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Live Attenuated Cold-Adapted Influenza Vaccines

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Live attenuated, cold-adapted influenza vaccines exhibit several desirable characteristics, including the induction of systemic, mucosal, and cell-mediated immunity resulting in breadth of protection, ease of administration, and yield. Seasonal live attenuated influenza vaccines (LAIVs) were developed in the United States and Russia and have been used in several countries. In the last decade, following the incorporation of the 2009 pandemic H1N1 strain, the performance of both LAIVs has been variable and the U.S.-backbone LAIV was less effective than the corresponding inactivated influenza vaccines. The cause appears to be reduced replicative fitness of some H1N1pdm09 viruses, indicating a need for careful selection of strains included in multivalent LAIV formulations. Assays are now being implemented to select optimal strains. An improved understanding of the determinants of replicative fitness of vaccine strains and of vaccine effectiveness of LAIVs is needed for public health systems to take full advantage of these valuable vaccines.

Although parenterally administered inactivated influenza vaccines induce a protective serum antibody response, there has been and continues to be an interest in live attenuated influenza vaccines because they offer the additional benefits of inducing mucosal and cell-mediated immunity, which confer greater breadth of protection against antigenic variant influenza viruses (Belshe et al. 2000a; Mendelman et al. 2004). Vaccine viruses bearing temperature-sensitive (ts) mutations were of particular interest because the temperature in the nasal passages tends to be a few degrees cooler than the core body temperature of the lower respiratory tract. Therefore, a ts live influenza virus vaccine ad-

ministered intranasally would replicate in the nasal mucosa and induce immunity but would be attenuated because of restricted ability to replicate in the lungs (Murphy and Coelingh 2002). The first licensed live attenuated influenza vaccines combined ts and genetic stability in cold-adapted (ca) vaccines and were developed in the United States and Russia in the 1970s (Maassab 1967, 1969; Ghendon et al. 1984; Kendal 1997). In both cases, the hemagglutinin (HA) and neuraminidase (NA) gene segments of the vaccine viruses are derived from circulating influenza viruses, because these two proteins are the targets of the protective immune response. The remaining six gene segments are derived from

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the vaccine donor strain that confers ts, ca, and attenuation phenotypes on the reassortant viruses. Thus, the live vaccine viruses replicate locally in the upper respiratory tract without causing clinical illness and induce protective immunity against the circulating influenza virus.

HISTORY

This review focuses primarily on the U.S.-based ca live attenuated influenza vaccine (LAIV) with comments on the Russian ca LAIV in specific areas. The key principles underlying the development of the licensed ca LAIVs are the following.

1. A vaccine backbone strain, now referred to as a master donor virus (MDV), bears one or more attenuating mutations in one or more of the internal protein gene segments to enhance the genetic stability of the MDV.
2. The “6:2” reassortant vaccine viruses are generated by co-infection or plasmid-based reverse genetics.
3. The reassortant seasonal influenza viruses are reproducibly and reliably attenuated by transfer of the internal protein gene segments of the MDV.
4. There are separate MDVs for influenza A and B viruses.

The history of the U.S. ca LAIV has been reviewed previously (Murphy 1993; Maassab and Bryant 1999; Murphy and Coelingh 2002; Jin and Subbarao 2015). An important early step in the development of LAIVs was the demonstration of three independent phenotypes of the LAIV (ts, ca, and attenuation) that were reproducibly transferred to 6:2 reassortant viruses. Preclinical studies in vitro and in animal models demonstrated the independent contribution of different gene segments to these phenotypes (Snyder et al. 1988; Subbarao et al. 1992). Much later, with reliable sequence data and the availability of reverse genetics techniques, the five critical mutations in three gene segments of the MDV-A strain (Jin et al. 2003) and the five critical mutations in three gene seg-

ments of the MDV-B strain (Hoffmann et al. 2005) were conclusively identified.

Preclinical studies, mainly in Golden Syrian hamsters or mice, focused on attenuation, genetic stability, immunogenicity, and efficacy of the LAIV. Hamsters were the preferred model because their body temperature was 38°C–39°C, which was more similar to that of the lower respiratory tract of humans than that of mice, which at 37°C was permissive for replication of the ca LAIV in the lower respiratory tract (Mills et al. 1971; Spring et al. 1977).

In the initial steps of clinical development, monovalent A/H1N1 and A/H3N2 LAIV were evaluated in studies that focused on safety, infectivity, assessing the 50% human infectious dose (HID_{50}) through dose escalation (or de-escalation), and immunogenicity starting in healthy adults and proceeding to successively younger subjects (Murphy 1993; Maassab and Bryant 1999; Murphy and Coelingh 2002). Subsequent studies were conducted with bivalent (A/H1N1 + A/H3N2) and later trivalent (inclusion of one influenza B vaccine virus) formulations that assessed safety, immunogenicity, interference between strains, genetic stability, and transmissibility of the vaccine virus following close contact between vaccinees and placebo recipients in childcare centers (Murphy 1993; Murphy and Coelingh 2002). The National Institutes of Health (NIH) supported the clinical evaluation of the LAIVs in a series of studies using different strains, tested in different age groups with an exploration of virus shedding and of mucosal and systemic immune responses (Murphy 1993; Murphy and Coelingh 2002).

Commercial development of the vaccine was undertaken by Wyeth and Aviron, the latter was acquired by MedImmune and subsequently by AstraZeneca. The pivotal trial that established the efficacy of the trivalent ca LAIV was a large, randomized, placebo-controlled trial in healthy, 15- to 71-mo-old children (Belshe et al. 1998, 2000a). In the first year of the study, the vaccine was well-tolerated and efficacy was 93% (95% CI 88–96) against culture-confirmed influenza. There was a 29% reduction in the incidence of febrile illness and 35% reduction in febrile otitis media with concomitant antibiotic use (Belshe

et al. 1998). In year 2 of the study, although the circulating epidemic strain influenza A/Sydney/97 (H3N2) was not well matched to the vaccine, the vaccine was 86% efficacious in preventing infection (Belshe et al. 2000a).

The key findings from this period of evaluation of LAIV were establishment of the efficacy of the vaccine in protection against influenza infection, including against a mismatched strain not contained in the vaccine (Belshe et al. 2000a; Mendelman et al. 2004), and vaccine effectiveness in practice, in children and adults (Nichol et al. 1999; Belshe et al. 2004; King et al. 2006; Vesikari et al. 2006; Piedra et al. 2007).

A trivalent LAIV was licensed by the U.S. Food and Drug Administration in 2003 for use in healthy individuals 5–49 yr of age. Licensure was subsequently revised to healthy individuals 2–49 yr of age, and LAIV was recommended as one of the vaccine options for healthy people in the appropriate age group by the Advisory Committee on Immunization Practices (ACIP) that advises the Centers for Disease Control and Prevention (Table 1). In 2013–2014, the U.K. Joint Committee of Vaccination and Immunisation (JCVI) recommended a single dose of LAIV to all children aged 2–16 yr, to directly protect the children themselves but also to protect other vulnerable members of the population by reducing their ability to spread influenza (Pebody et al. 2017a). The program was introduced in a phased approach and has demonstrated that vaccinating children of primary-school age is

associated with reductions in incidence for a range of surveillance indicators in England (Pebody et al. 2018b). In 2007, Norway offered influenza vaccination of 6- to 35-mo-old children following a formal cost-effectiveness analysis and introduced LAIV for 2-yr-olds in 2015/16 (Nohynek et al. 2016).

During the early studies, LAIV was not compared head-to-head with the licensed inactivated influenza vaccine (IIV), but in a postmarketing study, the two products were compared in nearly 8500 children aged 6–59 mo. There were 54.9% fewer cases of influenza in the LAIV group than in the IIV group; the relative efficacy of LAIV was 79% for influenza A/H3N2 and 89% for A/H1N1 infections (Belshe et al. 2007). Clinical data comparing LAIV and IIV in adults were reported in the U.S. military (Eick et al. 2009; Wang et al. 2009) and civilians (Ohmit et al. 2006, 2008; Monto et al. 2009). Unlike what was observed in children, IIV was more efficacious than LAIV in adults, although, notably, in the military, the efficacy of LAIV was more robust in vaccine-naïve service members (new recruits) than in annually revaccinated personnel (Wang et al. 2009). Potential explanations for age-specific differences in efficacy included reduced ability of LAIV to infect some adults because of their prior exposure to similar strains of influenza (Eick et al. 2009; Monto et al. 2009).

Several technical improvements in the vaccine occurred over the course of time, including

Table 1. A summary of ACIP recommendations regarding the use of LAIV

Year	Recommendation	Reference
2003	Recommended as an option for vaccination of healthy persons aged 5–49 yr of age.	Harper et al. 2003
2014	When immediately available, LAIV should be used for healthy children aged 2–8 yr who have no contraindications or precautions.	Grohskopf et al. 2014
2015	Preference of LAIV4 over IIV withdrawn.	Grohskopf et al. 2015
2016	LAIV4 should not be used for the 2016–2017 season.	Grohskopf et al. 2016
2017	LAIV4 should not be used for the 2017–2018 season.	Grohskopf et al. 2017
2018	LAIV4 is an acceptable option for vaccination of persons for whom it is appropriate.	Grohskopf et al. 2018a
2019	LAIV4 is an acceptable option for vaccination of persons for whom it is appropriate.	Grohskopf et al. 2019

(ACIP) Advisory Committee on Immunization Practices, (LAIV) live attenuated influenza vaccine.

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the generation of the reassortant vaccine viruses by reverse genetics instead of traditional co-infection, a transition from a trivalent to a quadrivalent vaccine (LAIV4) containing two influenza A and two influenza B strains in 2013–2014 in the United States, a reduction in the volume of the vaccine to 0.2 mL, with 0.1 mL administered in each nostril, and the development of a refrigerator-stable formulation in place of a product that required a freezer.

EXPERIENCE WITH THE CA LAIV DURING THE 2009 PANDEMIC

Following the public health decision that a vaccine should be produced against the 2009 H1N1 pandemic influenza virus, MedImmune Inc. rapidly developed a monovalent A/H1N1pdm09 vaccine virus based on the A/California/7/2009 (CA/09) virus (Chen et al. 2010) for use in the United States. Preclinical studies in mice and ferrets revealed that the CA/09 LAIV conferred complete protection from challenge with the wild-type virus (Chen et al. 2011). The safety of the vaccine was demonstrated in children and adults (Mallory et al. 2010), and a single dose of the vaccine was demonstrated to be 69% effective in preventing H1N1pdm09 infections in school-aged children (Uzicanin et al. 2012) and 82% effective in preventing H1N1pdm09-associated hospitalization in children aged 3–9 yr (Hadler et al. 2012).

CLINICAL EXPERIENCE POST-2009

CDC U.S. Influenza VE Network

For the last decade, the Centers for Disease Control and Prevention (CDC) have implemented a systematic assessment of vaccine effectiveness (VE) through a network of five clinical sites (U.S. Influenza VE network), using observational test-negative case-control (TNCC) (also called test-negative design or TND) studies that generate estimates of VE against medically attended acute respiratory illness while reducing bias associated with health-care-seeking behavior and misclassification of cases (Sullivan et al. 2016). Patients with influenza-like illness who

present to participating practices are tested for influenza; those testing positive for influenza virus are defined as cases, and those testing negative form the comparison group. VE is estimated by comparing the odds of testing positive for influenza among vaccinated and unvaccinated patients, adjusting for confounders.

VE data from three observational studies in the 2013–2014 season (the first season in which LAIV4 was available) revealed low effectiveness of LAIV against H1N1pdm09 in 2- to 17-yr-old children. Analysis of VE data from the U.S. Influenza VE network for 2010–2011 through 2013–2014 seasons revealed that children aged 2–17 yr had similar odds of influenza regardless of receipt of LAIV3 or IIV3 during 2010–2011 through 2012–2013. However, in 2013–2014, the first season of H1N1pdm09 predominance since the 2009 pandemic, the odds of influenza were significantly higher for 2- to 17-yr-old LAIV4 recipients (OR 2.88; 95% CI 1.62–5.12). The observation that VE of the LAIV against influenza A/H1N1pdm09 was consistently lower than that for IIV prompted the ACIP to change their recommendation for the preferential use of LAIV in children (Table 1; Campbell and Grohskopf 2018). Additionally, in 2014–2015, when an A/H3N2 drift variant circulated, the U.S. Influenza VE network found that neither LAIV4 nor IIV provided significant protection in 2- to 17-yr-old children and LAIV did not offer greater protection than IIV. A systematic review and meta-analysis of the VE against influenza A/H3N2 viruses, the dominant circulating strain during the 2016–2017 season, revealed comparable effectiveness of 51% and 46%, respectively, for LAIV4 and IIV (Mallory et al. 2020a).

In 2015–2016, the manufacturer (MedImmune/AstraZeneca) replaced CA/09 (H1N1pdm09) with A/Bolivia/559/2013 (H1N1pdm09), but in the subsequent season the U.S. Influenza VE network found no significant VE (3%; 95% CI –49–37) for LAIV4 against all influenza A and B viruses combined in 2- to 17-yr-olds or for influenza A/H1N1pdm09 (–21%; CI –108–30) (Grohskopf et al. 2016). A U.S. Department of Defense analysis similarly noted no VE in this age group for the 2015–2016 season. An obser-

vational study funded by the manufacturer reported a higher point estimate of VE, but it was not statistically significant (Poehling et al. 2018). In contrast, IIV was effective in all three studies (Table 2). The ACIP recommended that LAIV4 should not be used for the 2016–2017 and 2017–2018 seasons (Table 1).

Clinical Experience beyond the U.S. VE Network

Notably, VE estimates were not consistent among all studies in the United States (Poehling et al. 2018) and in countries outside the United States (Canada, United Kingdom, Norway, and Germany) that used LAIV (Table 2); VE estimates in the United Kingdom, Norway, and Canada were comparable to previous seasons and were markedly higher than those observed in the U.S. Influenza VE network (Pebody et al. 2016b, 2017a,b, 2018a, 2019; Buchan et al. 2018). These findings were summarized in a meta-analysis (Fig. 1; Caspard et al. 2017); although the point estimates varied, many were not statistically significant (<https://www.cdc.gov/flu/professionals/acip/immunogenicity.htm>). Although a satisfactory explanation has not yet been found for the inconsistent point estimates, the effectiveness of LAIV against H1N1pdm09 was lower than that of IIV (Caspard et al. 2017).

In addition to the observational studies, three randomized studies were conducted since 2009 and are summarized in Caspard et al. (2017).

1. A cluster-randomized, IIV-controlled study in school-aged children in Ontario, Canada, an open label study in 10 elementary schools conducted in 2013–2014 that demonstrated greater protection with LAIV3 than TIV for children and their household contacts in a season dominated by H1N1pdm09 (Kwong et al. 2015).
2. A community-randomized IIV-controlled study in Hutterite communities in Canada, conducted October 2012–May 2015, in which the incidence rate of influenza among vaccine recipients did not differ by vaccine type (Loeb et al. 2016).

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3. A placebo-controlled study in more than 1200 Japanese children aged 7–18 yr who were randomized to receive LAIV4 or placebo in 2014–2015, a season dominated by A/H3N2. LAIV4 efficacy against circulating A/H3N2 strains, all of which were mismatched to the vaccine, was 25.4% (95% CI 4.3–41.7) (Mallory et al. 2018).

The data from the randomized controlled trials were generally consistent with the observational studies: Efficacy against H1N1pdm09 was not consistently demonstrated, and although efficacy against mismatched H3N2 strains was significant, the point estimate was low.

POTENTIAL EXPLANATIONS FOR POOR VE AGAINST H1N1pdm09 VIRUSES AND SUBSEQUENT ACTION

Several potential explanations have been proposed for the observed lack of effectiveness of LAIV against H1N1pdm09 viruses (Ambrose et al. 2016; Penttinen and Friede 2016; Singanayagam et al. 2018; Belshe 2019). These include reduced stability and infectivity of the CA/09 (H1N1pdm09) vaccine virus, conferred by a single amino acid substitution in the HA stalk (Cotter et al. 2014), exposure of some vaccine lots to temperatures above those recommended for storage (Caspard et al. 2016), vaccine virus interference associated with the introduction of the fourth virus in LAIV4 (although the fact that low VE was seen with LAIV3 makes this unlikely), and differences in prior vaccine coverage among children contributing to differences in replicative fitness in different populations, leading to differences in VE, although the U.S. Influenza VE network found no significant effect of prior vaccination (Caspard et al. 2017; McLean et al. 2018).

A detailed analysis by the manufacturer concluded that reduced replicative fitness of both CA/09 and A/Bolivia/559/2013 (H1N1pdm09) strains was the primary root cause of poor VE against circulating H1N1pdm09 viruses. New assays using human nasal epithelial cells and tissue culture infectious dose 50 assays that require multiple rounds of virus replication were

Table 2. Vaccine effectiveness estimates from different locations

Location	Year	Age group (yr)	Versus	VE (95% CI)		Reference
				LAIV	IIV	
U.S. Influenza VE network	2015–2016	2–17	All influenza H1N1pdm09	5% (–47–39) –21% (–108–30)	60% (47–70) 63% (45–75)	Jackson et al. 2017
U.S. Department of Defense ICICLE (eight U.S. sites), funded by MedImmune/AZ	2015–2016 2015–2016		H1N1pdm09 All influenza	15% (–48–51) 46% (7–69)		Poehling et al. 2018
Finland	2015–2016	2	H1N1pdm09 All influenza A, presumed H1N1 B	50% (–2–75) 50.7% (28–66) 47.9% (22–65) 57.2% (0–82)	71% (51–82) 61% (31–78) 79.5% (50–92) –1% (–123–54)	Nohynek et al. 2016
Germany Canada	2012–2013 2012–2013 through 2015–2016	2–17	All influenza H1N1pdm09 H3N2 B	12% (–95–60) 59 (50–66) 69 (56–78) 36 (14–53)	90% (57–98) 60 (52–67) 79 (70–86) 43 (22–59)	Pebody et al. 2018a Buchan et al. 2018
UK	2015–2016	2–17	All influenza H1N1pdm09	74 (62–82) 57.6% (25–76)	56 (41–66) 77.8 (7–95)	Pebody et al. 2016a
UK	Three seasons (2013–2016)		All influenza H1N1pdm09 B	41.5 (–8–68) 81.4 (40–94)	100 (13–100) 56.3 (–122–91)	Pebody et al. 2016a
UK	2017–2018, medically attended laboratory-confirmed influenza	2–17	All influenza H1N1pdm09 H3N2 B	53.1 (31–68) 35.6 (–44–60) 46.7 (–7–73) 56.9 (61–96)	100 (16–100) –22 (–275–60) 24.8 (–153–78)	Public Health England ^a
UK	2018–2019 vs. hospitalization	2–17	All influenza H1N1pdm09	26.9 (–33–60) 90.3 (16–99) –75.5 (–290–21) 60.8 (8.2–83)	64.4 (29–82) 44.4 (–52–80)	Pebody et al. 2020

(VE) Vaccine effectiveness, (LAIV) live attenuated influenza vaccine, (IIV) inactivated influenza vaccine, (ICICLE) Influenza Clinical

Investigation for Children, (AZ) AstraZeneca, (UK) United Kingdom).

^a[https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/779474/](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/779474/Influenza_vaccine_effectiveness_in_primary_care_2017_2018.pdf)

Influenza_vaccine_effectiveness_in_primary_care_2017_2018.pdf.

Figure 1. Vaccine effectiveness (VE) of live attenuated influenza vaccine (LAIV). (Figure reproduced from Caspard et al. 2017 under the terms of the Creative Commons Attribution License.)

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introduced for assessment of potency, and A/Slovenia/293/2015 (H1N1pdm09) was selected for inclusion in the vaccine. The infectivity of the vaccine virus measured by shedding of the vaccine virus was assessed in 200 children aged 2–4 yr who received LAIV3 containing A/Bolivia/559/2013, LAIV4 containing A/Bolivia/559/2013, or LAIV4 containing A/Slovenia/293/2015. A/Slovenia/293/2015 was shed by a higher proportion of children than the comparators, and seroconversion rates were similar to pre-pandemic H1N1s when LAIV was effective (Mallory et al. 2020b).

The ACIP reviewed these data and also discussed combined individual patient level data of VE of LAIV4 and IIV during the 2013–2014 through 2015–2016 seasons, data pooled from five U.S. observational studies (Chung et al. 2019), a systematic review and meta-analysis of LAIV effectiveness for the 2010–2011 through 2016–2017 seasons from the United States and elsewhere (Grohskopf et al. 2018b). These analyses revealed that although LAIV4 was poorly effective or ineffective against H1N1pdm09 viruses in most studies, it was generally effective against influenza B viruses and generally no less effective than IIV against H3N2 viruses. For the 2018–2019 and 2019–2020 seasons, the ACIP recommended that LAIV4 was an acceptable option for vaccination of persons for whom it was appropriate (Table 1).

EXPERIENCE WITH RUSSIAN-BACKBONE LAIV

The effectiveness of the Russian LAIV was compared with IIV and placebo in school children in Novgorod, Russia, over a 2-yr period (Rudenko et al. 1993). In individuals and school-based comparisons, LAIV given in two doses was more protective than one dose of IIV. An indirect effect of vaccination on unvaccinated students and staff was found in schools in which students received LAIV (Rudenko et al. 1993). LAIV has since been widely used in Russia. Russian-backbone LAIV seed strains were provided to developing country manufacturers for pandemic preparedness, through an intellectual property program initiated by the World Health

Organization (WHO) (Rudenko et al. 2011). A monovalent H1N1pdm09 LAIV was manufactured by Serum Institute of India, Ltd (SIIL) and demonstrated VE of 76% (95% CI 42–90) in a case control study in 2010 (Kulkarni et al. 2014).

A seasonal trivalent LAIV manufactured by SIIL was tested in a randomized, placebo-controlled phase 2 study in 300 24- to 59-mo-old children in urban Bangladesh (Ortiz et al. 2015), in which the vaccine was well-tolerated and safe (Ortiz et al. 2015). No child shed the A/H1N1 vaccine strain, but 45% and 67% shed the A/H3N2 or influenza B vaccine viruses, respectively (Lewis et al. 2019). Serum and mucosal antibody responses to influenza A/H3N2 and B were observed, but only a mucosal IgA response to A/H1N1 was seen. Thus, the infectivity and immunogenicity of the H1N1pdm09 component of the vaccine were very low.

This was followed by a two-site randomized double-blind, placebo-controlled, parallel-group trial in which 1174 children aged 2–4 yr received a single dose of Russian-backbone LAIV and 587 received placebo. The vaccine had a good safety profile, and laboratory-confirmed influenza illness due to vaccine-matched strains was seen in 93 (15.8%) of children in the placebo group and 79 (6.7%) in the LAIV group, resulting in VE of 57.7 (95% CI 44–68). There was no efficacy against the mismatched influenza B strain (Brooks et al. 2016).

In striking contrast to the results from Bangladesh, a randomized double-blind, placebo-controlled, parallel-group, single-center trial conducted in Senegal in which 1174 children aged 2–5 yr received a single dose of Russian-backbone LAIV and 587 received placebo revealed no protection against symptomatic laboratory-confirmed influenza. Influenza incidence was high (~20% attack rate), with 210 (18%) endpoints of infection in the LAIV group and 105 (18%) among placebo recipients, resulting in VE of 0.0% (95% CI –26–21). Vaccine-mismatched influenza B was the dominant circulating strain; influenza A/H1N1pdm09 appeared midway through the trial and circulated extensively till the end of the study period. Analysis of secondary endpoints caused by vaccine-matched strains and by type and subtype or lin-

age also revealed no statistical evidence for vaccine efficacy (Victor et al. 2016). Shedding of at least one vaccine virus was reported in 55 (83%) of 66 LAIV recipients but immunogenicity was not assessed. Although nearly one-half of the participants in the Senegal study had been previously vaccinated with IIV, randomization ensured equal distribution in the LAIV and placebo groups. Potential explanations for the differences in the results of the studies in Bangladesh and Senegal that have been considered and largely discounted include differences in potency of the vaccine, previous history of influenza exposure, ecology of the nasopharynx, nutritional deficiencies that might differentially affect replication of the vaccine virus, or a history of recent receipt of oral polio vaccine (Isakova-Sivak et al. 2020).

The poor VE in the Senegal study was more consistent with that reported by the U.S. VE network with the U.S.-backbone LAIV than with that reported with the Russian-backbone LAIV in Bangladesh (Victor et al. 2016). The replicative fitness of the H1N1pdm09 vaccine strain (CA/09) in the LAIV was an ongoing concern. The vaccine strain was updated to an A/Michigan/45/2015-like strain (New York/15/5364; NY15), and in 2017, a prospective, open-label observational phase 4 study of the Russian-backbone trivalent LAIV with the updated H1N1pdm09 strain was undertaken in influenza vaccine-naïve children aged 24–59 mo in a peri-urban area of The Gambia (Lindsey et al. 2019). Vaccine virus shedding and immunogenicity were compared among children who received one dose of LAIV containing CA/09 or NY15 H1N1pdm09 strains. Only 16 (14%) of 118 who received the CA/09 LAIV shed the H1N1 vaccine virus and 6 (5%) seroconverted, whereas 80 (63%) of 126 who received the NY15 LAIV shed vaccine virus and 24 (19%) seroconverted. The NY15 strain also induced more robust T-cell immunity than CA/09 (Lindsey et al. 2019). These data are consistent with the improved shedding indicative of replicative fitness with the U.S.-backbone LAIV containing A/Slovenia/2015 in place of CA/09 and suggest that the poor VE with the Russian-backbone LAIV observed in

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Senegal may be overcome with a different vaccine strain.

CORRELATES OF PROTECTION

Immune markers that predict protection from infection or disease are referred to as correlates of protection. The definition is not always clearly stated, resulting in calls to clarify the terminology and build statistical or epidemiological frameworks for assessing correlates of protection (Qin et al. 2007; Plotkin 2008; Gilbert et al. 2019; Lim et al. 2019).

Neutralizing antibodies in the serum and at the mucosal surface are sufficient to prevent influenza infection; CD4⁺ T cells provide necessary help in B-cell development and CD8⁺ T cells mediate viral clearance. An experimental challenge study with influenza virus established that a serum hemagglutination inhibition (HAI) antibody titer of 1:32 (or 1:40, depending on the dilution series) was associated with a 50% reduction in risk of contracting influenza in a susceptible population and is referred to as the 50% protective titer (Hobson et al. 1972). Conventionally, an HAI titer of 1:40 is often referred to as “seroprotective” and is widely regarded as the correlate of protection for IIV. However, a model based on a meta-analysis of published studies demonstrated that the relationship between HAI antibody titer and protection is better described by a curve, rather than a simple threshold value (Plotkin 2008; Coudeville et al. 2010). A progressive increase in protection was noted with increasing HAI titer, particularly at titers of up to 1:100, irrespective of vaccination status or viral strain (Coudeville et al. 2010).

In contrast to IIV, it has been very difficult to identify a single correlate of protection for LAIV. In adults, resistance to wild-type virus infection following LAIV correlated with nasal wash IgA antibody and serum antibodies to NA (Clements et al. 1986), whereas in children the presence of any serum HAI antibody or nasal wash IgA antibody provided significant protection from virus shedding (Belshe et al. 2000b). Data from three prospective randomized studies comparing LAIV with placebo in children 6–36 mo of age revealed that nasal IgA contrib-

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utes to the efficacy of LAIV, although the inherent heterogeneity in nasal antibody and variability in nasal specimen collection hinders the precise evaluation of mucosal antibody responses (Ambrose et al. 2012).

Notably, however, even in the absence of detectable serum or nasal antibodies, LAIV-vaccinated children have fewer challenge infections than placebo recipients (4 of 16 vs. 16 of 35 in one study), indicating that they were protected by some other, unmeasured factor (Belshe et al. 2000b). In fact, in the 16 LAIV and 35 placebo recipients who lacked both serum HAI and nasal wash IgA antibodies, neutralizing antibodies were detected by microneutralization assay in the sera of 12 LAIV recipients but only one placebo recipient (Belshe et al. 2000b).

A detailed analysis that included 39 strain-specific assays of serum and mucosal immune responses to LAIV in children failed to identify a single measure that was predictive of protection (Wright et al. 2016). Data from a study comparing VE of IIV and LAIV in healthy adults were analyzed using a principal stratification/VE moderation framework, a statistical method for assessing how VE varies over subgroups defined by biomarkers measuring immune responses in vaccinees. The authors found no evidence that LAIV efficacy depended on previous vaccination, baseline HAI or NAI titer, or any post-vaccination titer markers, although there was limited precision in the study to learn about modifiers of LAIV VE (Gilbert et al. 2019).

A similar issue has been noted with the Russian-backbone LAIV (Brooks et al. 2016; Lewis et al. 2019). Vaccinees with lower baseline immunity to influenza had significantly higher odds of shedding a vaccine virus in the week following administration of the vaccine, and the magnitude of immune responses was most pronounced in children with detectable virus shedding (Brickley et al. 2019), but neither vaccine virus recovery nor immunologic response was fully predictive of a protective response to the Russian-backbone LAIVs (Brickley et al. 2019).

In summary, serum and mucosal antibodies are “co-correlates” of protection, defined as one of two or more correlates of protection, that may

be synergistic with other correlates (Plotkin 2008). Although high rates of seroconversion are associated with high efficacy, efficacy of LAIV is also observed with low rates of seroconversion (Bandell et al. 2011). No single assay predicts protection, but the range of available assays and sophisticated technical advances may lead to the identification of one, or an algorithm that incorporates different immunologic responses, that will predict LAIV-mediated protection.

PANDEMIC LAIV

Following the identification of avian H5N1 and H9N2 virus infections in humans, the NIH undertook a pandemic influenza vaccine development program in 2003 under a collaborative research and development agreement with MedImmune, using the U.S. LAIV platform. This approach was selected because the technology was licensed; seasonal LAIV was optimally balanced in attenuation and efficacy, LAIV-induced serum and mucosal antibody responses, T-cell responses, and broader cross-protection against antigenic drift variants, and seasonal LAIV had greater yield per egg than IIV (Rudraraju et al. 2019). The HA and NA gene segments of the pandemic LAIVs (pLAIVs) were derived from avian influenza viruses of different subtypes, including H2, H5, H6, H7, and H9, on a backbone of six internal protein genes of the influenza A MDV, that conferred ts and attenuation phenotypes. Known virulence motifs in the HA such as the multibasic amino acid cleavage site in H5 and some H7 viruses were removed by genetic engineering (Li et al. 1999; Min et al. 2010). The candidate pLAIVs were evaluated extensively in preclinical studies in mice and/or ferrets and, based on promising results, were advanced to phase I clinical trials for assessment of their safety in healthy adults. Eight pLAIVs representing five avian influenza subtypes were well-tolerated on clinical evaluation (Karron et al. 2009a,b; Talaat et al. 2009, 2011, 2013; Babu et al. 2014; Sobhanie et al. 2016). However, the vaccines were highly restricted in replication and were poorly immunogenic (Karron et al. 2009a,b; Talaat et al. 2009,

2011, 2013; Babu et al. 2014; Sobhanie et al. 2016). This was surprising for two reasons: first, because pLAIVs had replicated well and were immunogenic in mice and ferrets, and, second, because the healthy adults who received the pLAIV had no prior exposure to avian influenza viruses. Several potential explanations were investigated including the sialic acid receptor preference of the pLAIVs and inhibition of vaccine virus replication by anti-NA antibodies induced against human NAs cross-reacting with avian NAs or by cross-reactive T-cell responses (Peng et al. 2015), but none provided a satisfactory explanation for the restricted replication and immunogenicity in humans.

Russian-backbone pandemic LAIVs were also developed and evaluated in preclinical and clinical trials; some were 7:1 reassortant viruses bearing only the HA gene segment from a virus with pandemic potential (Rudenko and Isakova-Sivak 2015). Although they were not directly compared in a clinical trial, vaccine virus shedding and immunogenicity of the Russian-based pandemic LAIVs appeared to be greater than the U.S.-based pandemic LAIVs, a difference that was speculated to be due to differences in infectivity of the vaccine viruses. A direct comparison of H5N2 vaccines on the Russian and U.S.-based LAIV backbones in the ferret model showed no differences in infectivity, immunogenicity, and protective efficacy, suggesting that the differences in the clinical performance of the vaccines were due to factors other than the inherent biological properties of the two MDVs (Czakó et al. 2018).

In the meantime, several studies were published reporting that administration of a booster dose of subunit H5N1 pIIV to subjects previously primed with a variety of H5 vaccines (recombinant expressed H5 HA, DNA encoding H5 HA, or an H5N1 pIIV) resulted in a robust HAI and neutralizing antibody responses, even in individuals who had no detectable antibody response following initial vaccination (Rudraraju et al. 2019). A series of clinical trials was undertaken in which recipients of H5 or H7 pLAIVs were recalled or prospectively enrolled to receive sequential doses of matched pLAIV and pIIV (Babu et al. 2014; Talaat et al. 2014; Sobhanie

et al. 2016). A great majority (64%–79%) of the pLAIV-primed individuals had a robust and rapid antibody response (geometric mean HAI titer 119 to 175) to a booster dose of the corresponding pIIV administered 3 mo to nearly 5 yr later. The antibody was highly cross-reactive with other strains within the same subtype. These data proved that pLAIVs establish long-lasting immune memory that could be recalled with a single dose of pIIV (Rudraraju et al. 2019). These findings with pLAIV were consistent with reports of robust antibody responses in subjects boosted with pIIV following priming with DNA or adenovirus expressing the H5 HA (Ledgerwood et al. 2011; Gurwith et al. 2013; Khurana et al. 2013). Notably, similar observations have been reported with an H5 Russian-backbone pLAIV (Pitisuttithum et al. 2017).

The immunologic basis for the pLAIV/pIIV prime-boost phenomenon was investigated in an African green monkey model (Matsuoka et al. 2014; Jegaskanda et al. 2018), which revealed that intranasally administered pLAIV elicited a highly localized and somatically hypermutated germinal center B-cell response in the mediastinal lymph nodes, which was rapidly recalled following pIIV boost to germinal center reactions at distant immune sites, most notably the local draining axillary lymph node (Jegaskanda et al. 2018). It is plausible that the primary immune response to LAIV in humans is similarly restricted to a draining lymph node; this may explain why serum antibody is not a reliable correlate of immunity for seasonal and pandemic LAIVs (Jegaskanda et al. 2018).

The magnitude and quality of the antibody response following sequential administration of pLAIV and pIIV are superior to unadjuvanted pIIV (Babu et al. 2014; Talaat et al. 2014), and this approach merits further consideration and development for pandemic preparedness efforts because it can generate broad immunity within a novel avian influenza subtype.

CONCLUDING REMARKS

The attractive features of LAIV include ease of administration, stimulation of systemic and mucosal antibodies, a T-cell response that can con-

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fer greater breadth of protection than IIV, reduction in the spread of influenza conferring indirect protection to unvaccinated contacts, and cost-effectiveness. The WHO has recognized that LAIVs have the potential to address the unmet need for an influenza vaccine for young children that is programmatically suitable for low- and middle-income countries (Penttinen and Friede 2016). In the area of pandemic preparedness, LAIVs have additional advantages over IIVs, including the establishment of long-lasting B-cell memory that can be recalled rapidly, resulting in high-titer, high-quality, and cross-reactive antibodies; also, the yield of LAIV in embryonated eggs is greater than that of IIV, and this may be an important consideration in the event of a pandemic. However, despite the very impressive performance of LAIV in early clinical trials, some troubling observations have been made in the last decade with both the U.S. and Russian-backbone LAIVs that include the inconsistent VE of LAIV4 containing H1N1pdm09 viruses, inconsistent findings with the Russian-backbone LAIV in Bangladesh and Senegal, and the lack of reliable correlates of protection. It will be important to understand the biological basis for these findings and develop practical strategies to identify and avoid selecting LAIV strains that are over-attenuated (Belshe 2019) in order to take full advantage of these valuable vaccines and implement them in our public health systems.

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