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LETTER

Immunotherapeutic effects of specific and nonspecific mRNA-lipid nanoparticles in a mouse model of HDM-induced airway inflammation

To the Editor,

Epidemiological data evidenced large sensitization levels to group 1 and 2 HDM allergens worldwide in HDM allergic patients.[1] The efficacy of HDM extract-based allergen-specific immunotherapy (AIT) is dependent on the selection of patients sensitized to these allergens.[2] We recently evidenced that a bivalent vaccine combining synthetic nucleoside-modified mRNA encoding Der p 1 (pDp1, ProDer p 1)/Der p 2 (Dp2K96A) and formulated with lipid nanoparticles (LNPs) promoted Th1-biased immune responses characterized notably by potent blocking specific IgG responses.[3] Whereas a pioneer study highlighted the efficacy of mRNA encoding allergens in immunoprophylaxis,[4] mRNA-based AIT was not investigated so far.

Here, we evaluated the immunotherapeutic capacity of our bivalent vaccine in a mouse model of HDM-induced airway inflammation (Figure 1A). To reflect the natural exposure to HDM allergens, mice were intranasally sensitized and challenged with unadjuvanted HDM extracts. Sensitized animals were intramuscularly vaccinated with PBS (allergic group), Der p 1-encoding mRNA-LNP/Der p 2-encoding mRNA-LNP (mRNA-HDM) at two doses (10 μg/10 μg) (High, H) or 1 μg/1 μg (Low, L) or with a firefly luciferase-encoding mRNA-LNP (control group, mRNA-Cont) at 20 μg (H) or 2 μg (L). A typical HDM-specific allergic response characterized by induction of specific IgE, IL-5 and airway eosinophilia was developed in mice from the allergic group. Compared to the allergic group, mRNA-Cont-H/−L but not mRNA-HDM-H/−L decreased the total BALF cell number (Figure 1B). Der p 1/2-specific and control mRNA-LNP formulations similarly reduced macrophage and eosinophil infiltration (Figure 1C), suggesting that these cellular changes are mediated by complementary innate sensing of mRNA and/or LNPs.[5] Of note, ionizable lipid was shown to be responsible for the adjuvant activity of LNP formulations with mRNA or protein.[6] In contrast and contrary to both allergic and mRNA control groups, HDM-specific mRNA-LNP at both tested doses up-regulated the recruitment of lymphocytes and neutrophils (Figure 1C). Histological analyses evidenced that the largest significant reduction of peribronchiolar and perivascular inflammation was mediated by the mRNA-LNP control at high dose (Figure S1) whereas these effects were less marked following vaccinations with mRNA-HDM. Cytokine measurements in BALF showed that IL-5 levels from allergic group were drastically blunted by any mRNA-LNP treatment (Figure 2A). A significant reduction in IL-4 levels in the BALF was also mediated by the mRNA vaccines. The Th2 cytokine reduction was accompanied with IFNγ induction, the highest production being mediated with mRNA-HDM-H/−L. Significant reductions in IL-17A BALF levels were also observed except for vaccinations with mRNA-Con-L. In the supernatants from restimulated splenocytes, IL-5 was significantly reduced in each mRNA-LNP-treated group and compared with allergic mice (Figure 2B). Dose-dependence was only observed with mRNA-Cont. Compared with IL-5, very low levels of IL-4 were measured whatever the groups (Figure 2C). However, a significant higher IL-4 level was only measured in Der p 1-restimulated splenocytes from mRNA-HDM-H/−L groups. Vaccinations with mRNA-LNP formulations elicited high and moderate dose-dependent production of IFNγ and IL-17A respectively (Figure 2D,E). However, these cytokine inductions were much more pronounced with mRNA-HDM. Vaccination of sensitized mice with mRNA-HDM or mRNA-Cont were similarly capable to reduce Der p 1-/Der p 2-specific IgE antibody levels in a dose-dependent manner (Figure 2F,G). However, lower specific IgE antibody levels were measured in the HDM-specific mRNA-treated groups. The highest downregulation of the specific IgE responses in mRNA-HDM groups measured after intranasal challenges confirmed the antigen specificity of the anti-IgE effects of mRNA-LNP. mRNA-HDM but not mRNA-Cont triggered potent dose-dependent specific IgG1/IgG2a responses which persisted following the challenges (Figure 2H,I). Affinity-purified Der p 1-/Der p 2-specific IgG of post-challenge pooled sera from mRNA HDM-treated mice inhibited the IgE binding to Der p 1/Der p 2 in a dose-dependent manner. At the IgG/allergen molar ratio of 200, the inhibition of IgE binding to Der p 1 and Der p 2 reached 85% and 70% in the 10 μg/10 μg dose group whereas 65% and 40% of inhibition were obtained in the 1 μg/1 μg dose group respectively (Figure S2).

In conclusion, our results highlighted that specific and non-specific mRNA-LNP-based AIT were able to reduce key allergic parameters (IL-5, specific IgE, eosinophilia). In our present animal model, the potent blocking IgG antibody activities only mediated by mRNA HDM-LNP appears dispensable for the downregulation of the HDM allergic response. Further vaccination studies in other mouse models of HDM allergy are needed to confirm the blocking IgG-independent effects of mRNA-LNP. In contrast, mRNA HDM-LNP switched HDM-specific airway eosinophilia into airway neutrophilia. We speculate, from our T-cell assay data, that mRNA HDM-LNP but not mRNA control induces the development of allergen-specific Th17 cells, which, in turns, recruit neutrophils. Together with their blocking function, allergen-specific IgG induced by specific mRNA-LNP AIT could simultaneously elicit IgG-mediated allergic response through the engagement of Fcγ receptors by allergen-IgG complexes leading to the recruitment of neutrophils. Although preclinical studies evidenced that ionizable lipid-containing LNPs have adjuvant activity,[5,6] the downregulation of the HDM-induced allergic response by aspecific mRNA-LNP remains unexplained. Interestingly, MyD88-dependent innate immune signaling pathways can be activated by modified mRNA-ionizable LNP vaccines.[5,6] As ionizable LNPs display MyD88-/MAVS-independent adjuvant activity, it is possible that modified mRNA and its degradative products can be sensed by a TLR receptor mediating MyD88-dependent innate signaling as TLR7. Of note, several TLR ligands were shown to be able to downregulate the HDM-induced allergic response.[7] Further studies will be critical to characterize in detail the distinct immunological pathways mediated by HDM-specific and non-specific mRNA-LNP vaccines.

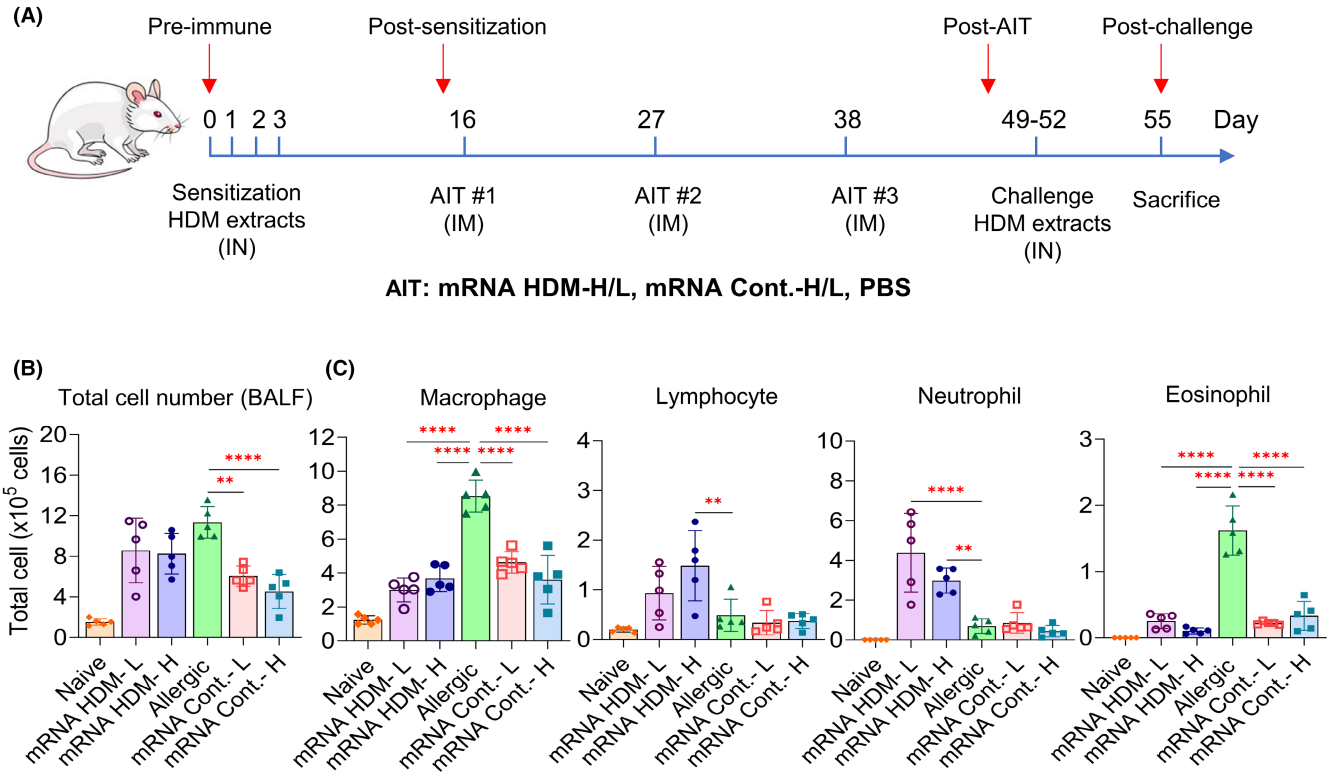
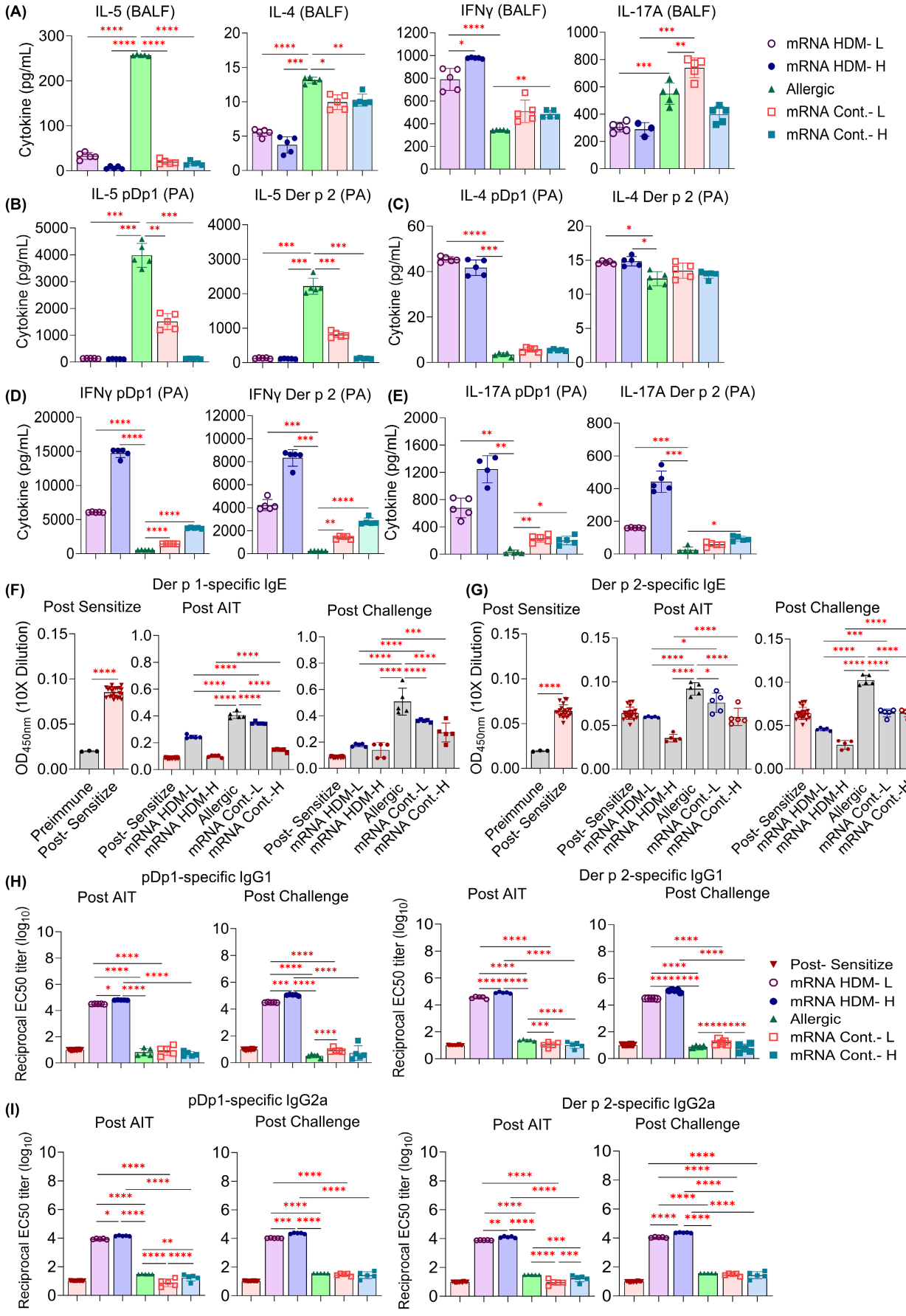


FIGURE 1 Effects of mRNA-LNP AIT on experimental HDM-induced airway inflammation. (A) Adjuvant-free mouse model of HDM-induced airway inflammation. The schedules of sensitization, AIT and challenges and bleedings are shown; (B) Total BALF cell number; (C) Number of macrophages, lymphocytes, neutrophils and eosinophils in BALF. $N = 5$ mice per experimental group. One representative of 2 similar experiments is shown. P values were calculated in the Mann Whitney *t*-test or one-way ANOVA, * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$. mRNA HDM H or L: mix of pDp1-Dp2K96A mRNA-LNP at 10 μg/10 μg or 1 μg/1 μg dose; mRNA Cont H or L: Luciferase mRNA-LNP at 20 or 2 μg dose; Allergic: no AIT (PBS).

FIGURE 2 Modulation of the cytokine and antibody response by mRNA-LNP AIT. (A) Levels of IFNγ, IL-4, IL-5, and IL-17A in the BALF; (B–E) Levels of IL-5, IL-4, IFNγ IL-17A in supernatants of splenocytes restimulated with pDp1 or Der p 2 (PA: proliferation assay); (F,G) Der p 1- and Der p 2-specific IgE levels (OD_{450nm} at 1/10 serum dilution) in preimmune sera or in sera from post-sensitization, post-AIT and post-challenge bleedings. $N = 25$ for post-sensitization levels, $N = 5$ for the other time points; (H–I) pDp1- and Der p 2-specific IgG1 and IgG2a antibody titers at the post-sensitization, post-vaccination and post-challenge time points. No specific antibodies were detected in sera from naïve mice (data not shown). One representative of two similar experiments is shown. P values were calculated in the Mann Whitney *t*-test or one-way ANOVA, * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$. mRNA HDM H or L: mix of pDp1-Dp2K96A mRNA-LNP at 10 μg/10 μg or 1 μg/1 μg dose; mRNA Cont H or L: Luciferase mRNA-LNP at 20 or 2 μg dose; Allergic: no AIT (PBS).



AUTHOR CONTRIBUTIONS

A.J., L.F. and F.B. designed the study; P.J.C.L. formulated mRNA with LNP; S.C., C.T., L.V. and P.P. performed the experiments on the design of the animal model and immunotherapy. A.J. provided supervision and analyzed the data. A.J. drafted the manuscript. All authors contributed to and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

P.J.C.L is an employee at Acuitas Therapeutics. The other authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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