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SUPPLEMENT ARTICLES

How Live Attenuated Vaccines Can Inform the Development of Broadly Cross-Protective Influenza Vaccines

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There are 2 approaches to achieving the goal of a universal influenza vaccine that will protect against all influenza A viruses (IAVs) or possibly against both IAV and influenza B virus (IBVs). One is to use novel approaches, which are discussed by others in this issue. The other is to improve existing vaccines, to extend their breadth of protection to cover all IAVs within a subtype or across subtypes. Four classes of licensed influenza vaccines are available in different parts of the world: unadjuvanted inactivated influenza vaccines (IIVs), adjuvanted IIVs, live attenuated influenza vaccines (LAIVs), and recombinant hemagglutinin vaccines. An ideal influenza vaccine will be easy to administer and will induce cellular immune responses and lasting mucosal and systemic antibody responses that protect against a broad range of influenza viruses, across all subtypes or at least within subtype. Intranasally administered LAIVs meet several of these desirable traits, and in this article we focus on how LAIVs that are currently licensed or in development can inform the design of a broadly cross-protective influenza vaccine.

Seasonal LAIVs were developed in the United States and Russia and are now licensed in several countries. Both vaccines are based on the development of a master donor virus (MDV) with temperature-sensitive and attenuating mutations in different internal protein gene segments that reproducibly confer the attenuation phenotype on reassortant viruses that derive their hemagglutinin (HA) and neuraminidase (NA) gene segments from circulating wild-type influenza viruses [1–3]. The underlying principle is that the temperature-sensitive LAIVs replicate at the colder temperatures of the nasal passages (the upper respiratory tract) and induce an immune response, but their replication is shut off at the warmer, core body temperature of the lungs, thus limiting their ability to cause lower respiratory tract infection. The influenza A/Ann Arbor/6/60 cold-adapted virus is the MDV of the US LAIV for IAV, and B/Ann Arbor/1/66 is the MDV for IBV [1–3]. The MDVs for the Russian LAIV are A/Leningrad/134/47/57 and B/USSR/60/69 [4, 5]. The attenuation mutations in the US and Russian LAIVs have been identified [5–8], and both viruses are genetically and phenotypically stable following manufacture in embryonated eggs and following replication in vaccine recipients [9–12], likely because they bear several mutations in different gene segments, reducing the likelihood of reversion.

LESSONS FROM SEASONAL LAIV

A key lesson from the clinical development of the US LAIV was the importance of mucosal immunity. IIV reliably induces a strain-specific serum antibody response against the HA, which is measured by hemagglutination inhibition (HAI) assays. A serum HAI titer of 1:40 is an accepted correlate of protection for IIV [13]. However, seroconversion rates and titers of serum antibody following LAIV are lower than after IIV [14–16]. Furthermore, LAIV has been shown to be effective in the absence of a robust serum HAI antibody response, indicating that serum HAI antibody is not an accurate correlate of protection for this vaccine [17].

In addition to serum antibodies, intranasally administered LAIV induces mucosal antibodies. In a study comparing immune responses to LAIV and trivalent IIV, 83% of LAIV recipients developed influenza virus–specific immunoglobulin A (IgA) mucosal antibodies, compared with only 38% of trivalent IIV recipients [18]. LAIV-induced IgG and IgA antibodies in nasal wash samples correlated with protection from virus replication, and either antibody in serum samples or IgA in nasal wash specimens were predictors of protection in human challenge studies [15, 19, 20]. Significantly higher

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vaccine-specific nasal IgA antibody titers were reported in a subset of children who received LAIV as compared to placebo in 3 prospective, 2-year randomized clinical trials [21], although the precise role of mucosal antibody in vaccine efficacy remains to be elucidated.

The contribution of the different arms of the immune system to LAIV-induced protection has been evaluated in mice and ferrets [22–25]. The body temperature of ferrets is about 39°C; therefore, ferrets are used to test the attenuation phenotype conferred by the temperature-sensitive mutations of the Ann Arbor cold-adapted virus, with replication limited to the upper respiratory tract. In contrast, the body temperature of mice is closer to 37°C, making them permissive hosts for the replication of the Ann Arbor cold-adapted virus in the upper and lower respiratory tract. In mice, both cellular and humoral immunity contribute to LAIV-mediated protection, and their relative contribution to viral clearance depends on the location and replication of the vaccine virus [24]. Neutralizing antibody confers optimal protection against wild-type virus challenge in mice; the magnitude of the antibody response and the access of antibodies to the respiratory tract are critical determinants of protection [25]. However, the relevance of the findings from the mouse model to the human experience are not clear because LAIV strains administered intranasally to anesthetized mice replicate in the lower respiratory tract, but they do not replicate in the lower respiratory tract of humans.

The second important lesson from the development and implementation of seasonal LAIV was that it offered greater breadth of protection against antigenic drift variants than IIV in naive individuals. In randomized clinical trials in young children, there was evidence that LAIV offered protection against antigenic drift variants [26, 27]. This was a remarkable advantage over IIV because the latter generally induces strain-specific immunity, and an increase in the breadth of cross-protection is highly desirable in the event that an antigenic drift variant emerges after the vaccine has been manufactured or implemented. However, it should be noted that, for reasons that are not clear, LAIV has not provided superior protection against antigenic drift variants in recent years and that IIV was more effective than LAIV in adults [28, 29].

PANDEMIC LAIV

Laboratory of Infectious Diseases at the National Institutes of Health undertook a pandemic influenza vaccine development program in 2003, the LAIV platform was selected because it used licensed technology; seasonal LAIV viruses were optimally balanced in attenuation and efficacy; LAIV induced serum and mucosal antibody responses and T-cell responses and had broader cross-protection against antigenic drift variants; and LAIV viruses had greater yield per egg than IIV. Under a collaborative research and development agreement with MedImmune, we developed pandemic LAIV for several IAV subtypes, including H2, H5, H6, H7, and H9, and evaluated them extensively in preclinical and clinical settings.

Similar to seasonal LAIV, pandemic LAIVs are 6:2 reassortants that contain the 6 internal protein gene segments from MDV-A bearing the temperature-sensitive and attenuation phenotypes and the 2 surface glycoproteins (HA and NA) from a selected avian or animal IAV. All of the pandemic LAIV viruses except the H9N2 virus were generated by reverse genetics [30–35]. The polybasic cleavage site in the HA of highly pathogenic avian influenza H5 and H7 viruses was removed and replaced with the motif seen in low-pathogenicity avian influenza viruses [34–36] because the polybasic cleavage site makes the HA cleavable by intracellular and extracellular proteases and is a virulence determinant for chickens. Pandemic LAIV candidates were tested in mice, ferrets, and nonhuman primates [30–35, 37–39].

In mice, the pandemic LAIV viruses were nonlethal and were highly restricted in replication in the lungs and failed to spread to the brain [30, 31, 33–35]. Animals that received a single dose of vaccine developed only low levels of neutralizing and HAI antibodies against the homologous wild-type viruses and little to none against antigenically and genetically drifted (heterologous) strains [33–35]. Despite low or undetectable neutralizing antibody titers, all pandemic LAIV–recipient mice survived challenge with homologous and heterologous influenza viruses [33–35]. Two doses of pandemic LAIV induced robust antibody responses and protected mice from challenge virus replication in the lungs [33–35].

Following intranasal inoculation in ferrets, the replication of the pandemic LAIV was restricted to the upper respiratory tract, with no detectable replication in the lungs or extrapulmonary sites [30, 32–35]. Pandemic LAIV–recipient animals were completely protected against challenge virus replication in the lungs, with limited replication in the upper respiratory tract [30, 32–35].

On the basis of promising data on infectivity, immunogenicity, and efficacy in mice and ferrets, 8 pandemic LAIV viruses (2 H5N1 strains and 1 each of H2N2, H6N1, H7N7, H7N3, H7N9, and H9N2) were evaluated for safety and immunogenicity in healthy adults in open-label phase 1 clinical trials. The vaccines were safe and well tolerated, but the vaccine viruses were highly restricted in replication, and, with the exception of the H7N3 pandemic LAIV, the vaccines were uniformly poor in inducing a detectable serum antibody response [40–46]. The highly restricted replication was surprising because the vaccine viruses replicated reasonably well in mice and ferrets and because the healthy adults who were vaccinated had no prior exposure to avian IAV. The discrepancy in the findings in small-animal models and humans prompted us to evaluate the pandemic LAIVs in nonhuman primates. We found that African green monkeys recapitulated the highly restricted replication of the pandemic LAIV viruses in the upper respiratory tract, and a single dose of pandemic LAIV did not elicit a serum antibody

response [39]. Although the wild-type parent avian IAV could be detected in nasal wash samples, the pandemic LAIV viruses were not detected in secretions. However, on necropsy performed at days 2 and 4 after inoculation, the vaccine viruses were detected in nasal turbinate tissue specimens, suggesting that infection was highly cell associated. African green monkeys were more permissive than humans because monkeys developed a serologic response following 2 doses of pandemic LAIV, while humans did not [39].

PRIME-BOOST VACCINATION WITH PANDEMIC LAIV AND PANDEMIC IIV

Several investigators reported that administration of a booster dose of subunit H5N1 pandemic IIV to subjects previously primed with a variety of H5 vaccines (recombinant expressed H5 HA, DNA encoding H5 HA, or an H5N1 pandemic IIV) resulted in a robust HAI and neutralizing antibody responses, even in individuals who had no detectable antibody response following initial vaccination [47–50]. In light of these findings, we hypothesized that prior receipt of pandemic LAIV would prime for a higher antibody titer and greater frequency of seroconversion to a subsequent dose of pandemic IIV. In a series of separate trials, we recalled subjects who were previously vaccinated with an H5N1 or H7N7 pandemic LAIV 2–4 years earlier and vaccinated them with H5N1 or H7N7 pandemic IIV, respectively [40, 51], or prospectively enrolled subjects to receive sequential immunization with H7N9 pandemic LAIV and pandemic IIV [43]. A majority (64%–79%) of the pandemic LAIV–primed individuals had a rapid and robust antibody response (geometric mean HAI antibody titer, 119–175) to a booster dose of the corresponding pandemic IIV administered 3 months to almost 5 years later [40, 43, 51]. The antibody was of high affinity and cross-reacted with several distinct clades or antigenically and geographically distinct viruses within the same subtype. These data prove that pandemic LAIVs establish long-lasting immune memory that can be recalled with a single dose of pandemic IIV.

Similar findings have been reported in clinical trials in which subjects were primed with DNA or an adenovirus expressing the H5 HA and were boosted with pandemic IIV [47, 52, 53]. These strategies should be compared head to head because the immune mechanism and quality and longevity of the immune response with the different platforms may not be the same.

Investigation of the immunologic basis for the pandemic LAIV/pandemic IIV prime-boost phenomenon in the African green monkey model has revealed that intranasally administered pandemic LAIV elicits a highly localized and somatically hypermutated germinal center B-cell response in the mediastinal lymph nodes that is rapidly recalled following pandemic IIV boost to germinal center reactions at distant immune sites, most notably the local draining axillary lymph node [54]. If the primary immune response to LAIV in humans is similarly restricted to a draining lymph node, we may be closer to understanding why the serum antibody response is not a reliable correlate of immunity for seasonal and pandemic LAIVs [54].

LESSONS FROM THE EVALUATION OF PANDEMIC LAIV

Three important lessons from the evaluation of pandemic LAIV that could be of relevance in the development of broadly neutralizing or universal influenza vaccines are as follows: (1) data from ferrets and mice did not predict the experience in humans, while the findings from experiments in nonhuman primates were much closer to what was observed in humans; (2) even when they fail to replicate well and fail to induce a detectable antibody response on primary immunization, pandemic LAIV viruses establish durable long-lasting immune memory that can be recalled rapidly; and (3) the combination of the LAIV and IIV platforms in a prime/boost strategy gives excellent results in terms of seroconversion rates and antibody titers.

WHAT ARE SOME OF THE PROBLEMS THAT HAVE EMERGED WITH SEASONAL LAIV?

LAIV was preferentially recommended for use in children in the United States by the Advisory Committee for Immunization Practices in 2014, but the recommendation was withdrawn for 2 years because seasonal LAIV was not effective against 2009 pandemic influenza A(H1N1) virus (A[H1N1]pdm09), while IIV was [55]. Vaccine effectiveness against A(H1N1)pdm09 was strikingly lower than what had been observed in earlier studies. A single root cause of the reduced vaccine effectiveness against A(H1N1)pdm09 has not been identified, and it is likely that several factors contributed to the poor vaccine effectiveness [56]. Notably, some A(H1N1)pdm09 viruses have an unstable HA, and the IBV components of the quadrivalent LAIV replicate more efficiently than A(H1N1)pdm09 and may interfere with its ability to infect the host. The manufacturers have refined their ability to identify an A(H1N1)pdm09 vaccine virus that meets the performance characteristics of pre-2009 seasonal H1N1 vaccine strains, and the ACIP reinstated seasonal LAIV as one of the recommended vaccines in 2018 [57]. This cautionary experience suggests that the HA has a significant bearing on the performance of LAIV strains, particularly in multivalent vaccine formulations; these findings may be relevant to other live influenza virus vaccines that are under development.

In randomized clinical trials (RCTs) in the 1990s, the efficacy of the US LAIV was established in children and adults [20]. The vaccine was licensed in the United States in 2003 and was subsequently available for annual immunization. Over the years, the method by which vaccine effectiveness is measured has changed, and the test-negative design is now widely used [58]. By this method, the effectiveness of LAIV is much lower than in the RCTs leading to licensure. While methodologic differences, such as observational studies versus RCTs and efficacy versus test-negative studies, are likely to explain some of the difference,

it is also possible that the LAIV is less effective in children who are vaccinated every year than it was when it was first evaluated in vaccine-naive children. Seroconversion rates in adults who received LAIV were often similar to those in placebo recipients, presumably because adults are immunologically primed by repeated prior exposure to influenza viruses [59–61]. Vaccine virus shedding was also reduced in adults, compared with young children [59]. LAIV may have similarly reduced infectivity and immunogenicity in annually revaccinated children, because of their repeated exposure to influenza viruses. If this is the case, it will be important to establish whether the duration of vaccine-mediated protection would allow for a longer interval than 1 year for revaccination with LAIV.

The success of any live attenuated vaccine lies in achieving a balance between attenuation and immunogenicity. The take-home message for the development of new live vaccines is that annual revaccination may alter this balance, as may have happened with seasonal LAIV.

HOW SHOULD OUR THINKING ABOUT A BROADLY PROTECTIVE OR UNIVERSAL INFLUENZA VACCINE DIFFER FROM THAT ABOUT A SEASONAL INFLUENZA VACCINE?

Depending on the immunogen used, vaccination may not prevent infection but may prevent severe disease and its complications. It will be important to be clear about this distinction in public health messaging. Additionally, it is likely that ≥2 doses of a novel vaccine will be needed to immunize the population successfully. This is generally true for HA head–based IIVs, which are only immunogenic in primed subjects. Unexpectedly, when A(H1N1)pdm09 emerged in 2009, all age groups except children <3 years of age responded well to a single dose of IIV [62, 63]. This suggested that most of the population had cross-reactive immunity that was recalled with a single dose of IIV. If 2 doses of vaccine are needed, it is worth considering priming with a vaccine that induces broad immunity, such as a pandemic LAIV followed by a boost with a vaccine that induces strain-specific immunity, such as a pandemic IIV, to optimally combine the benefits of the breadth of immunity with highly strain-specific immunity. Currently, pandemic LAIVs are administered to study participants who are admitted to an inpatient unit. They are discharged from the unit only when it is clear that they are not shedding vaccine virus, to obviate the risk of introducing a novel influenza virus subtype into the community. Practical use of a pandemic LAIV prime and IIV boost strategy can only be considered if the pandemic LAIV prime can be offered in an outpatient setting.

REPLICATION INCOMPETENT OR SINGLE-CYCLE REPLICATION VIRUSES

Several innovative vaccine design strategies are being explored to attenuate IAVs, including engineered viruses that lack a functional HA, M2, or NS1 protein or rewriting the genetic code by codon pair deoptimization, expansion of the genetic code of the viral genome [64], and genome-wide elimination of interferon modulating functions [65]. We discuss the single-cycle replication virus with a disabled HA in greater detail below.

An influenza vaccine that induces a $CD8^+$ T-cell response is an attractive strategy because this is the primary mechanism for viral clearance [66], and in the absence of detectable neutralizing antibodies, T cell–mediated immunity cross-protects across IAV subtypes. $CD8^+$ T cells recognize conserved peptides in the internal viral proteins, such as NP and M [67–69], that are processed in the cytosol during infection. Targeting the highly conserved internal influenza virus proteins to induce a $CD8^+$ T cell–mediated protective immune response therefore constitutes a valid and promising strategy for the design of a broadly protective universal influenza vaccine.

Townsend et al developed a novel universal influenza vaccine called "Signal Minus FLU" ("S-FLU") [70]. S-FLU is a pseudotyped IAV in which the viral RNA encoding HA is inactivated and functional HA proteins that are necessary to form infectious particles are provided in trans in cells that are stably transduced to express a full-length HA. Thus, the pseudotyped S-FLU virus is coated with HA from the transduced cells and expresses functional NA and internal viral proteins but cannot express the genetically silenced HA glycoprotein. The S-FLU virus infects Madin-Darby canine kidney cells or animal models as efficiently as wild-type virus [70], but it is replication deficient and only undergoes a single round of replication. Expression of the highly conserved internal viral proteins in the cytosol of S-FLU virus–infected cells enables antigen presentation for induction of cross-reactive $CD8^+$ T cell–mediated immunity.

To date, H1-, H3-, H5-, and H7-pseudotyped S-FLU viruses have been evaluated in mice, ferrets, and pigs [70–73]. The viruses were not pathogenic in any animal model, and immunization reduced lethality, pathology, and viral replication upon homosubtypic or heterosubtypic virus challenge [70]. S-FLU induced strong cross-reactive $CD8^+$ T-cell responses to the conserved NP protein [70, 71]. Furthermore, delivering the vaccine to the lower respiratory tract of pigs through aerosol administration induced a large population of lung-tissue-resident memory T cells [72], which play a role in cross-protective immunity against influenza virus infection [74]. S-FLU viruses express and display NA on their surface as efficiently as wild-type influenza viruses [70], and immunization via the respiratory tract induced a strong, anti-NA antibody response but not an HA-specific neutralizing antibody response in mice and ferrets [70, 71]. Taken together, S-FLU vaccines induce broadly protective T cell–mediated heterosubtypic immunity that is effective against virus challenge in several animal models.

S-FLU has some potential advantages over LAIV in terms of the risk of reassortment with circulating IAV. First, the HA viral RNA is fully inactivated, and several additional safeguards

reduce the risk of reversion to the wild-type HA sequence so a viable HA protein cannot be donated to a circulating seasonal influenza virus; and second, because S-FLU virus undergoes only a single round of infection, the likelihood of coinfection with circulating seasonal strains is minimized [70]. Overall, S-FLU vaccines represent a promising strategy for universal influenza immunization by inducing broadly cross-reactive T cell–dependent immunity in the lung, accompanied by systemic antibody-mediated protection. Thus, evaluation in humans is warranted.

WHAT DOES THE FUTURE HOLD?

There is great interest in improving current influenza vaccines, to increase the breadth of immunity and protection they confer, and in developing new broadly protective or universal influenza vaccines. It is likely that both approaches will be pursued for now. LAIVs embody several of the desired characteristics of such vaccines, and the development of LAIVs offer several lessons in vaccine development that can inform new strategies. A combination of LAIV and IIV, with the latter administered with or without adjuvant, may achieve an intermediate goal of providing broad immunity to IAV subtypes.

Notes

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