



A Message of Tolerance: Turning Down the Heat on Allergic Asthma

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Asthma affects over 300 million people around the world, and while current therapies control airway inflammation and relieve symptoms to improve patients' quality of life, they don't address the immunological defects that drive allergic asthma. In allergic asthma, the immune system fails to maintain tolerance to harmless environmental antigens.¹ It misidentified otherwise harmless substances as a danger and triggers airway inflammation, the overproduction of mucus, and bronchial hyperresponsiveness. Although current therapies like corticosteroids and targeted biologic therapies have greatly improved disease management, these treatments mainly suppress downstream inflammation and usually require lifelong administration. Most current therapies do not fully correct the underlying immune dysregulation responsible for allergic asthma.

In recent years, mRNA–lipid nanoparticle technology has emerged as a powerful platform for nucleic acid delivery as a powerful platform for vaccination and immune activation.^{2–4} The applications of mRNA systems extend beyond the induction of protective immunity against infectious diseases. Rather than activating immunity, mRNA–LNP systems can also be engineered to dampen immune responses and promote.⁵ This work reframes mRNA therapies as tools for immune re-education. The central challenge in developing such a therapy against asthma is to selectively reprogram the immune system to tolerate allergens without causing broad systemic immunosuppression. Although existing therapies effectively control airway inflammation, they fail to eliminate immune memory. Furthermore, current allergen immunotherapies require extended treatment, vary in efficacy, and may produce adverse reactions. As a result, only about 5% of people with allergic rhinitis, asthma, or both in the United States undergo this treatment.⁴ To overcome these limitations, Wang *et al.* tested a spleen-targeted approach using tolerogenic mRNA–LNPs incorporating long-chain saturated fatty acids to reprogram allergen-specific immu-

nity and suppress allergic asthma through tolerance mechanisms.^{5,6} This work represents a new approach to using programmable mRNA therapeutics to address the upstream immunologic cause rather than simply managing downstream symptoms. Previous work with an mRNA–LNP vaccine platform incorporated the long-chain saturated fatty acid stearic acid into the lipid nanoparticles to produce stearic acid-doped LNPs (SA-LNPs). Stearic acid alters the physicochemical properties of the particles and had previously been shown to promote preferential targeting of the spleen and splenic dendritic cells.⁵ The SA-LNPs were used to co-deliver nucleoside-modified ovalbumin (OVA) mRNA and celastrol, an anti-inflammatory compound that suppresses dendritic cell activation.⁷ Using luciferase mRNA, fluorescently labeled nanoparticles, and LNP formulations doped with natural long-chain saturated fatty acids, the researchers showed that the modified particles preferentially accumulated in the spleen, with particularly high uptake by splenic dendritic cells.⁵ Uptake of the tolerogenic mRNA–LNPs by splenic dendritic cells was significantly greater than uptake by other immune-cell populations. Importantly, the addition of celastrol did not impair mRNA delivery efficiency, nanoparticle stability, or protein expression. These findings indicate that the tolerogenic modification preserved the delivery capabilities of the LNP while changing its immunological behavior.

The next set of experiments examined whether the mRNA–LNPs could induce a tolerogenic phenotype among DCs. Bone marrow-derived DCs generated *in vitro* were exposed to nanoparticles and then stimulated with lipopolysaccharide, which would normally induce inflammatory maturation. DCs treated with control mRNA–LNPs lacking celastrol showed increased expression of costimulatory molecules, consistent with immunogenic activation. In contrast, the DCs treated with celastrol-loaded tolerogenic mRNA–LNPs reduced the expression of activation markers and maintained the DCs in an immature,

tolerogenic state. RNA sequencing revealed that celastrol fundamentally changed the transcriptional state of treated cells, promoting immune regulation while blocking inflammatory signals. DCs treated with sLNP-OVA/celastrol or sLNP-celastrol produced higher levels of anti-inflammatory cytokines IL-10 and TGF- β while reducing pro-inflammatory cytokines IL-6 and IL-1 β (Figure 1A). Western blot analysis showed inhibition of the NF- κ B signaling pathway through reduced phosphorylation of IKKB and p65 (Figure 1B), providing evidence that celastrol actively rewired intracellular inflammatory signaling within antigen-presenting cells.

After establishing tolerogenic DC induction, Wang *et al.* investigated whether the altered antigen-presenting cells could generate regulatory T cells (Tregs). In co-culture experiments, bone marrow-derived DCs treated with tolerogenic vaccine induced higher numbers of CD4⁺ Foxp3⁺ Tregs compared with standard mRNA–LNP formulations. The Tregs also expressed elevated levels of Foxp3, IL-10, and TGF- α , indicative of a suppressive phenotype. *In vivo* experiments showed that vaccination increased OVA-specific Treg accumulation in the spleen and lungs. This suggests that immune tolerance initiated in the spleen could influence other sites of allergic inflammation, linking systemic immune reprogramming to local suppression of airway pathology.

Finally, Wang tested whether the tolerogenic vaccine could therapeutically suppress allergic asthma in mice sensitized and challenged with the OVA allergen. Histological analysis of the lung tissue following treatment showed marked reductions in peribronchial inflammation and mucus secretion in treated mice. Flow cytometry revealed decreased eosinophil infiltration in the lungs, and bronchoalveolar lavage fluid contained fewer inflammatory cell populations including eosinophils, neutrophils, and lymphocytes. Additional immunofluorescence and flow cytometric analysis demonstrated fewer inflammatory monocytes and greater Treg infiltration in lung tissue following treatment. Together, these experiments in this mouse model that spleen-targeted tolerogenic mRNA–LNP vaccination not only altered systemic immune signaling but also alleviated the cardinal pathological features of experimental allergic asthma. This supports the idea that antigen-specific immune tolerance can be



therapeutically induced through programmable mRNA nanomedicine.

This article extends the potential for applications of mRNA therapeutics and allergic disease by showing that mRNA-LNP systems are programmable platforms that can be engineered to induce antigen-specific immune tolerance. By demonstrating that spleen-targeted delivery can reprogram DCs towards a regulatory phenotype, promote Treg responses, and reduce features of allergic airway inflammation in vivo. The study expands the versatility of mRNA therapeutics, which can be formulated either to stimulate protective immune responses or to promote antigen-specific immune tolerance.^{8,9} This presents new possibilities for treating allergic asthma and other autoimmune diseases rather than simply suppressing their symptoms.

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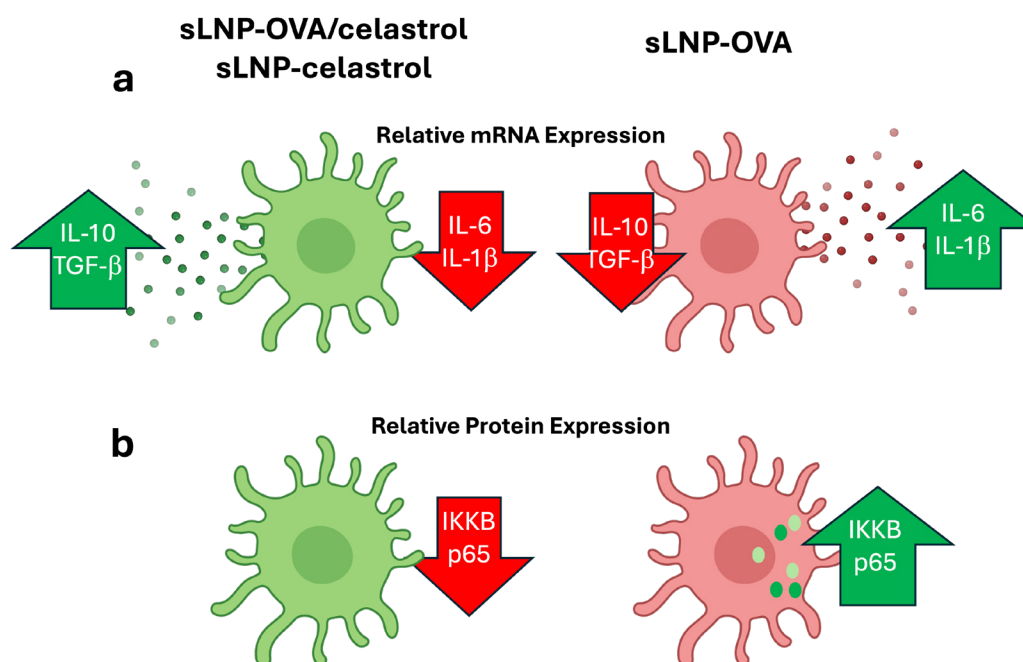


Figure 1 | Comparative analysis of relative mRNA and protein expression in bone marrow derived DCs. Cells were treated with sLNP-celastrol, sLNP-OVA/celastrol vs. sLNP-OVA A) Relative mRNA expression of anti-inflammatory cytokines IL-10 and TGF-β, and pro-inflammatory cytokines IL-6 and IL-1β in bone marrow-derived DCs. B) Relative protein expression of NF-κB pathway proteins, IKKB and p65.