CLINICAL IMPLICATIONS OF BASIC RESEARCH

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An mRNA Influenza Vaccine — Could It Deliver?

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The development of a universal influenza vaccine that confers broad and durable protection against a diverse range of circulating and emerging influenza viruses is a long-standing goal for public health and pandemic preparedness.[1,2] Influenza A viruses are zoonotic viruses that infect multiple animal species and have the potential to spill over from the animal reservoir and cause human pandemics. The major target for protective antibody responses elicited by conventional influenza virus vaccines is hemagglutinin (HA), a surface glycoprotein critical for the attachment of the virus to the respiratory epithelium. However, there are 18 known influenza A HA subtypes (H1 to H18) and two influenza B HA subtypes. Developing a vaccine that protects against all 20 HA subtypes poses formidable technical, immunologic, and regulatory challenges. For context, conventional seasonal inactivated influenza vaccines include three (trivalent) or four (quadrivalent) HA subtypes and elicit largely strain-specific immune responses against the HA subtypes included in the vaccine. Unfortunately, seasonal vaccines provide little to no protection from new influenza subtypes and are therefore unsuited to pandemic preparedness.[1,2]

The Covid-19 pandemic has driven innovation in vaccine development, and mRNA technology rose to prominence as a critical public health intervention. Nucleic acid–based mRNA vaccines are composed of a synthetic mRNA template encoding a specific viral glycoprotein antigen (e.g., the spike protein of SARS-CoV-2) that is translated once the mRNA is delivered into a cell. To achieve efficient in vivo delivery, the mRNA is chemically modified to improve its stability, prevent its degradation, and enhance translation and then is encapsulated within a lipid nanoparticle (LNP). The rapid scalability of mRNA-based vaccines, in addition to their demonstrated safety profile, robust immunogenicity, and high efficacy, has permitted a nimble response to the Covid-19 pandemic. The ability to quickly incorporate new variants, as shown by the bivalent (ancestral virus plus B.1.1.529 [omicron]) formulations, is an additional strength of the platform.

A study by Arevalo and colleagues suggests that an mRNA vaccine against influenza is feasible (Fig. 1).[3] They designed a multivalent mRNA-based formulation incorporating the 20 known influenza virus HA subtypes. Intramuscular immunization of mice with this cocktail of mRNAs, each encoding a single full-length HA, successfully elicited high levels of binding antibodies against each HA regardless of whether the mice had previously been exposed to influenza virus. Testing in a subset of vaccinated mice showed neutralizing antibodies against homologous (matched) HAs. To determine the mechanism of the antibody response, the authors depleted antibodies that bind specifically to H1 or H3 HA in the serum of mice that had received the 20-HA mRNA-LNP vaccine. Depletion of the H1- or H3-reactive antibodies eliminated reactivity against H1 and H3, respectively, but did not substantially affect the binding of antibody to the other 19 HA subtypes. These data suggest that the 20-HA mRNA-LNP formulation induces strain-specific immune responses against each subtype rather than eliciting antibodies that are broadly cross-reactive against a range of HAs. This distinction is important, because other universal influenza vaccine strategies involve conserved influenza antigens, such as the HA stalk domain, for induction of cross-reactive immunity.[4]

Cross-reactive antibodies have a role in protecting against seasonal influenza, given the continuous evolution of influenza viruses. Moreover, although the 20-HA mRNA-LNP conferred complete protection against a lethal challenge with a matched H1 challenge virus (A/California/07/2009), a single dose did not provide sterilizing protec-

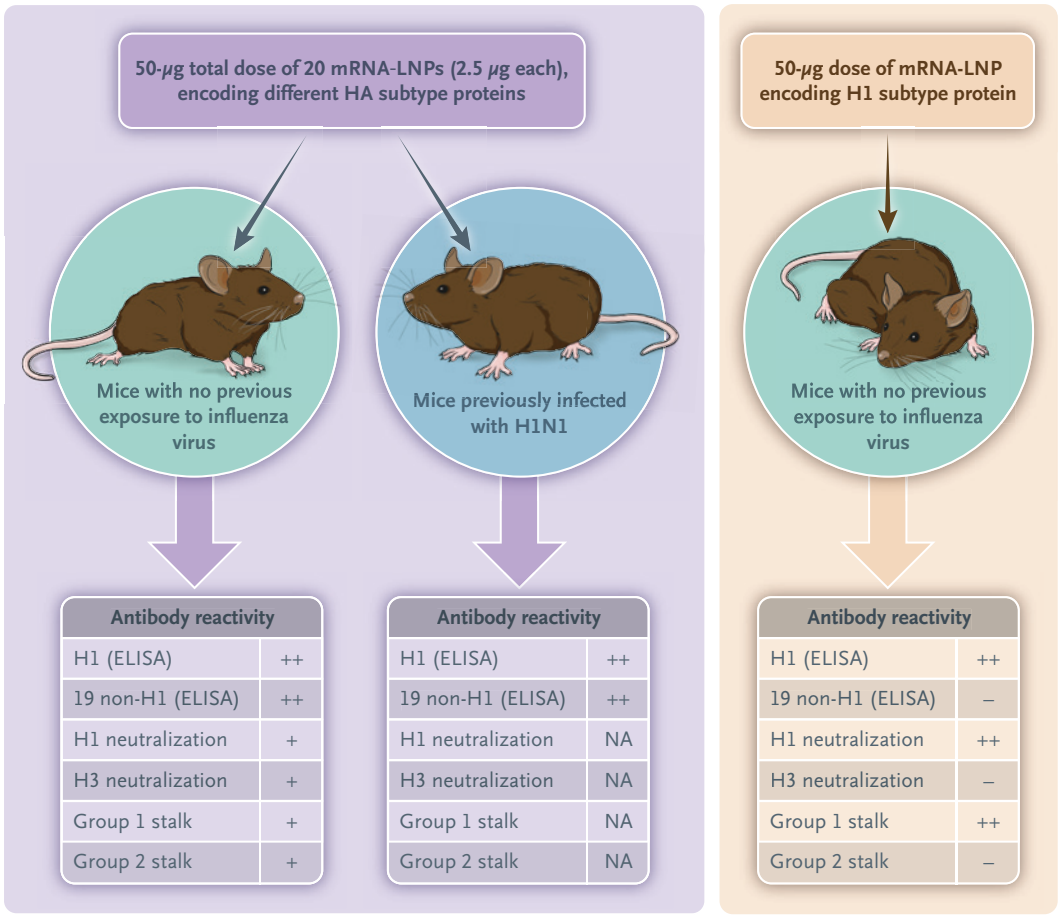

Figure 1. Vaccine Valency and Breadth of Immune Response.

Arevalo and colleagues[3] showed that a 20-valent mRNA vaccine administered to mice provided immune protection against 20 different strains of influenza. They vaccinated previously unexposed mice and H1N1-primed mice with an mRNA–lipid nanoparticle (LNP) formulation. Mice that received a single dose of the 20–hemagglutinin (HA) mRNA-LNP (2.5 μg for each HA; total dose of 50 μg) showed development of robust levels of binding antibodies against all strains of influenza virus represented in the vaccine: previous exposure to H1N1 virus did not diminish the broad responses elicited. In the previously unexposed mice that received the 20-HA mRNA-LNP, neutralization and stalk antibodies to influenza groups 1 and 2 were elicited. In previously unexposed mice that received a monovalent 50-μg dose of H1 mRNA-LNP, strain-specific and group-specific neutralization and stalk antibodies were elicited; the higher strain-specific and group-specific responses in these mice than in the mice that received the 20-HA mRNA-LNP are probably due to the higher antigen content of the H1 component of the vaccine. ELISA denotes enzyme-linked immunosorbent assay, and NA not applicable.

tion in the lung (where viral replication was substantial), nor did it provide absolute protection in mice challenged with a heterologous (mismatched) H1 challenge virus (A/Puerto Rico/8/1934): 20% of these mice died. These data support the conclusion that the 20-HA mRNA-LNP does not protect against a heterologous challenge. However, the authors subsequently administered two vaccine doses to ferrets, a "gold standard" model for influenza virus pathogenesis. Immunization with the 20-HA mRNA-LNP elicited multistrain-specific antibodies, modified disease severity, and protected the ferrets from lethal challenge with a mismatched H1N1 virus.

The application of mRNA technology to influenza vaccines would permit the design of vaccines that incorporate mRNAs matched to multiple influenza strains, a rapid adaptive response to virus evolution, and the manufacture of combination vaccines that include influenza

and noninfluenza proteins, which would facilitate delivery to populations. Arevalo et al.[3] included all 20 known HA antigens in a single vaccine — similar to the inclusion of multiple serotypes in pneumococcal vaccines, in which each vaccine antigen protects against an individual serotype. However, this is not an "either–or" scenario: mRNA technology could be harnessed to deliver mRNA encoding conserved antigens (such as HA stalk or neuraminidase) to achieve more broadly reactive immunity.

Clinical trials of mRNA influenza vaccines representing both options are under way or in the late planning stages.[5] Careful attention to safety evaluations will be critical. It will be important to determine whether side effects, such as the myocarditis that has been associated with the Covid-19 mRNA vaccines, are associated with the mRNA platform or are specific to the Covid-19 mRNA vaccine.[6] It will also be important to understand whether side effects differ with the type or number of mRNAs included in a vaccine.

A major goal for pandemic preparedness is to extend manufacturing capacity to countries and regions that have traditionally relied on outside suppliers. For Covid-19, the countries with early vaccine rollout were largely those with the capacity to produce the vaccines. The inequity of vaccine access for populations in countries without manufacturing capacity has persisted. Thus, the mRNA platform provides an opportunity for more equitable distribution of vaccines for influenza and other vaccines of regional importance.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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