

REVIEW ARTICLE

CURRENT CONCEPTS

Influenza Vaccines for the Future

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EACH YEAR, SEASONAL EPIDEMICS OF INFLUENZA CAUSE SERIOUS ILLNESS and death throughout the world. In the United States, the annual burden of disease is estimated to be 25 million to 50 million cases of influenza, resulting in an average of 225,000 hospitalizations. Over the past three decades, the estimated number of influenza-associated deaths per year in the United States has ranged from 3349 to 48,614. The majority of deaths (>90%) occur among elderly persons, usually those with chronic underlying health conditions.¹⁻³ The World Health Organization uses these estimates to extrapolate a likely global disease burden from influenza of up to 1 billion infections, 3 million to 5 million cases of severe disease, and between 300,000 and 500,000 deaths annually.¹ Pandemics of influenza with varying rates of illness and death have occurred throughout history; the most notable was the 1918–1919 pandemic, which claimed an estimated 50 million to 100 million lives worldwide.⁴

First isolated from humans in 1933,⁵ influenza viruses contain 8 single-stranded RNA segments encoding 11 proteins (Fig. 1). There are three types of influenza viruses: A, B, and C, with types A and B causing annual human epidemics. A key feature of the influenza virus is its error-prone polymerase, which results in an accumulation of genetic mutations that are selected for in hemagglutinin (HA) and to a lesser extent neuraminidase (NA) — the major surface glycoproteins of the virus. This antigenic drift of the HA protein renews our susceptibility to influenza viruses and is the basis for frequent updating of the composition of seasonal influenza vaccines. Protection after natural infection is primarily mediated by HA-specific antibodies in serum and mucosa, with the presence of antibodies against NA, conserved influenza proteins, and T-cell responses correlating with reduced disease severity.

A novel virus can emerge in humans either through direct interspecies transmission or as a result of molecular exchanges between influenza viruses that already infect humans. Because the influenza virus genome is segmented, coinfection of a single host cell with two or more different influenza viruses can result in a reassortment (or shuffle) of their genetic material. The antigenic shift can lead to a pandemic if the resulting progeny virus contains an HA protein to which humans have no preexisting immunity, if it has an efficient replication-competent set of internal genes, and if it can readily spread from human to human — as was the case with the 2009 H1N1 virus.

VACCINES FOR INFLUENZA CONTROL

Vaccination is the primary strategy for the prevention and control of influenza.^{8,9} Although both inactivated vaccines and the live attenuated vaccine are effective in preventing influenza and its associated complications, the protection they confer varies widely, depending on the antigenic match between the viruses in the vaccine

and those that are circulating during a given influenza season and on the recipient's age and health status.¹⁰ More effective vaccination options are needed, especially for persons who have a reduced immunologic response to vaccination, including the elderly and those with chronic underlying disease. A step toward this goal is the recently approved high-dose, inactivated influenza vaccine.¹¹

Seasonal influenza vaccines are trivalent. Each dose is formulated to contain three viruses (or their HA proteins) representing the influenza A H3N2, influenza A H1N1, and influenza B strains considered to be the most likely to circulate in the upcoming influenza season. The strains for Northern Hemisphere vaccines are generally selected in February for use in the following season. Inactivated-vaccine production begins with the generation of vaccine reference strains — hybrid viruses with the HA and NA genes from the drifted variant combined with other genes from a laboratory strain adapted to grow well in eggs. This process can take several weeks or longer.^{12,13} Manufacturers sometimes find that the new strain still grows poorly in eggs or yields low levels of HA protein and needs to be further “egg-adapted” through serial passage. In contrast, plasmid-based reverse-genetics technology is now being used to reliably generate reference strains within a shorter time frame and to improve their growth in eggs.^{14,15} Traditionally, from February to late summer, manufacturers amplify the vaccine viruses in hundreds of millions of embryonated chicken eggs and inactivate or purify them. These vaccines are then formulated, packaged, and distributed beginning early in the fall for administration before the peak of the influenza season, which usually occurs after December.

CHALLENGES TO PRODUCING H1N1 VACCINE

When human 2009 H1N1 viruses were identified in the spring of 2009, vaccine manufacturers were well into their annual production of seasonal influenza vaccine for the 2009–2010 season.¹⁶ Owing to the uncertainty of the evolving outbreak, a decision was made to continue the seasonal vaccine production and to begin separate production of a vaccine against the new virus. The persistence and dominance of the 2009 H1N1 virus became evident throughout the summer, and the number

of cases of 2009 H1N1 virus–related influenza increased in August and September, compressing the vaccine production timeline further, by several months. An additional challenge for the inactivated-vaccine manufacturers was the substantially lower-than-expected yields of HA protein, resulting in fewer doses being available initially. For the immediate future, priorities have been established for overcoming the rate-limiting steps in the production of inactivated vaccines. These include wider implementation of technologies, such as reverse genetics, to generate vaccine reference strains optimized to grow well in eggs, and new methods to accelerate vaccine potency and sterility testing, which would substantially shorten the time from strain selection to release of vaccine.¹⁷

The live attenuated 2009 H1N1 viruses reached very high titers in eggs, allowing this vaccine to be the first one distributed. However, several barriers need to be overcome for broader use of the live attenuated vaccine in a future pandemic, including its approval for use in age groups other than those for which it is currently indicated (i.e., only healthy persons 2 to 49 years of age) and the development of formulations that can be administered without a special nasal-spray device (i.e., nose drops). For both inactivated and live attenuated vaccines, the approval of preservative-free multidose vials could further accelerate their availability and use.¹⁸

NEW TECHNOLOGIES IN VACCINE PRODUCTION

The limitations of currently available vaccines, the complex manufacturing process, and the compressed production times underscore the need for more effective vaccines and more rapid, efficient, and reliable vaccine-production technologies, as well as considerably more surge capacity in the event of a pandemic. Multiple efforts are under way to address these areas, and new approaches to influenza-vaccine production as well as existing technologies are summarized in Table 1.

To be licensed, a new influenza vaccine must be shown to be safe and effective, to elicit antibodies, and to prevent influenza infection. Additional studies may include correlating efficacy with less traditional immune responses (e.g., antibodies against NA or M2 or cellular responses) and comparing the efficacy of the new vaccine with that of a vaccine that has already been approved.¹⁹

INTERIM APPROACHES*Cell-Culture Techniques*

Our current egg-based vaccine-manufacturing process is vulnerable because of an insufficient egg supply in the event of a zoonotic outbreak of avian influenza or other diseases affecting chicken flocks and a lack of capacity for a surge in production. To better prepare us for seasonal influenza and the next pandemic, substantial resources have been invested in developing mammalian cell cultures as an alternative substrate for the production of influenza vaccines with the goal of U.S. licensure in the near future.²⁰ Although a shift from eggs to cell culture would have several advantages — allowing manufacturers to work directly with wild-type viruses, avoiding the generation of egg-adaptive mutations in the HA protein, increasing surge capacity in the event of a pandemic, and providing better manufacturing control through a closed-system fermentation process — limitations remain.^{21,22} For inactivated vaccines, large quantities of the viruses yielding sufficient HA protein would still need to be produced. In addition, the cell-grown viruses need to be processed in a manner that is similar to the processing of viruses grown in eggs, so it remains to be seen whether cell-based technologies would substantially shorten the time needed to produce inactivated vaccines. In the United States, a cell-culture-based, live attenuated vaccine is also in late-stage preclinical development; however, since the viruses are not inactivated and are only minimally purified, studies to assess residual cell-substrate DNA are needed before they can progress to clinical testing.²³

Adjuvants

Adjuvants amplify the immune response to an antigen by enhancing the delivery and presentation of antigen as well as the recruitment of inflammatory and immunocompetent cells to the area of antigen deposition, by directly activating an innate immune response, or both. Several HA-based seasonal influenza vaccines with adjuvants have been approved and used in Europe, including those formulated with phospholipids or oil-in-water emulsions.^{24,25} In 2009, H1N1 vaccines containing oil-in-water adjuvants were used in Europe and other countries.²⁶⁻²⁸ Despite the approval and widespread use of such vaccines abroad and their excellent safety record, there were reserva-

Figure 1 (facing page). Structure and Replication Cycle of Influenza A Virus and Well-Characterized Adaptive Immune Responses.

Eight gene segments code for 11 proteins, including hemagglutinin (HA) and neuraminidase (NA), which account for most of the known antigenic determinants. The portion of the matrix 2 (M2) protein outside the viral envelope is also antigenic. Adaptive immune responses are shown in Panels A through D. In Panel A, the influenza HA protein mediates attachment of the virus to its host cell receptor. Antibodies directed against the HA protein block attachment of the virus to the host cell receptor or block fusion of the virus and host membrane. Antibodies generated against HA are correlated with vaccine protection against infection. In Panel B, antibodies generated against the NA protein do not prevent infection but limit the release of virus from infected cells. Antibodies directed against NA have been correlated with a reduction in disease severity. In Panel C, antibodies generated against the highly conserved external domain of the M2 protein epitope interfere with virus assembly or constrain proton transport and are highly cross-reactive across virus subtypes. In Panel D, CD8+ T-cell responses to conserved influenza virus components have been correlated with enhanced clearance of virally infected cells; however, the exact degree to which they contribute to a reduction in illness remains uncertain. NP denotes nucleoprotein, PA polymerase acidic protein, and PB2 polymerase basic protein 2. Adapted from Kaiser⁶ and Subbarao et al.⁷

tions about adopting them in the United States. These concerns were expressed against a backdrop of caution toward vaccines in general among certain segments of the public. Although it was fortunate that adjuvants were not needed to enhance the immune response to the 2009 H1N1 vaccine, clinical trials have shown that oil-in-water adjuvants are needed to stimulate high levels of antibodies against influenza viruses that have novel HAs (e.g., H5N1 viruses), and these adjuvants may be critically important in future vaccination programs.²⁹⁻³² Purified bacterial outer-membrane proteins, toll-like receptors, and a variety of toll-like-receptor agonists (bacterial carbohydrates, lipids, proteins, and nucleic acids) have also shown promise as next-generation adjuvants for influenza vaccines, and several of these adjuvants are in the early stages of clinical testing.³³⁻³⁷

Novel Live Attenuated Vaccines

Efforts also are under way to develop live influenza vaccines based on the influenza NS1 protein, a nonstructural, multifunctional protein involved

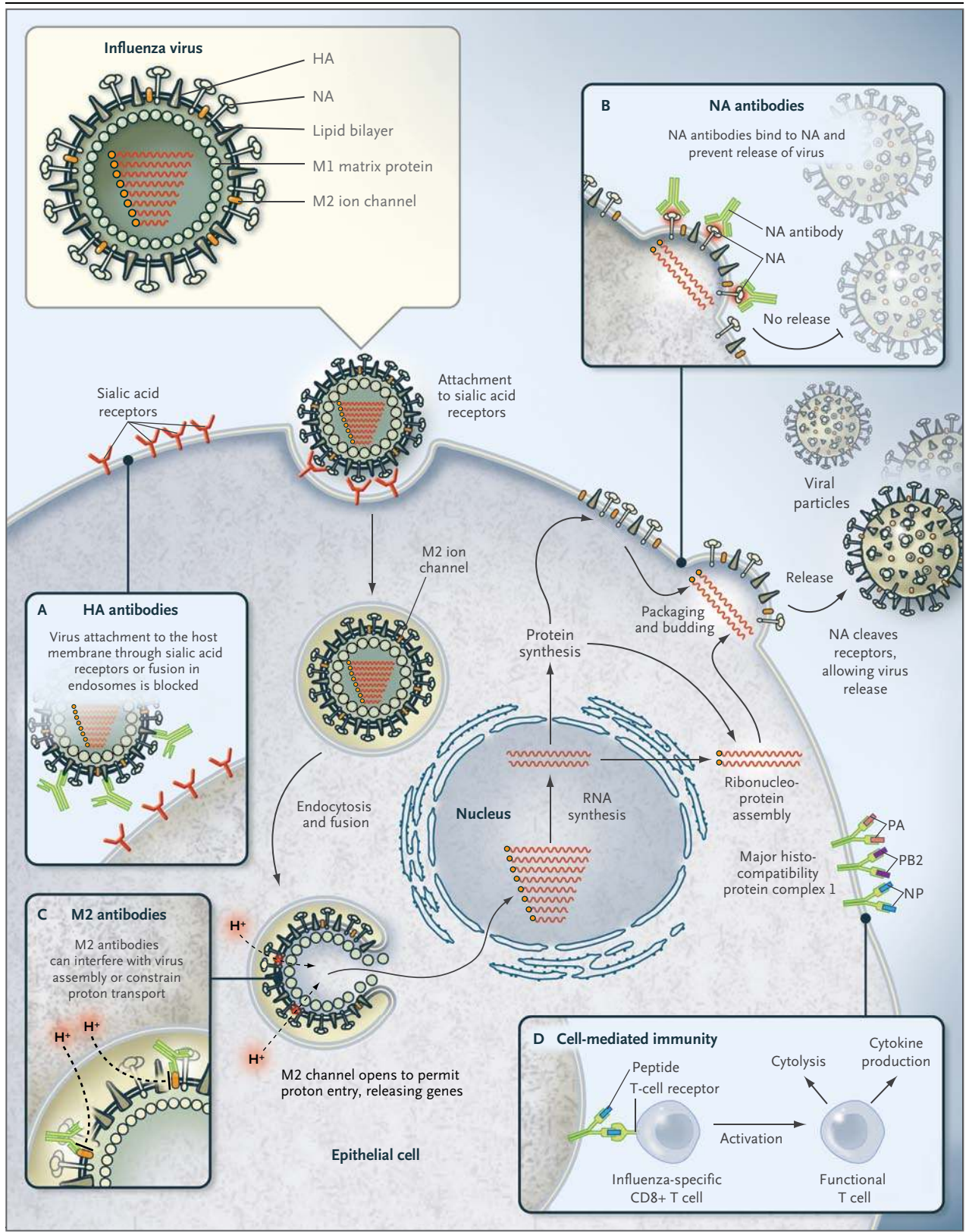


Table 1. Current and New Approaches to Influenza-Vaccine Production.

Vaccine	Stage of Development			
	Preclinical Development	Phase 1 and 2 Clinical Testing	Phase 3 Clinical Testing	Licensed or Approved
Inactivated vaccines				
Egg-based	Yes	Yes	Yes	Yes
Cell-based	Yes	Yes	Yes	In Europe but not in the United States
With adjuvant	Yes	Yes	Yes	In Europe but not in the United States
Live attenuated vaccines				
Egg-based	Yes	Yes	Yes	Yes
Cell-based	Yes	Yes	No	No
Next generation				
Recombinant proteins	Yes	Yes	Yes	No
Viruslike particles	Yes	Yes	No	No
Viral vectors	Yes	Yes	No	No
DNA-based vaccines	Yes	Yes	No	No
Universal vaccines	Yes	Yes	No	No

in viral replication and inhibition of the host's innate immune responses. Preclinical studies have shown that infection with viruses containing an altered or deleted NS1 protein blocks viral replication and stimulates both humoral and cellular immune responses.^{38,39} Early clinical data have shown that an intranasal NS1 vaccine is well tolerated and generates neutralizing HA antibodies.⁴⁰

NEXT GENERATION OF INFLUENZA VACCINES

Although cell-based influenza vaccines and vaccines containing adjuvants are likely to expand the capacity to produce influenza vaccines within several years, recombinant DNA techniques are facilitating new production strategies by allowing vaccine candidates to be generated as soon as the genetic sequence of the influenza virus HA is known; this approach would eliminate the need to handle pathogenic viruses or to adapt viruses to grow in eggs or cells (Fig. 2). These technologies, which are still mostly in early stages of development, may substantially reduce production timelines.

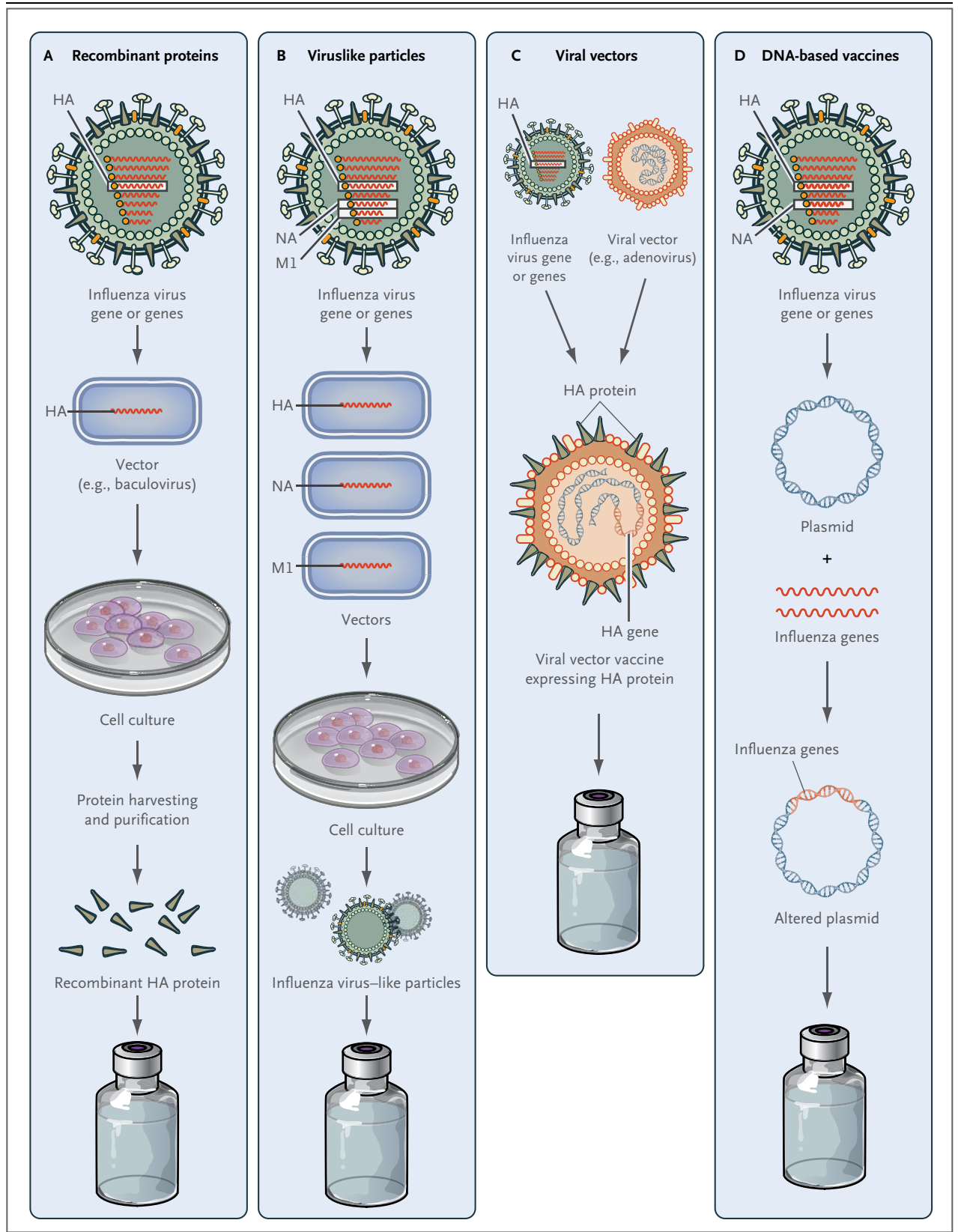
Recombinant Proteins

A recombinant trivalent HA protein-based influenza vaccine is in the late stages of clinical development in the United States (Fig. 2A). As soon as the influenza vaccine strains are selected, the genes

encoding the HA proteins are cloned into baculovirus vectors. Insect cells infected with these vectors express HA proteins, which are then further purified and formulated into a trivalent vaccine.⁴¹ The safety, immunogenicity, and efficacy profile of this vaccine has been reported, and an application has been submitted to the Food and Drug Administration for its approval

Figure 2 (facing page). New Approaches to Influenza-Vaccine Production.

Steps in the production of vaccines using recombinant proteins (Panel A), viruslike particles (Panel B), viral vectors (Panel C), and DNA-based methods (Panel D) are shown. Once the sequence of an influenza virus genome is determined, recombinant DNA technology enables the rapid generation of influenza vaccines with the use of a variety of production strategies. In Panel A, the HA gene is cloned into a vector, which expresses the recombinant HA protein in infected cells, and the protein is subsequently purified. In Panel B, the simultaneous infection of cells with individual vectors containing the HA, NA, and M1 genes, respectively, results in self-assembling viruslike particles that contain HA and NA proteins on their surface but lack influenza gene segments. In Panel C, the influenza HA protein is expressed on the surface of a "carrier" virus that presents it to the immune system but that cannot itself cause disease. In Panel D, a DNA vaccine consists of a DNA plasmid into which one or more influenza virus genes are inserted. HA denotes hemagglutinin, M1 matrix 1 protein, and NA neuraminidase.



for use against seasonal influenza in healthy persons 18 years of age or older.⁴²

Viruslike Particles

The use of noninfectious viruslike particles is another promising approach to the production of influenza vaccines (Fig. 2B). Recombinant viral vectors that express HA, NA, and the influenza matrix (M1) protein — a structural protein lining the inside of the viral envelope that is involved in viral assembly and budding — are used to infect cultured cells. The expressed influenza proteins spontaneously self-assemble at the plasma membrane and bud from the infected cells, forming particles that structurally resemble wild-type viruses. Other influenza proteins or immune-enhancing molecules can be incorporated into the budding particle. Multiple viruslike particle candidates have shown promise in studies in animals, and at least one has advanced to phase 2 clinical trials.^{43,44}

Viral Vectors

A variety of viruses that are incapable of replicating or that replicate but cannot cause disease are being evaluated as new ways of delivering influenza virus proteins to the immune system (Fig. 2C). Influenza HA genes from seasonal or H5N1 viruses, or both, have been cloned into so-called carrier viruses, including vaccinia virus, alphaviruses, adenoviruses, Newcastle disease virus, baculoviruses, and vesicular stomatitis virus. Cellular and antibody responses that provide protection against the vaccine virus and antigenically drifted strains have been shown to develop in animals vaccinated with these viral vectors.⁴⁵⁻⁵⁰ Early clinical trials evaluating the safety and immunogenicity of intranasal and orally administered adenovirus-based HA vaccines have been completed or are under way, with encouraging results.⁵¹⁻⁵³

DNA-Based Vaccines

Influenza vaccines comprising DNA sequences have been studied for more than 20 years. DNA encoding the HA or NA protein, injected intramuscularly either alone or in combination with internal gene segments in animals, has elicited protective responses against drifted influenza viruses (Fig. 2D).^{54,55} Although DNA vaccines administered alone have shown promising results in animals, the results of clinical trials have not

been as encouraging.⁵⁶⁻⁵⁸ Whether this approach will ultimately lead to the development of a viable commercial strategy remains to be seen.

“Universal” Vaccines

The ideal influenza vaccine would be one that is safe, elicits humoral and cellular responses identical to those triggered by a natural infection, provides long-lasting and cross-strain protection, and can be manufactured rapidly in large amounts under well-controlled conditions. Major targets in the search for a “universal,” or “common-epitope,” vaccine have been the highly conserved external domain of the influenza matrix 2 (M2) protein and conserved epitopes from the influenza NP, matrix 1 (M1), and HA proteins.⁵⁹⁻⁶¹ Preclinical studies have shown that these candidate vaccines stimulate broadly cross-reacting antibody responses when administered either alone or in combination with adjuvants or carrier proteins, and several of these vaccines are now undergoing clinical testing.⁶¹⁻⁶⁴ Optimism about the ability to develop a universal vaccine is based in part on recent studies in animal models that used two-step vaccination strategies — priming with a DNA-based HA vaccine followed by a second dose or boost with an inactivated, attenuated, or adenovirus-vector-based vaccine — which resulted in the generation of broadly cross-neutralizing antibodies.^{65,66} A truly universal vaccine that provides lifelong protection against any strain of influenza with one or more vaccinations may not be achievable, but some variant of this concept should be considered. For example, the strategy of periodic vaccinations, given every few years, with a product that expresses several immunogenic common epitopes and that would induce full or partial protection against drifting strains, as well as against newly emerging pandemic strains, is certainly a goal that is worth pursuing.

CONCLUSIONS

Although the past decade has witnessed considerable improvements in our approach to the development of influenza vaccines, much still needs to be done. Although converting from egg-based to cell-based systems and adding adjuvants to enhance protective immune responses are important steps in the right direction, it is the new vaccine

technologies that hold the promise of revolutionizing influenza vaccinology. Over the next decade, advances are anticipated that will substantially decrease vaccine production time, provide enhanced protection (especially in populations at greatest risk), and end mismatches between vaccine strains and circulating viruses. To fully reap the benefits as progress is made in developing new vaccines, we will need both clear regulatory guidance on

pathways for their approval and a robust and agile infrastructure to ensure their timely production and equitable distribution.

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

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