzed a liposomal vaccine containing unmodified or modified RNA (called 'RNA-LPX') used in a human clinical trial for metastatic tumors (ClinicalTrials.gov identifier NCT03289962) and showed that RNA-LPX induced a large amount of IL-1β in the blood and in cultures of peripheral blood mononuclear

cells. These data showed that monocytes were activated and that the two-step inflammasome pathway was involved in inducing pro-IL-1β, followed by secretion of mature IL-1β, which suggests that the mRNA and lipid individually activate these pathways. LPX is composed of lipids different from those used in mRNA vaccines against COVID-19, but they produce results similar to those obtained with the ionizable lipid SM102, which is used in the Moderna mRNA-1273 vaccine against COVID-19. The key finding of this paper is that the LNP induced IL-1Ra, a member of the IL-1 family that blocks the binding of IL-1 to its receptor, IL-1R, which results in inhibition of IL-1-mediated inflammation and IL-6. Curiously, when Tahtinen et al. compared the induction of IL-1Ra by LNP in patients, NHPs and mice, they found that only mice had constitutively high levels of IL-1Ra in the serum, which was further elevated by RNA-LPX treatment; this reduced the sensitivity to IL-1-mediated inflammation by ~1,000-fold compared with that in humans and NHPs[2]. This finding indicates that the pre-clinical data obtained with mice or NHPs might not translate correctly to humans, especially in the case of reactogenicity, and possibly even immunogenicity. In fact, *Il1ra*-deficient mice had more systemic inflammation than that of wild-type control mice after administration of RNA-LPX, equivalent to the extent in humans and NHPs, which

might be more predictive of the potential reactogenicity of mRNA vaccination in humans.

Given the data from both Alameh et al.[7] and Tahtinen et al.[2], it seems the combination of LNP with mRNA functions as a built-in adjuvant that induces IL-1 and regulatory cytokines (such as IL-1ra) to control immunogenicity and reactogenicity. IL-1α and IL-1β are essential for the production of IL-6, another cytokine needed for both optimal immunogenicity (involving antigen-specific CD4+ T$_{FH}$ cells and GC B cells) and reactogenicity, such as systemic inflammation by blood monocytes via inflammasome activation. In addition to IL-1, other members of the IL-1 family, such as IL-18 and IL-33, known to activate MyD88-dependent innate immune signaling, are unique in that they can be induced both by PAMPs and by DAMPs and thereby contribute to the immunogenicity and reactogenicity of LNP-mRNA vaccination.

LNP-mRNA vaccines, composed of two types of lipid-forming nanoparticles encapsulating antigen-encoding modified mRNA, are a hallmark of the innovation that has occurred in COVID-19 and other vaccine research and development. However, there seems to be more room to improve the immunogenicity and reduce the reactogenicity of LNP-mRNA vaccine formulations by further study of immunization methods (including delivery

systems and devices) and their built-in adjuvanticity. ❐

Kouji Kobiyama[1,2,3] and Ken J. Ishii [1,2,3] ✉
¹Division of Vaccine Science, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. ²International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. ³Center for Vaccine Adjuvant Research, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan.
✉e-mail: kenishii@ims.u-tokyo.ac.jp

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HEMATOPOIESIS

B cells regulate hematopoietic stem cells via cholinergic signaling

B-cell-derived acetylcholine initiates a circuit that prompts bone marrow stromal cells to secrete factors that restrain the blood-forming activity of hematopoietic stem cells in response to cardiovascular dysfunction.

Sweta B. Patel and Eric M. Pietras

Acetylcholine is a neurotransmitter that is associated with the parasympathetic nervous system, which governs a wide range of autonomic functions, including immune cell activity. In response to numerous immune stimuli, including activation of Toll-like receptors, immune cells can express choline acetyltransferase (ChAT),

the enzyme that catalyzes the synthesis of acetylcholine, and/or its receptors[1]. The activity of hematopoietic stem cells (HSCs) themselves is tightly constrained by cholinergic signaling. Acetylcholine acts together with neurotransmitters produced by the sympathetic nervous system, such as dopamine and norepinephrine, to regulate HSC trafficking in and out of the bone

marrow (BM)[2–4]. This system coordinately governs HSC mobilization from the bone BM to the peripheral blood in response to various stimuli, including cytokine signals, such as G-CSF, and daily light–dark cycles[4]. However, the role of cholinergic signaling and the relevant player(s) that regulate hematopoietic responses to inflammation or regenerative cues remain open questions.