VACCINES

A multivalent nucleoside-modified mRNA vaccine against all known influenza virus subtypes

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Seasonal influenza vaccines offer little protection against pandemic influenza virus strains. It is difficult to create effective prepandemic vaccines because it is uncertain which influenza virus subtype will cause the next pandemic. In this work, we developed a nucleoside-modified messenger RNA (mRNA)–lipid nanoparticle vaccine encoding hemagglutinin antigens from all 20 known influenza A virus subtypes and influenza B virus lineages. This multivalent vaccine elicited high levels of cross-reactive and subtype-specific antibodies in mice and ferrets that reacted to all 20 encoded antigens. Vaccination protected mice and ferrets challenged with matched and mismatched viral strains, and this protection was at least partially dependent on antibodies. Our studies indicate that mRNA vaccines can provide protection against antigenically variable viruses by simultaneously inducing antibodies against multiple antigens.

There are at least 18 different influenza A virus (IAV) subtypes that circulate in animal reservoirs, and these viruses occasionally enter the human population and cause a pandemic (*1*). Currently, H1N1 and H3N2 IAVs, as well as one or two antigenically distinct lineages of influenza B viruses (IBVs), circulate seasonally in the human population. Although surveillance programs and modeling studies have increased our knowledge of pandemic risk (*2*, *3*), we cannot accurately predict which influenza subtype will cause the next pandemic. Several universal influenza vaccines are in development to provide protection against diverse influenza virus subtypes (*4*). Most universal influenza vaccines include a limited number of antigens that have epitopes that are conserved across different influenza virus subtypes (*5*–*7*). An alternative approach for inducing universal immunity is to design multivalent vaccines that encode antigens from every known influenza virus subtype. This approach may be impractical using conventional influenza vaccine technologies but is now feasible with nucleic acid–based vaccine platforms (*8*). We had previously developed nucleoside-modified mRNA–lipid nanoparticle (LNP) vaccines expressing hemagglutinin (HA) antigens from single influenza virus subtypes and had found that these vaccines elicit antibodies against both the HA head and stalk in mice and ferrets (*9*, *10*). In this study, we generated a nucleoside-modified mRNA-LNP vaccine expressing HA antigens from all known influenza virus subtypes and found that this multivalent vaccine elicits diverse antibodies that protect mice and ferrets against matched and mismatched viral strains.

We prepared 20 different HA-encoding nucleoside-modified mRNAs encapsulated in LNPs as previously described (*9*), ensuring that a representative HA from each IAV subtype and IBV lineage was included (fig. S1, A to C). We vaccinated groups of mice intramuscularly with a low dose (3 μg) of each individual HA mRNA vaccine to verify that each mRNA vaccine component was immunogenic. Each individual HA mRNA vaccine elicited antibodies that reacted more efficiently to the encoded HA compared with other HAs that we tested (fig. S1D). There was a low level of cross-reactivity among antibodies elicited by single-HA mRNA vaccinations, which is consistent with our previous work (*9*) that has demonstrated that higher doses of vaccines are required to elicit antibodies targeting conserved epitopes, such as the HA stalk.

We then vaccinated mice with all 20 HA mRNA-LNPs simultaneously with a combined dose of 50 μg of HA mRNA (2.5 μg of each individual HA mRNA-LNP). As controls, we vaccinated mice with a 50-μg dose of mRNA-LNPs encoding single HAs from H1N1, H3N2, IBV, or mRNA-LNPs expressing luciferase. Mice vaccinated with the 20-HA mRNA-LNPs produced antibodies that reacted to all 20 encoded HAs (Fig. 1A and fig. S2) [$P < 0.05$, comparing vaccinated versus phosphate-buffered saline (PBS) controls], whereas mice vaccinated with single-HA mRNA-LNPs (Fig. 1, B to D), PBS (Fig. 1E), or mRNA-LNPs expressing luciferase (fig. S3) did not. Antibody levels in mice immunized with the 20-HA mRNA-LNP vaccine remained largely unchanged 4 months post-vaccination (Fig. 1F). We created a vaccine containing 50 μg of 20 different recombinant HA proteins (2.5 μg per protein) so that we could compare the 20-HA mRNA-LNP vaccine with a more conventional protein vaccine platform. Mice immunized with the multivalent protein vaccine produced low levels of anti-HA antibodies (fig. S4). We also tested the 20-HA mRNA-LNP vaccine in mice that were previously infected with either antigenically matched or mismatched H1N1 viruses (fig. S5). Animals infected with H1N1 viruses had antibodies that reacted to H1 and other group 1 HAs before vaccination (fig. S5, A to C). H1 antibodies in preexposed animals were boosted by the 20-HA mRNA-LNP vaccine, but this did not come at the expense of generating de novo antibody responses against the other HA components (fig. S5, D to F). Thus, the 20-HA mRNA-LNP vaccine elicits high levels of antibodies against all 20 encoded HAs in mice with and without prior exposures to influenza virus.

We completed absorption assays to determine the level of cross-subtype reactivity of antibodies elicited by vaccination. mRNA-LNPs encoding single HAs elicited antibodies that were efficiently depleted by beads coupled with the corresponding encoded HA (Fig. 2, A to C). H1-coupled beads efficiently depleted H1-reactive antibodies, and H3-coupled beads efficiently depleted H3-reactive antibodies in the serum of mice vaccinated with the 20-HA mRNA-LNP vaccine, but these absorptions did not substantially decrease the binding of antibodies reactive to other HAs in our testing panel (Fig. 2D). Thus, the 20-HA mRNA-LNP vaccine elicits antibodies that are reactive to distinct HAs rather than purely cross-reactive antibodies capable of recognizing all HA subtypes. The 20-HA mRNA-LNP vaccine elicited group 1 (H1N1 and H5N1) and group 2 (H3N2 and H7N9) neutralizing antibodies (Fig. 2, E to H) as well as group 1 and 2 HA stalk–reactive antibodies (Fig. 2, I and J). As expected, H1 and H3 neutralizing antibodies were elicited at lower levels in mice receiving the 20-HA mRNA-LNP vaccine (which contained only 2.5 μg of H1 mRNA and 2.5 μg of H3 mRNA) compared with mice receiving 50 μg of H1 or H3 mRNA-LNPs (Fig. 2, E and F). Thus, mRNA vaccines can successfully deliver at least 20 distinct HA antigens that elicit antibodies targeting both variable and conserved epitopes.

We challenged mice 28 days after vaccination with an H1N1 virus (A/California/07/2009) that was similar (97.2% HA amino acid homology) to the H1 component of the vaccine

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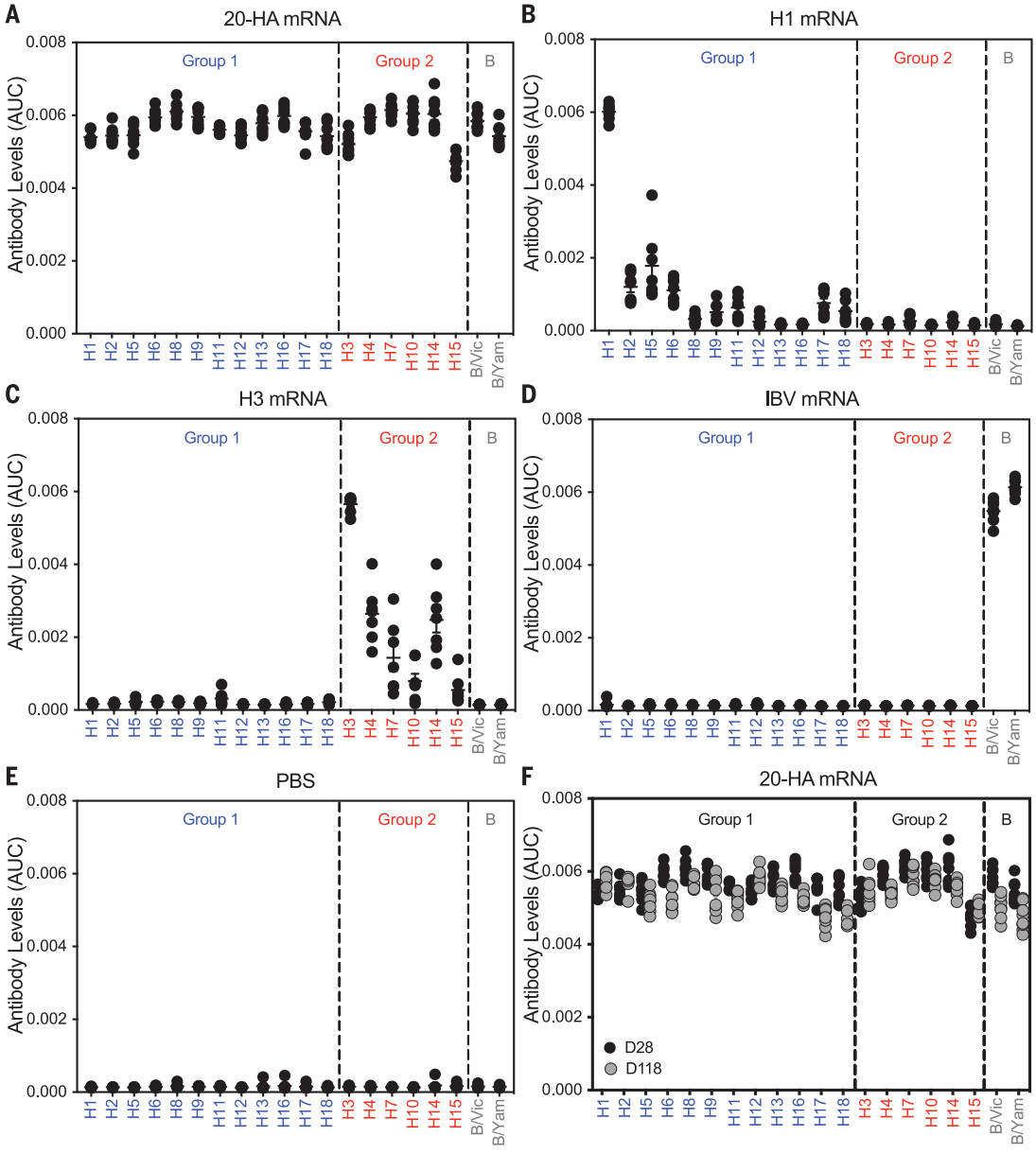

Fig. 1. The 20-HA mRNA-LNP vaccine elicits long-lived antibody responses that react to all 20 HAs. (**A**) Mice were simultaneously vaccinated intramuscularly (i.m.) with 20 different HA mRNA-LNPs (a combined total dose of 50 μg of mRNA-LNP, including 2.5 μg of each individual HA mRNA-LNP). AUC, area under the curve. (**B** to **E**) Other groups of mice were vaccinated i.m. with 50 μg of H1 mRNA-LNP (B), 50 μg of H3 mRNA-LNP (C), 50 μg of IBV HA mRNA-LNP (D), or PBS (E). Sera were collected 28 days (D28) [(A) to (E)] or 118 days (**F**) later, and antibody reactivities to different HAs were quantified using enzyme-linked immunosorbent assays (ELISAs) coated with recombinant proteins. Seven or eight mice were included for each experimental group, and in some instances, data points overlap. Group 1 HAs are shown in blue, and group 2 HAs are shown in red. Data are representative of two independent experiments and are shown as means ± SEMs. Raw ELISA data curves are shown in fig. S2.

(A/Michigan/45/2015) or an H1N1 virus (A/Puerto Rico/08/1934) that was antigenically distinct (81.8% HA amino acid homology) compared with the H1 component of the vaccine. Antibodies elicited by the 20-HA mRNA-LNP vaccine and the H1 mRNA-LNP vaccine bound to HAs from A/California/07/2009 and A/Puerto Rico/08/1934 (fig. S6, A and B) but only neutralized the A/California/07/2009 virus (fig. S6, C and D). Mice vaccinated with H3 mRNA-LNP, IBV mRNA-LNP, or an mRNA-LNP expressing luciferase rapidly lost weight, displayed severe clinical signs of disease, and died between 7 and 9 days after infection with either A/California/07/2009 (Fig. 3, A to C) or A/Puerto Rico/8/1934 (Fig. 3, F and G). Mice vaccinated with H1 mRNA-LNP or the 20-HA mRNA-LNP did not lose as much weight after infection with A/California/07/2009 (Fig. 3A), displayed few clinical signs of disease (Fig. 3B), and survived the viral challenge (Fig. 3C). A/California/07/2009 viral titers in the lungs of mice vaccinated with H1 mRNA-LNP or the 20-HA mRNA-LNP were undetectable 2 and 5 days after infection (Fig. 3, D and E). Mice vaccinated with H1 mRNA-LNP or the 20-HA mRNA-LNP initially lost weight after infection with the mismatched A/Puerto Rico/08/1934 virus (Fig. 3F), displayed clinical signs of disease (Fig. 3G), but then began recovering 7 to 8 days after infection (Fig. 3G), and most of these mice survived (Fig. 3H). A/Puerto Rico/08/1934 viral titers in the lungs were similar between the experimental groups 2 and 5 days after infection (Fig. 3, I and J). Thus, the 20-HA mRNA-LNP vaccine provided mice different degrees of protection against matched and mismatched viral strains.

To determine whether the 20-HA mRNA-LNP vaccine requires all 20 HA components, we vaccinated mice with a combination of every HA mRNA-LNP except H1 mRNA-LNP (19-HA mRNA-LNP vaccine) and then challenged these animals with A/California/07/2009 or A/Puerto Rico/08/1934 H1N1 viruses (fig. S7). Mice vaccinated with the multivalent 19-HA mRNA-LNP lacking the H1 mRNA-LNP rapidly lost weight (fig. S7A), displayed clinical signs of disease (fig. S7B), and frequently died (fig. S7C) after infection with A/California/07/2009 H1N1. This suggested that the H1 component of the 20-HA mRNA-LNP vaccine was critically important for eliciting protective responses against the A/California/07/2009 H1N1 strain. Mice vaccinated with the 19-HA mRNA-LNP lost weight (fig. S7A), displayed some clinical signs of disease (fig. S7B), but

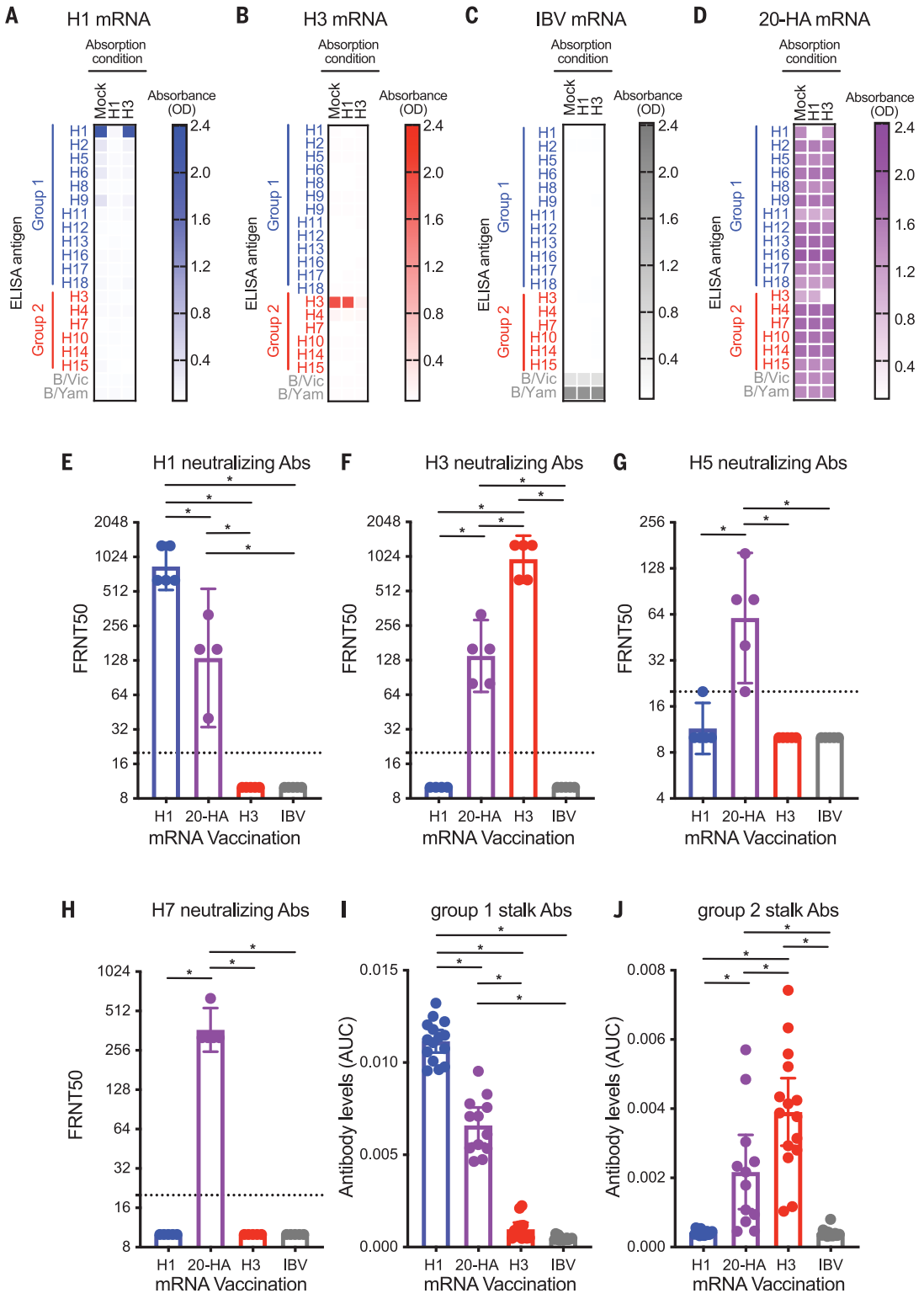

Fig. 2. The 20-HA mRNA-LNP vaccine elicits diverse antibodies targeting both conserved and variable epitopes. (**A** to **D**) Serum samples were collected from mice 28 days after H1 (A), H3 (B), IBV (C), or 20-HA (D) mRNA-LNP vaccination. Samples were absorbed with magnetic beads coupled to recombinant H1, H3, or no HA (mock), and antibody levels remaining in the unabsorbed fraction were quantified by ELISA (A to D). OD, optical density. (**E** to **H**) Focus reduction neutralization tests (FRNTs) were completed using A/Michigan/45/2015 H1 (E), A/Singapore/INFIMH-16-0019/2016 H3 (F), A/Vietnam/1203/2004 H5 (G), or A/Shanghai/02/1013 H7 (H). Titers are reported as the inverse of the highest dilutions of serum amount required to inhibit 50% of virus infections. Abs, antibodies. (**I** and **J**) HA stalk–reactive antibodies were quantified by ELISA using "headless" group 1 (H1) (I) and group 2 (H3) (J) recombinant proteins. (A to H) Six mice were included for each experimental group. (I and J) Twelve mice were included for each experimental group. Data are representative of two or three independent experiments. Data in (A) to (D) are shown as means. Data in (E) to (H) are shown as geometric means ± 95% confidence intervals (CIs), and values were log-transformed before statistical analysis. Data in (I) and (J) are shown as means ± 95% CIs. Data in (E) to (J) were compared using a one-way analysis of variance (ANOVA) with Tukey's post hoc test. *$P < 0.05$.

survived after A/Puerto Rico/08/1934 infection (fig. S7C). Although the immunological basis of this finding is unclear, the HA of A/Puerto Rico/08/1934—but not that of A/California/07/2009—likely shares a conserved epitope with a non-H1 immunogen in the 20-HA mRNA-LNP vaccine.

To further probe the mechanism(s) by which the 20-HA mRNA-LNP vaccine provides protection against different H1N1 virus strains, we depleted $CD4^+$ and $CD8^+$ T cells from mice vaccinated with the 20-HA mRNA-LNP vaccine and then challenged these animals with A/California/07/2009 or A/Puerto Rico/08/1934 (fig. S8, A to D). Vaccinated mice lacking $CD4^+$ and $CD8^+$ T cells had similar survival rates compared with vaccinated mice with intact T cells. Mice that received a passive transfer of serum from 20-HA mRNA-LNP–vaccinated mice survived A/California/07/2009 H1N1 infection but were not fully protected

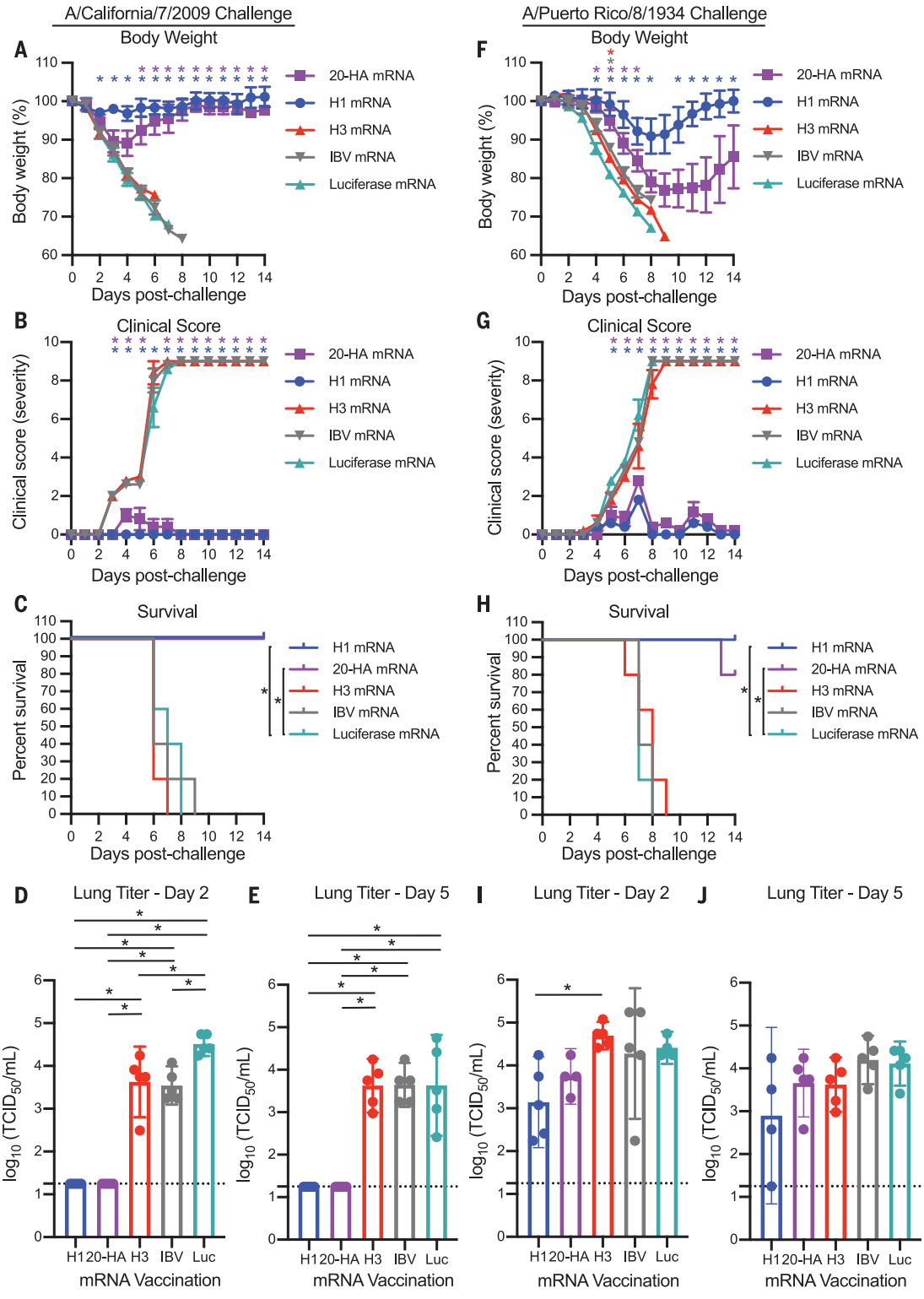

Fig. 3. The 20-HA mRNA-LNP vaccine protects mice from challenge with antigenically matched and mismatched distinct H1N1 strains. Mice were vaccinated with mRNA-LNPs encoding H1 (blue), H3 (red), IBV (gray), luciferase (Luc) (green), or 20 HAs (purple). Twenty-eight days later, they were infected intranasally (i.n.) with A/California/7/2009 [5 median lethal doses (LD_{50})] or A/Puerto Rico/8/1934 H1N1 (2 LD_{50}) influenza virus. (**A** to **C**) Weight loss (A), clinical scores (B), and survival (C) were monitored for 14 days after A/California/7/2009 infection. (**D** and **E**) Virus levels in lung homogenate samples isolated 2 days (D) and 5 days (E) after infection were quantified using median tissue culture infectious dose ($TCID_{50}$) assays. (**F** to **H**) Weight loss (F), clinical scores (G), and survival (H) were monitored for 14 days after A/Puerto Rico/8/1934 H1N1 infection. (**I** and **J**) Virus levels in lung homogenate samples isolated 2 days (I) and 5 days (J) after infection were quantified using $TCID_{50}$ assays. Horizontal dotted lines in (D), (E), (I), and (J) denote limit of detection of the assay, and samples with no detectable titers were assigned a titer at this limit of detection. Five mice were included per group. Data in (A), (B), (F), and (G) are shown as means ± SEMs and were analyzed by mixed-model ANOVA with Greenhouse-Geisser correction and Sidak's multiple comparisons test. The homologous viral challenge [(A) to (E)] was performed once. The heterologous viral challenge [(F) to (J)] was repeated at 3 months post-vaccination (instead of 28 days postvaccination) with similar results. The viral titers shown in (D), (E), (I), and (J) are from one experiment. For animals that died, their weight on the day before death was carried forward for statistical analyses. Differences compared with luciferase mRNA vaccination are indicated in (A), (B), (F), and (G); $*P < 0.05$. Data in (C) and (H) were analyzed using a log rank test; $*P < 0.05$. Data in (D), (E), (I), and (J) are shown as means ± 95% CIs, and titers were compared using a one-way ANOVA with Tukey's post hoc test; $*P < 0.05$.

against A/Puerto Rico/08/1934 H1N1 infection (fig. S8, E and F). We hypothesized that antibodies elicited by the 20-HA mRNA-LNP vaccine contribute to protection against mismatched viral strains through nonneutralizing mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) (*11*). Antibodies elicited by the 20-HA mRNA-LNP vaccine efficiently mediated ADCC with cells expressing either matched or mismatched HAs (fig. S9). Antibodies elicited by the H1 mRNA-LNP vaccine were able to mediate ADCC with cells expressing a mismatched H1 antigen but not with a matched H1 antigen (fig. S9). This was likely because the H1 mRNA-LNP

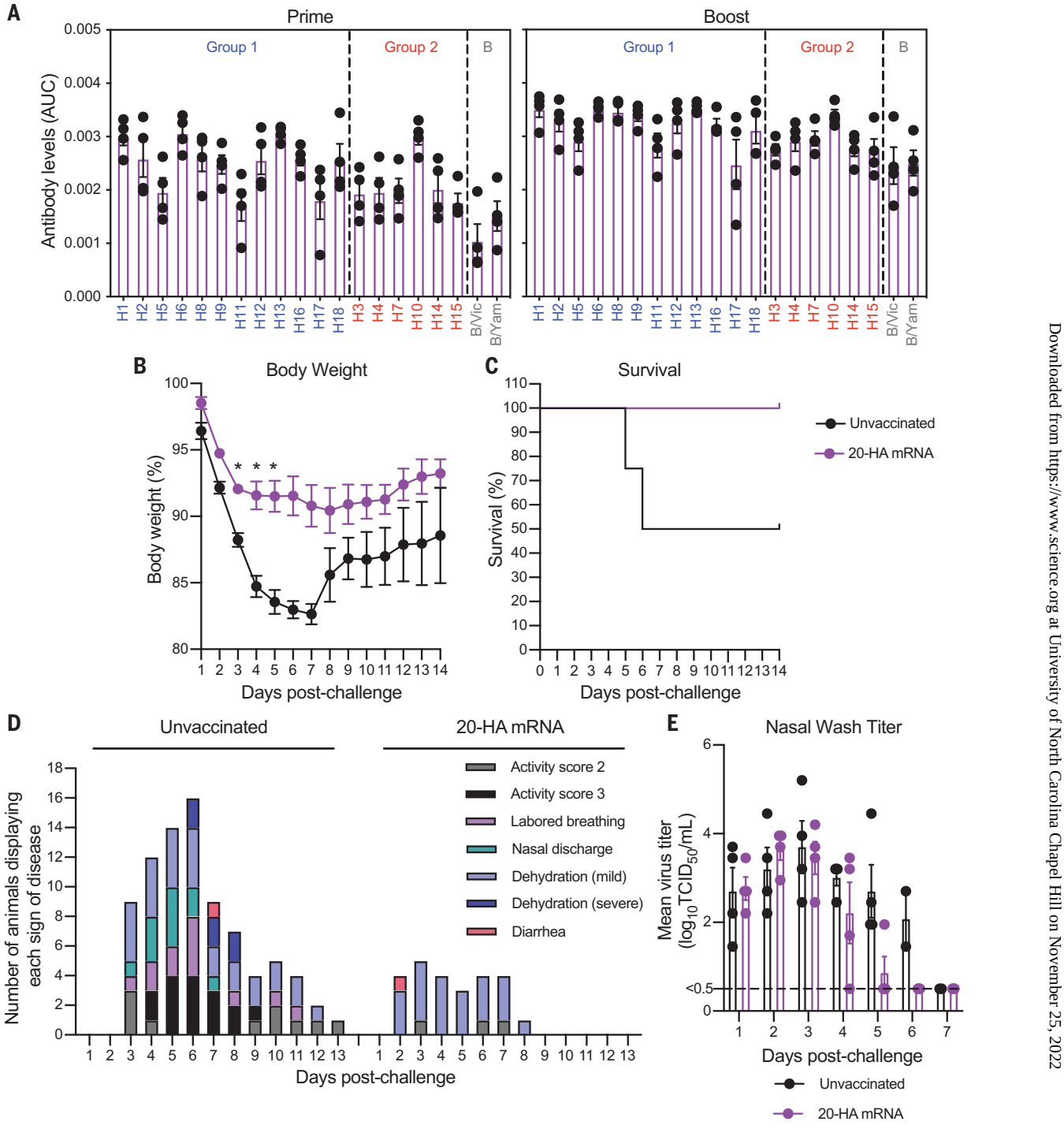

Fig. 4. Twenty-HA mRNA-LNP vaccination protects ferrets from challenge with an antigenically distinct H1N1 strain. Ferrets were primed with 60 μg of the 20-HA mRNA-LNP vaccine (3 μg of each HA mRNA-LNP) and were then boosted with the same vaccine dose 28 days later. (**A**) Sera were collected 28 days after the first and second vaccinations, and antibody reactivities to different HAs were quantified using ELISAs coated with recombinant proteins. Twenty-eight days after the second vaccination, ferrets were infected i.n. with 10^6 $TCID_{50}$ of A/Ruddy turnstone/Delaware/300/2009 H1N1 influenza virus. As a control, unvaccinated animals were also infected with the virus. (**B** to **D**) Weight loss (B), survival (C), and signs of disease (D) were monitored for 14 days after infection. The same animal could make multiple contributions to the graph in (D). (**E**) Virus levels in nasal wash samples isolated 1 to 7 days after infection were quantified using $TCID_{50}$ assays. The horizontal dashed line indicates limit of detection. Four ferrets were included for each experimental group, and the experiment was performed once. Data shown are means ± SEMs [(A), (B), and (E)]. Data in (B) and (E) are shown as means ± SEMs and were analyzed by mixed-model ANOVA with Greenhouse-Geisser correction and Sidak's multiple comparisons test; *P < 0.05. For animals that died, their weight on the day before death was carried forward for statistical analyses. Data in (C) were analyzed using a Mantel-Cox log rank test.

vaccine elicited high levels of H1N1 neutralizing antibodies (fig. S6), which have previously been found to inhibit ADCC activity mediated by nonneutralizing antibodies (*12*, *13*).

Finally, we completed a prime-boost vaccination experiment in ferrets to mimic the dosing schedule initially used for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines (*14*, *15*). Each ferret produced antibodies reactive to all 20 HAs after a single vaccination, and antibody levels increased after a booster vaccination delivered 28 days later (Fig. 4A). We challenged vaccinated and unvaccinated ferrets with an avian H1N1 virus (A/Ruddy turnstone/Delaware/300/2009) that was distinct from the H1 HA that was included in the vaccine (81.8% HA amino acid homology with vaccine H1 component) to mimic a pandemic featuring an unknown viral strain. Unvaccinated animals lost >16% of their initial weight by 5 days after infection and two out of four animals died, whereas vaccinated ferrets lost only ~8.5% of their initial weight by 5 days after infection and all animals survived (Fig. 4, B and C). Unvaccinated animals displayed more clinical signs of disease relative to vaccinated animals after infection (Fig. 4D). Viral titers in nasal washes were similar in unvaccinated and vaccinated animals at days 1 to 4 after infection, but the virus was cleared more efficiently in vaccinated animals at days 5 and 6 after infection (Fig. 4E). Thus, the 20-HA mRNA-LNP vaccine protected ferrets against an antigenically mismatched avian H1N1 virus.

In this Report, we present an alternative strategy for inducing universal immunity against distinct influenza virus strains. We had previously demonstrated that nucleoside-modified mRNA-LNP vaccines expressing HA and conserved influenza virus antigens are immunogenic in mice (*9*, *14*). Instead of focusing on immunogens to elicit antibodies against epitopes that are conserved among many different influenza virus strains, we designed a vaccine that encodes separate immunogens from all known IAV subtypes and IBV lineages. Previous studies have shown that cocktails of virus-like particles encoding antigens from four different influenza virus subtypes are immunogenic in mice when delivered intranasally (*16*). We found that antigens from at least 20 distinct influenza viruses can be simultaneously delivered through mRNA-LNPs. The production and standardization of different antigens expressed by mRNA-LNP vaccines is simpler compared with other vaccine approaches (*17*, *18*) and there may be specific properties of mRNA vaccines that allow for the induction of immune responses to multiple antigens without noticeable immunodominance biases, even in the context of preexisting immune responses. For example, we had previously reported that mRNA-LNP vaccines induce long-lived germinal center reactions in mice (*19*), a finding that has recently been found to occur in SARS-CoV-2 mRNA–vaccinated humans as well (*20*, *21*). Long-lived germinal centers may facilitate the simultaneous induction of immune responses against multiple epitopes, including epitopes that are usually subdominant.

Further studies will be required to fully elucidate the mechanisms by which the 20-HA mRNA vaccine provides protection. Our present findings suggest that protection against antigenically matched strains is mediated by neutralizing antibodies, whereas protection against mismatched viral strains may occur through nonneutralizing mechanisms, such as ADCC. Over the course of our studies, we used antigenically matched as well as antigenically mismatched challenge strains to mimic the emergence of a novel pandemic influenza virus strain. It is likely that mRNA influenza vaccines that are imperfectly matched to novel pandemic influenza virus strains will not provide sterilizing immunity but will instead limit disease severity and protect against death through nonneutralizing mechanisms. A similar phenomenon may be occurring with SARS-CoV-2 variant infections in humans immunized with SARS-CoV-2 mRNA vaccines that were developed using spike sequences obtained from viral strains isolated early in the pandemic. In most cases, symptoms and severity are greatly reduced and virus is cleared faster in vaccinated individuals infected with antigenically drifted SARS-CoV-2 variants (*22*, *23*).

Our overall approach will likely be useful for infectious diseases other than influenza viruses. Multivalent mRNA-LNP vaccines may be applied against other variable pathogens, such as coronaviruses and rhinoviruses. For example, SARS-CoV-2 mRNA vaccines are being updated to include multiple spike components to combat antigenically distinct strains (*24*). Additional studies will be required to determine the maximum number of antigens that can be simultaneously delivered through mRNA-LNP vaccines and the underlying immunological mechanisms that allow for the induction of responses against multiple antigens.

REFERENCES AND NOTES

1. F. Krammer *et al.*, *Nat. Rev. Dis. Primers* **4**, 3 (2018).
2. M. I. Nelson *et al.*, *Epidemics* **26**, 116–127 (2019).
3. W. N. Harrington, C. M. Kackos, R. J. Webby, *Exp. Mol. Med.* **53**, 737–749 (2021).
4. E. J. Erbelding *et al.*, *J. Infect. Dis.* **218**, 347–354 (2018).
5. H. M. Yassine *et al.*, *Nat. Med.* **21**, 1065–1070 (2015).
6. K. S. Corbett *et al.*, *mBio* **10**, e02810-18 (2019).
7. R. Nachbagauer *et al.*, *Nat. Med.* **27**, 106–114 (2021).
8. B. S. Graham, J. R. Mascola, A. S. Fauci, *JAMA* **319**, 1431–1432 (2018).
9. N. Pardi *et al.*, *Nat. Commun.* **9**, 3361 (2018).
10. E. Willis *et al.*, *Sci. Transl. Med.* **12**, eaav5701 (2020).
11. D. J. DiLillo, G. S. Tan, P. Palese, J. V. Ravetch, *Nat. Med.* **20**, 143–151 (2014).
12. W. He *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11931–11936 (2016).
13. P. E. Leon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **113**, E5944–E5951 (2016).
14. A. W. Freyn *et al.*, *Mol. Ther.* **28**, 1569–1584 (2020).
15. E. J. Topol, *Cell* **184**, 1401 (2021).
16. L. M. Schwartzman *et al.*, *mBio* **6**, e01044-15 (2015).
17. N. Pardi, M. J. Hogan, D. Weissman, *Curr. Opin. Immunol.* **65**, 14–20 (2020).
18. N. Pardi, M. J. Hogan, F. W. Porter, D. Weissman, *Nat. Rev. Drug Discov.* **17**, 261–279 (2018).
19. N. Pardi *et al.*, *J. Exp. Med.* **215**, 1571–1588 (2018).
20. J. S. Turner *et al.*, *Nature* **595**, 421–425 (2021).
21. K. Lederer *et al.*, *Cell* **185**, 1008–1024.e15 (2022).
22. A. Sheikh, J. McMenamin, B. Taylor, C. Robertson, Public Health Scotland and the EAVE II Collaborators, *Lancet* **397**, 2461–2462 (2021).
23. J. Lopez Bernal *et al.*, *N. Engl. J. Med.* **385**, 585–594 (2021).
24. G. McLean *et al.*, *mBio* **13**, e02979-21 (2022).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S9
References (*25*–*39*)
MDAR Reproducibility Checklist
Data S1

View/request a protocol for this paper from *Bio-protocol*.

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Science

A multivalent nucleoside-modified mRNA vaccine against all known influenza virus subtypes

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A cornucopia of antigens

Vaccines serve as an indispensable tool for the control and prevention of influenza, but several challenges remain. Some populations, for example, the elderly, respond poorly to vaccination. Furthermore, the highly variable nature of influenza viruses can make targeting optimal antigens difficult. Broadly neutralizing antibodies have been proposed as a solution to such disadvantages, but they present their own pitfalls, including limited cross-reactivity to both influenza A and B strains and the need for repeated injections during flu season. Arevalo *et al*. developed a nucleoside-modified messenger RNA-lipid nanoparticle vaccine encoding hemagglutinin antigens from all 20 known influenza A and B virus subtypes (see the Perspective by Kelvin and Falzarano). Such vaccines may provide protection against antigenically variable viruses by simultaneously inducing antibodies against multiple antigens. —STS

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