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LETTER

Nucleoside-modified mRNA vaccines yield robust blocking antibody responses against major house dust mite allergens

To the Editor,

The capacity of nucleoside-modified mRNA vaccines to generate potent neutralizing antibody responses against viruses,[1] including SARS-CoV-2, could be harnessed for the design of new allergen-specific immunotherapy (AIT) protocols to promote high levels of blocking antibodies.[2] In the present study, two lipid nanoparticle (LNP)-formulated nucleoside-modified mRNAencoding secreted hypoallergenic forms of house dust mite (HDM) allergen Der p 1[3] (ProDer p 1, pDp1) and Der p 2[4] (Dp2K96A) were synthesized (Figures S1 and S2). BALB/c mice were intramuscularly (i.m.) immunized with 20 μg of each mRNA-LNP at weeks 0, 3 and 6 and antibody responses were followed for 15 total weeks (Figure 1A).

Both mRNA-LNP formulations triggered potent allergen-specific IgG1/IgG2a titers (Figure 1B), with the highest binding magnitude reached after the first boosting dose ($p < .05$, week 6). A second booster vaccination did not increase the antibody levels ($p > .05$). At the time of animal necropsy (week 15), the decrease in specific antibody titers was not significant for Der p 1-specific IgG1 and Der p 2-specific IgG2a but was significant for Der p 1-specific IgG2a and Der p 2-specific IgG1 ($p < .05$). Remarkably, Der p 1- or Der p 2-specific IgE were not detectable in any tested sera (data not shown).

Allergen-specific IgGs were capable to inhibit the binding of human specific IgE (Table S1) to coated natural Der p 1 (nDer p 1) or rDer p 2 (Figure 1C). The magnitude of blocking capacity of IgG antibodies to Der p 1 or Der p 2 peaked at week 9 (three immunizations), with inhibition percentage of around 85%–90% and 60%–75% for 1/40 and 1/200 immune serum dilution, respectively. At the time of animal necropsy (week 15), the binding of IgE to coated allergen could still be reduced by around 70% and 20% at the same tested serum dilutions. The antibodies at week 9 were able to inhibit the degranulation of RBL-SX38 cells triggered by nDer p 1 or rDer p 2, the highest magnitude of inhibition (around 80%) being observed with the highest dilution of mice sera (1/200 dilution) (Figure 1D).

As HDM allergics are predominantly co-sensitized with Der p 1 and Der p 2,[5] we next characterized the immunogenicity of a bivalent vaccine (a 1:1 mix of pDp1 - Dp2K96A mRNA-LNP) and investigated the difference in antibody responses between three different doses (10, 2 or 0.4 μg) (Figure 2A). Statistically significant difference in specific IgG1 and IgG2a titers was observed between the different dose groups after the first and the second immunization (Figure 2B). Strikingly, a third immunization largely attenuated the dose effect as comparable antibody titers were detected at all tested dose (with the exception of Der p 2-specific IgG1 at week 6) up to week 13 ($p > .05$). Two immunizations with 10 or 2 μg but not with 0.4 μg mRNA mix induced Der p 1- and Der p 2-specific IgG capable to inhibit IgE binding to natural Der p 1 by around 75% and 55% and to rDer p 2 by around 50% and 30%, respectively (Figure 2C). The blocking IgG responses peaked after the second boosting dose (around 85% inhibition in the 10 μg dose group, $p < .05$) and the levels of inhibition were maintained up to week 13. Der p 1-/Der p 2 specific antibodies, after three immunizations, displayed dose-dependent RBL degranulation inhibitory capacities (Figure 2D). The basophil activation was reduced by more than 90% and 80% with the Der p 1- and Der p 2-specific IgG, respectively, in the 10 μg group (1/200 dilution, $p < 0.05$). Finally, splenocyte restimulations evidenced that mRNA vaccinations triggered strict Th1 response as judged by IFNγ production and the absence of detectable levels of IL-5 (Figure 2E). The trend for a dose-dependent IFNγ secretion was not stastistically significant.

Finally, we evaluated the reactogenicity of mRNA-LNP in BALB/c mice intraperitoneally (i.p.) co-sensitized with nDer p 1/rDer p 2 adsorbed to alum. Two weeks post-sensitization, animals were intramuscularly injected with a mix of 10 μg pDp1-Dp2K96A mRNA-LNP or Poly(C) control mRNA-LNP. As a positive control, sensitized mice were challenged intraperitoneally with unadjuvanted nDer p 1/rDer p 2 (Figure 2F). The i.p. nDer p 1/rDer p 2 challenge performed in sensitized mice induced, within 20 min, an anaphylactic response characterized by a drop in body temperature, symptom development and high MCPT-1 serum levels (Figure 2G–I). These cardinal features of anaphylaxis were not observed in mRNA-LNP-challenged animals. Mouse monitoring performed 5 h post-mRNA-LNPs injection, a time point where the mRNA translation to antigen production commonly peaks,[7] did not evidence any change in mouse behavior and body

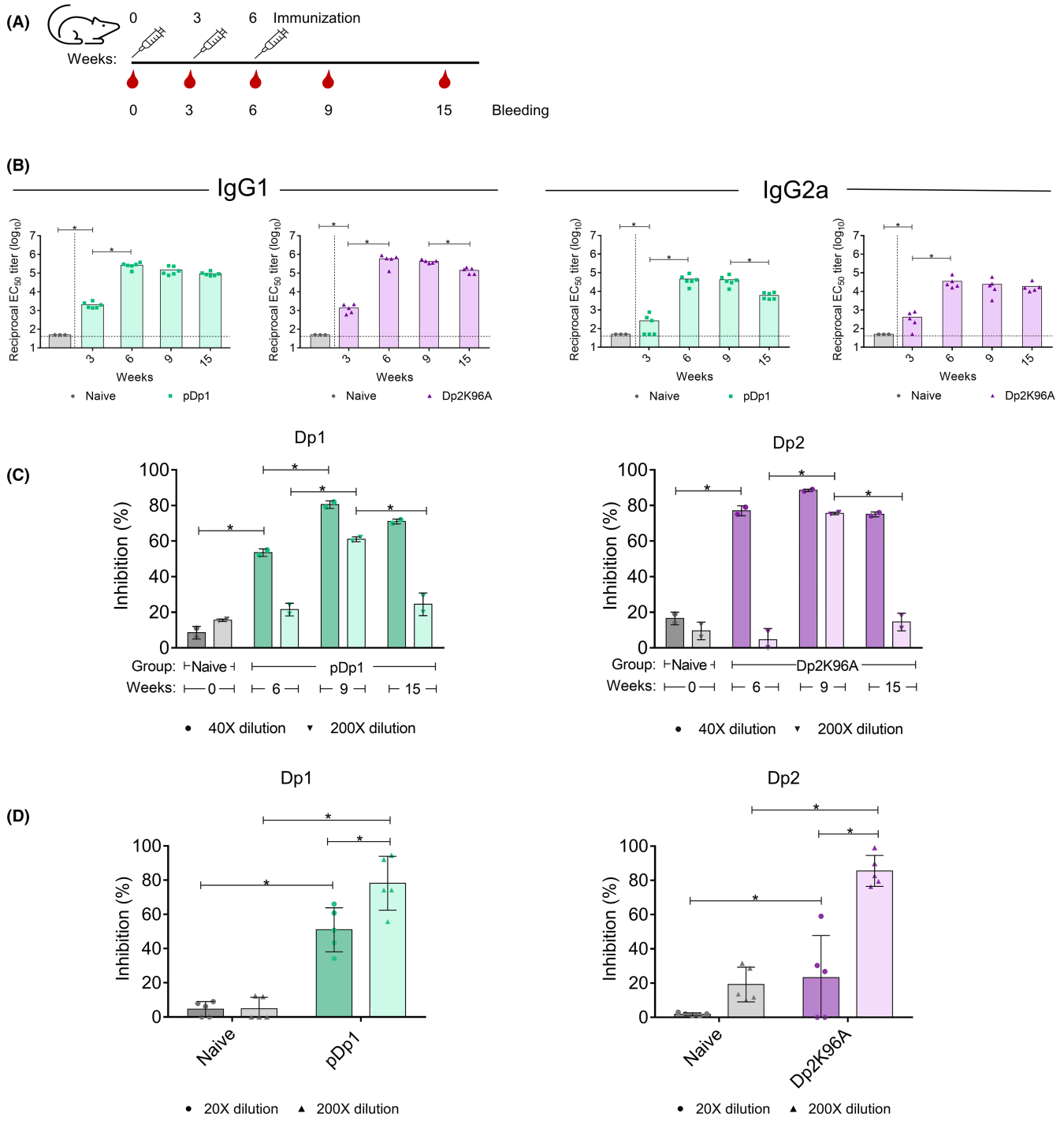

FIGURE 1 Immunogenicity and blocking IgG antibody capacity of monovalent mRNA-pDp1-LNP and mRNA-Dp2K96A-LNP. (A) Immunization and bleeding schedule; (B) pDp1- and Dp2-specific IgG1 and IgG2a antibody titers; the dotted horizontal lines represent the lowest serum dilution tested. * $p < .05$; (C) Blocking capacity of specific IgG triggered by mRNA-pDp1-LNP or mRNA-Dp2K96A-LNP at 1/40 or 1/200 dilution. * $p < .05$; (D) Inhibition of RBL-SX38 cell degranulation by specific IgG induced by mRNA-pDp1-LNP or mRNA-Dp2-LNP immunization. RBL-SX38 cells, primed with five Der p 1- or Der p 2-positive sera, were activated with 0.01 μg/ml nDer p 1 or rDer p 2 preincubated or not with pooled mouse sera (preimmune or week 6) diluted 20 or 200 times. *$p < .05$. One representative of two similar experiments is shown. $N = 6$ animals per experimental group. p values were calculated using the Mann–Whitney t-test or Two-way ANOVA.

temperature. Mice remained as well in good health conditions at time points 24 and 48 h following mRNA-LNP injection.

In conclusion, our results show the great potential of the synthetic nucleoside-modified mRNA-LNP platform for the development of potent allergen-specific blocking IgG responses. Moreover, our first preclinical safety data, combined with the short-lived in-vivo antigen expression commonly observed in mRNA-LNP-immunized mice,[7] suggest that AIT based on mRNA-LNP administration could

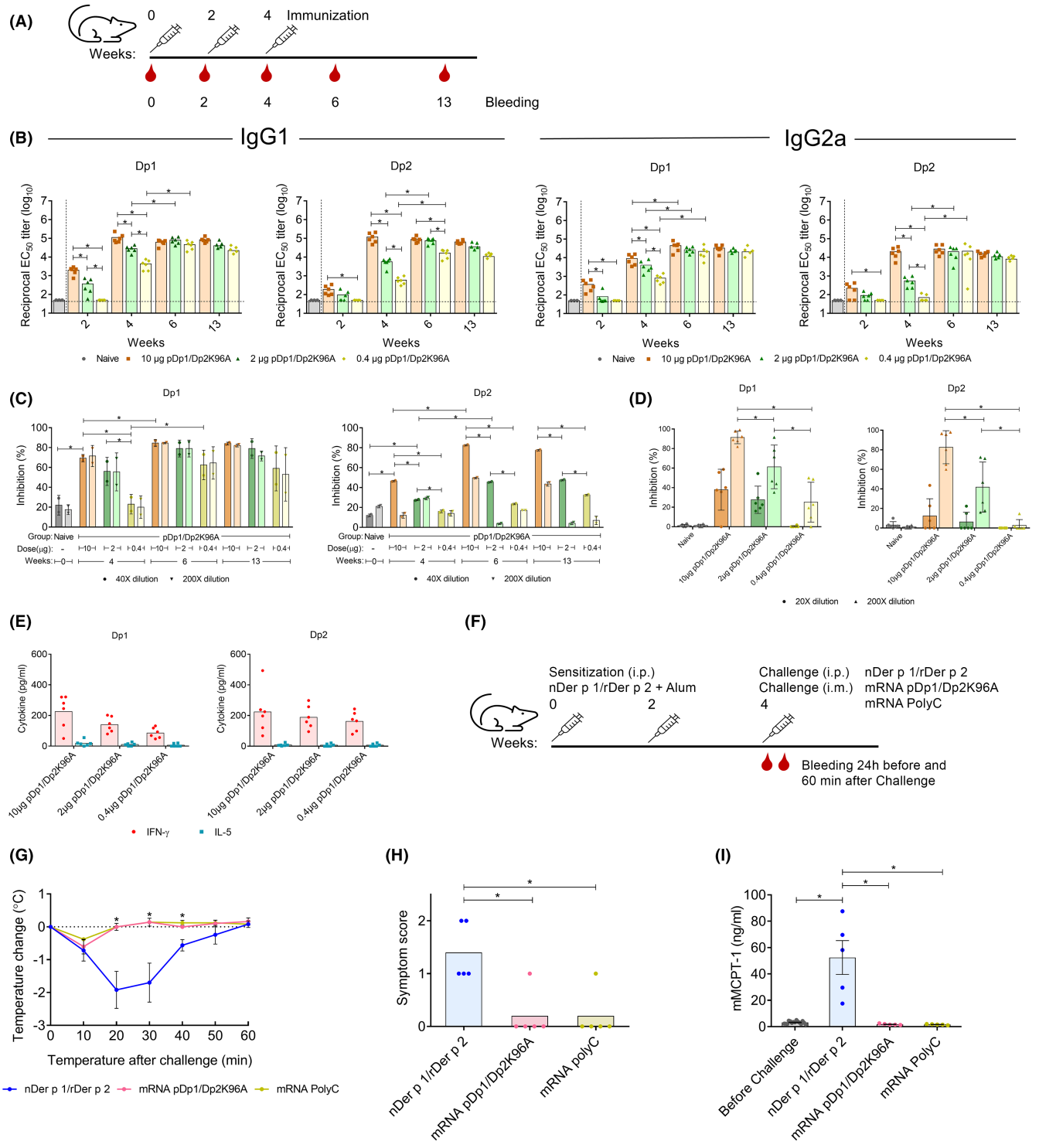

FIGURE 2 Immunogenicity, blocking IgG antibody capacity and reactogenicity of bivalent mRNA-pDp1-LNP/mRNA-Dp2K96A-LNP. (A) Immunization and bleeding schedule; (B) pDp1- and Dp2-specific IgG1 and IgG2a antibody titers; the dotted horizontal lines represent the lowest serum dilution tested. * $p < .05$; (C) Blocking capacity of specific IgG triggered by bivalent mRNA-LNP at week 6 and at 1/40 or 1/200 dilution. * $p < .05$; (D) Inhibition of RBL-SX38 cell degranulation by specific IgG induced by bivalent mRNA-LNP. RBL-SX38 cells, primed with five Der p 1- or Der p 2-positive sera, were activated with 0.01 µg/ml nDer p 1 or rDer p 2 preincubated or not with pooled mouse sera (preimmune or week 6) diluted 20 or 200 times. * $p < .05$; (E) IFNγ and IL-5 secretion by splenocytes from immunized mice restimulated with rpDp1 or rDp2. (F) Sensitization and challenge schedule to test the reactogenicity of mRNA-LNP; (G) Body temperature change (Mean and SEM) monitored by infrared thermometer for 60 min following the challenge. * $p < .05$; (H) Anaphylactic symptom score observed 20 min following the challenge. * $p < .05$; (I) Serum levels of mMCPT-1 (Mean and SD) measured before and 60 min following challenge. * $p < .05$. One representative of two similar experiments is shown. $N = 6$ animals per experimental group. p values were calculated using the Mann–Whitney t-test or Two-way ANOVA.

be safe. Future studies will aim to measure the efficacy of AIT protocols based on mRNA-encoding Der p 1 and Der p 2 in mouse models of HDM-induced allergic airway inflammation.[6]

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CONFLICT OF INTEREST

N.P. and D.W. are named as co-inventors on a patent describing the use of nucleoside-modified mRNA in lipid nanoparticles as a vaccine platform (WO 2016/176330 A1). P.J.C.L is employee at Acuitas Therapeutics. The remaining authors declare that they have no conflicts of interest.

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