PNAS COMMENTARY OPEN ACCESS

mRNA, the beginning of a new influenza vaccine game

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Licensed seasonal influenza vaccination results in variable effectiveness, and the substantial residual medical need calls for novel vaccines to induce better and broader immunity against this everchanging virus. In PNAS, McMahon et al. (1) report a new quadrivalent vaccine containing lipid nanoparticle (LNP)-formulated nucleoside-modified messenger RNA (mRNA). This vaccine codes for headless hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), and matrix-2 ion channel (M2) antigens and protects mice against a range of influenza strains.

Vaccine-Induced Protective Immunity to Influenza Virus

Influenza virus, one of the most common respiratory pathogens, is still a global public health concern. Every year, 3–5 million severe flu cases are estimated to occur (2), with high incidence of medically attended illness in all age groups (3). Periodically, influenza viruses cause pandemics infecting billions of people and causing millions of deaths. Influenza vaccines made by inactivated egg-grown viruses have been available for over 60 y. Recently, vaccines made by cell culture grown viruses, recombinant vaccines, and adjuvanted vaccines have also been licensed. They are recommended for all ages in a quadrivalent formulation representative of two A and two B virus subtypes (2).

Current vaccines primarily target the viral surface glycoprotein HA, characterized by an extensive mutation rate to counteract immune pressure (antigenic drift) and by the genetic reassortment between different strains (antigenic shift). The level of antibody response to HA has been identified as correlate of protection. Vaccines also elicit immunity toward a second less abundant surface antigen, NA, which evolves and drifts independently of HA. Given the constant immune escape, influenza vaccines need to be updated annually to antigenically match the circulating strains. When a new strain emerges, either from evolution or zoonosis, with no antibody-mediated protection in the population, it can result in devastating influenza pandemics, such the one caused by the 1918 H1N1 virus with over 40 million deaths (4). Preexisting immunity also profoundly affects protection to new influenza viruses. The evidence of immune imprinting, or original antigenic sin, suggests that memory B cells generated during the first encounter with HA lead to the recall of antibody-producing B cells to strain-specific epitopes even during subsequent encounters with different virus strains later in life. The boosting of antibodies to nonprotective epitopes effectively limits the capacity of the immune system to mount immunity to new epitopes and impacts susceptibility to infection (5). Thus, improved antigen design and immune focusing to conserved antigens might improve vaccine efficacy. Production of vaccines is also not optimal. The experience with 2009 pandemic H1N1 showed that vaccines were available in large quantities only after pandemic peak and only in high-income countries (6). Vaccine production technology was a bottleneck in 2009 and continues to be today. Of the licensed vaccines, most are still produced in eggs, limiting scalability and introducing differences between the viruses in the vaccine and the ones that are circulating.

In spite of the recent advances, the effectiveness of current generation vaccines is still limited, especially in the elderly where protection usually is in the 40 to 60% range, but it can be as low as 20% in case of strain mismatch. Mutations caused by adapting the viruses to grow in eggs, poor prediction of circulating strains, virus antigenic shifts, and the need to vaccinate people with imprinted preexisting nonprotective immunity are some of the causes of the poor performance of influenza vaccines. Clearly, we need better influenza vaccines.

mRNA Enters the Influenza Vaccine Game. Given the global success demonstrated during the COVID-19 pandemic, the mRNA platform is entering the influenza vaccine challenge with the potential to deliver a new generation of high-efficacy vaccines for multiple reasons. First, strain match may be more accurate because there will be no need to grow the virus in eggs. This can also improve manufacturing, because making mRNA is less cumbersome compared to recombinant technology, facilitating vaccine approval and distribution. Second, vaccine immunity may be better or broader because the viral proteins will be expressed at high fidelity by human cells likely preserving the natural structure. Third, mRNA makes it easier to incorporate a larger number of antigens, which may stimulate cellular immunity or expand protection beyond HA and NA.

mRNA influenza vaccines have been in the making for the past decade. The first clinical data of H10N8 and H7N9 vaccines in healthy adults achieved proof-of-concept. However, despite demonstrating safety and immunogenicity, only the higher vaccine doses induced high seroconversion rates: 100 μg dose induced hemagglutination inhibition titers in 100% and 50 μg dose in 89.7% participants of H10N8 and H7N9 studies, respectively (7). More recently, Moderna's quadrivalent flu vaccine mRNA-1010 successfully boosted antibody titers against all four strains in healthy adults. Also in this

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Author contributions: S.P. and R.R. wrote the paper.

Competing interest statement: S.P. is a full time employee and stockholder of the GSK group of companies. R.R. is stockholder of Novartis and the GSK group of companies.

See companion article, "Assessment of a quadrivalent nucleoside-modified mRNA vaccine that protects against group 2 influenza viruses," 10.1073/pnas.2206333119.

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Published December 5, 2022.

case, however, seroconversion was not robust across the strains, with lower influenza B neutralizing antibody titers (2–3-fold increase) as compared to influenza A (6–10-fold increase) and lower immunogenicity in older adults (8). This suggests that, in comparison with current technologies (9), mRNA vaccines containing only HA might not be particularly better than other influenza vaccines. Pfizer/BioNTech also released preliminary clinical data of their quadrivalent mRNA vaccine, reporting ≥twofold expansion of CD4 and CD8 T cells in older adults against all four vaccine strains (10). This, on the contrary, could be an advantage of the mRNA platform. As previously reported for SARS-CoV-2 vaccines, T cell response elicited by mRNA were less susceptible to viral immune escape and contributed to protect from severe disease (11). Both Moderna and Pfizer have begun phase 3 clinical trials of their influenza vaccines. Other companies working on mRNA influenza vaccines, Sanofi/Translate Bio and GlaxoSmithKline (GSK)/CureVac, both in phase 1, have not released preliminary data yet.

> **"In PNAS, McMahon et al. (1) report a novel vaccination approach and show that a single dose of a quadrivalent vaccine containing nucleoside modified mRNA coding for a headless HA, NA, NP and the M2 antigen formulated with LNP protects mice from weight loss and death against a range of influenza strains."**

Aside from expressing HA from seasonal strains, mRNA technology allows the combination of multiple antigens. In mice, a combination of conserved H1N1 antigens (HA stalk, NA, M2, and NP) induced strong breadth and potency, protecting animals from challenge with a panel of influenza A group 1 viruses (12). In this PNAS issue, McMahon et al. expanded this approach to a quadrivalent influenza A group 2 mRNA formulation. Mice were protected from a broad panel of influenza A viruses, with improved protection of quadrivalent compared to monovalent formulations. Interestingly, HA stalk immunogenicity was low in this model, requiring prime-boost regimen, while NP consistently improved mice survival, likely due to elicitation of protective T cell immunity (1).

Alternative Approaches and Challenges for Broad Application. In an effort to develop broadly protective vaccines, elicitation of high-affinity antibodies to conserved epitopes not subject to antigen drift could allow pan-influenza or universal vaccination, to protect against emergent pandemic or zoonotic strains. In particular, the stem (or stalk) region of HA, included in McMahon's study, is highly conserved, immuno subdominant, and recognized by broadly protective antibodies (13). Efforts to induce such protective responses led to the development of chimeric adjuvanted vaccines, which by sequential vaccination elicited anti-stalk antibodies in humans previously primed to group 1 HA (14). In another study, H1 and H2 stalk antibodies were highest in the AS03 adjuvanted group as compared to AS01 and nonadjuvanted groups and absent in the inactivated quadrivalent vaccine control group. These results suggest that optimal elicitation of broadly reactive antibodies in humans might require careful prime-boost evaluation and that adjuvant might be an important contributor. Taking advantage of the plasticity of the mRNA platform, multiple immunization strategies could be tested in humans, rapidly advancing this concept. Moreover, mRNA LNP adjuvanticity is currently being investigated (15), and finetuning LNP-induced innate immune responses could be used to improve HA stalk immunogenicity, although lack of adjuvant in mRNA formulation might be a limitation.

Immune response to influenza vaccines can also be improved by multimerization of HA. When the four HAs of licensed quadrivalent influenza vaccines were codisplayed on nanoparticles, antibody responses in animals against vaccine-matched strains were equivalent to or better than commercial quadrivalent vaccines and simultaneously induced broadly protective antibodies to heterologous viruses by targeting the HA stem (16). When tested in humans exposed only to group 1 influenza viruses, group 2 nanoparticle vaccine elicited broadly neutralizing antibodies to seasonal H1 and avian H5 subtypes by targeting the HA stem (17). These results suggest that optimal antigen presentation could improve antibody-mediated immunity limiting antigenic shift and the effects of original antigenic sin. mRNA was previously used to express SARS-CoV-2 nanoparticle vaccines eliciting broadly neutralizing antibodies in mice (18), suggesting that this could be applied to influenza vaccines as well.

Conclusions

Overall, mRNA technology holds the promise to change influenza vaccination by improving strain match, vaccine production, and immunity to additional antigens in high-valent formulations to cover additional viral strains and improve cell-mediated immunity (Fig. 1). However, available data suggest that immunogenicity of mRNA-expressed HA could be improved, and alternative technologies demonstrated that immunity to subdominant conserved HA domain might require careful antigen design, multimerization on nanoparticles, and use of adjuvants in order to escape the original antigenic sin. Thus, mRNA influenza vaccines might require additional improvement to truly revolutionize current and recombinant protein vaccines. This might be the beginning of a vaccine revolution driven by mRNA technology, but the game has just started.

ACKNOWLEDGMENTS. This work was sponsored by GlaxoSmithKline Biologicals SA. The authors wish to thank GiorgioCorsi for the artwork.

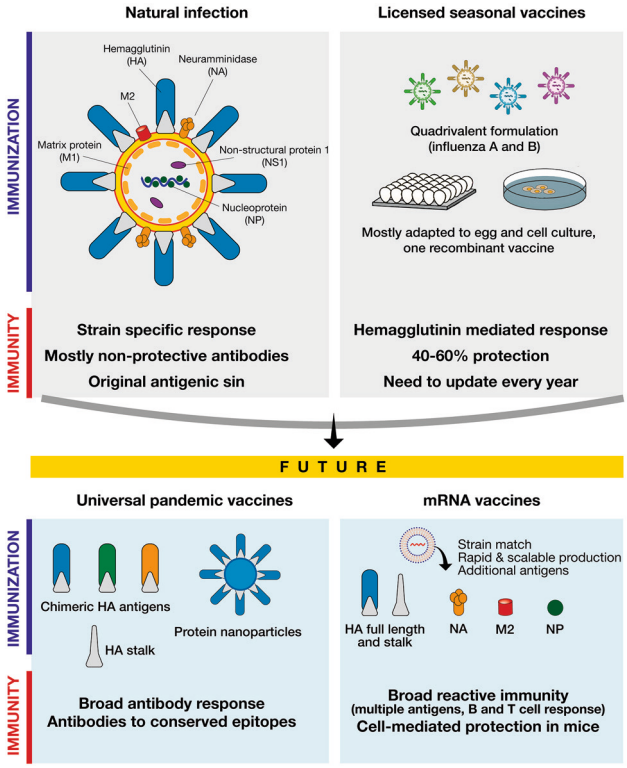

Fig. 1. From current influenza vaccine strategies to future universal and mRNA approaches.

1. M. McMahon *et al.*, Assessment of a quadrivalent nucleoside-modified mRNA vaccine that protects against group 2 influenza viruses. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2206333119 (2022).
2. World Health Organization (WHO), Influenza (seasonal). https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal). Accessed 16 October 2022.
3. M. L. Jackson *et al.*, Incidence of medically attended acute respiratory illnesses due to respiratory viruses across the life course during the 2018/19 influenza season. *Clin. Infect. Dis.* **73**, 802–807 (2021).
4. P. Palese, Influenza: Old and new threats. *Nat. Med.* **10**, S82–S87 (2004).
5. H. L. Dugan *et al.*, Preexisting immunity shapes distinct antibody landscapes after influenza virus infection and vaccination in humans. *Sci. Transl. Med.* **12**, eabd3601 (2020).
6. R. Rappuoli, P. R. Dormitzer, Influenza: Options to improve pandemic preparation. *Science* **336**, 1531–1533 (2012).
7. R. A. Feldman *et al.*, mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials. *Vaccine* **37**, 3326–3334 (2019).
8. Moderna Press Release, Moderna announces positive interim phase 1 data for mRNA flu vaccine and provides program update. December 10, 2021. https://investors.modernatx.com/news/news-details/2021/Moderna-Announces-Positive-Interim-Phase-1-Data-for-mRNA-Flu-Vaccine-and-Provides-Program-Update/default.aspx. Accessed 16 October 2022.
9. F. S. Dawood *et al.*, Comparison of the immunogenicity of cell culture-based and recombinant quadrivalent influenza vaccines to conventional egg-based quadrivalent influenza vaccines among healthcare personnel aged 18–64 years: A randomized open-label trial. *Clin. Infect. Dis.* **73**, 1973–1981 (2021).
10. Pfizer, Second quarter 2022 earnings teleconference. July 28, 2022. https://s28.q4cdn.com/781576035/files/doc_financials/2022/q2/Q2-2022-Earnings-Charts-FINAL.pdf. Accessed 16 October 2022.
11. E. J. Wherry, D. H. Barouch, T cell immunity to COVID-19 vaccines. *Science* **377**, 821–822 (2022).
12. A. W. Freyn *et al.*, A multi-targeting, nucleoside-modified mRNA influenza virus vaccine provides broad protection in mice. *Mol. Ther.* **28**, 1569–1584 (2020).
13. D. C. Ekiert *et al.*, Antibody recognition of a highly conserved influenza virus epitope. *Science* **324**, 246–251 (2009).
14. R. Nachbagauer *et al.*, A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial. *Nat. Med.* **27**, 106–114 (2021).
15. M. G. Alameh *et al.*, Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. *Immunity* **55**, 1136–1138 (2022).
16. S. Boyoglu-Barnum *et al.*, Quadrivalent influenza nanoparticle vaccines induce broad protection. *Nature* **592**, 623–628 (2021).
17. K. V. Houser *et al.*, Safety and immunogenicity of a ferritin nanoparticle H2 influenza vaccine in healthy adults: A phase 1 trial. *Nat. Med.* **28**, 383–391 (2022).
18. W. Sun *et al.*, The self-assembled nanoparticle-based trimeric RBD mRNA vaccine elicits robust and durable protective immunity against SARS-CoV-2 in mice. *Signal Transduct. Target. Ther.* **6**, 340 (2021).