Effect of Heterosubtypic Immunity on Infection with Attenuated Influenza A Virus Vaccines in Young Children

MARK C. STEINHOFF,^{1*} LOUIS F. FRIES,¹ RUTH A. KARRON,¹ MARY LOU CLEMENTS,¹ AND BRIAN R. MURPHY²

Center for Immunization Research, Department of International Health, School of Hygiene and Public Health, and Departments of Pediatrics and Medicine, School of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205,¹ and Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20814²

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Resistance to infection with an influenza A virus conferred by previous infection with an influenza A virus belonging to another subtype is called heterosubtypic immunity. Heterosubtypic immunity is demonstrable in laboratory animals but is believed to be weak in humans. The present study examined whether heterosubtypic immunity from previous influenza virus infection induced resistance to infection with an attenuated influenza A vaccine virus. Two groups of vaccinees consisting of young infants and children who received either influenza A H1N1 or H3N2 attenuated virus were studied. Influenza A H3N2 virus vaccine recipients were classified by their preexisting H1N1 heterosubtypic antibody level induced by prior infection with wild-type virus, and the H1N1 vaccinees were classified by their history of infection with H3N2 vaccine virus. For both groups of vaccinees, the rates of seroconversion and virus shedding and the level of vaccine virus replication were compared in subjects with and without heterosubtypic immunity. In 48 influenza A H3N2 virus and 39 H1N1 virus vaccinees, heterosubtypic immunity had no demonstrable effect on infectivity, immunogenicity, or replication of attenuated vaccine virus. These observations confirm the weak nature of heterosubtypic immunity in humans and suggest that it will not limit the utility of live attenuated influenza A viruses in young infants and children.

Currently two influenza A virus subtypes are epidemic in humans, specifically those with H1N1 and H3N2 hemagglutinin and neuraminidase surface glycoproteins, which are the major protective antigens of the influenza A virus (2). Since humans can be sequentially infected with more than one subtype of influenza A virus in the same season (1, 8), it is clear that the resistance conferred by infection with one wild-type influenza A virus (e.g., H1N1) to infection with a second wild-type influenza A virus belonging to a different subtype (e.g., H3N2) is both weak and transient (2). Nonetheless, this type of immunity, referred to as heterosubtypic immunity, is readily demonstrable in mice and ferrets (3, 9). Even though heterosubtypic immunity in humans appears to be largely ineffective against a wild-type virus, it is possible that such immunity could have a more pronounced effect against infection with an attenuated vaccine virus. Therefore, we sought to determine the effect of heterosubtypic immunity on the level of replication and immunogenicity of live attenuated influenza A virus vaccines that are being developed for the prevention of disease in adult and pediatric populations.

Live attenuated H1N1 and H3N2 influenza A virus vaccines have previously been evaluated in our center as monovalent vaccines in infants and children selected as seronegative for the vaccine virus by hemagglutination inhibition assay (HAI) (5, 6). Some of the seronegative vaccinees had been infected previously with a wild-type or an attenuated influenza virus belonging to another subtype; i.e., some H1N1 vaccinees were previously infected with H3N2 virus and vice versa. Therefore, it was possible to examine

whether heterosubtypic immunity induced by previous infection with a wild-type or an attenuated influenza A virus could induce resistance to subsequent infection with an attenuated influenza vaccine virus. We have compared the levels of replication of vaccine virus and the serum antibody responses in vaccinees with and without evidence of previous infection with a heterosubtypic influenza A virus.

MATERIALS AND METHODS

Guidelines for human experimentation of the U.S. Department of Human Services, The Joint Committee for Clinical Investigation of the Johns Hopkins University School of Medicine, and the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases were followed in the conduct of this study.

In previous evaluations of avian-human (ah) and cold-adapted (ca) live attenuated influenza A/Bethesda/85 (H3N2) and A/Kawasaki/86 (H1N1) reassortant virus vaccines, we enrolled 231 healthy infants and children aged 6 to 36 months who were seronegative (titer of <1:8 by HAI) for the vaccine virus. The details of these placebo-controlled vaccine evaluations have been published previously (5, 6). Evaluation of the H3N2 vaccine preceded that of the H1N1 vaccine. Although the ah H1N1 vaccine was more reactogenic than the ca H1N1 vaccine, the overall levels of replication and immunogenicity were similar for both ah and ca H1N1 reassortants (6). The ah and ca H3N2 reassortants did not show a difference in reactogenicity, immunogenicity, or level of viral replication (5). As a consequence, data from ah and ca vaccine recipients of each subtype were combined for this report. The findings from 48 of the H3N2 vaccinees and 39 of the H1N1 vaccinees were further analyzed in this report. This subset of vaccinees was selected for this anal-

* Corresponding author.

TABLE 1. Effects of heterosubtypic immunity to influenza A virus in children who receive live attenuated influenza A virus vaccine^a

Vaccine virus	Preimmunization status	No. of vaccinees	% Infected	Vaccine shedding		Antibody response to vaccine virus		
				%	Score	% with seroconversion	Mean ELISA antibody titer ^b	
							Prevaccination	Postvaccination
H3N2	H1N1 HAI antibody titer							
	≥1:8 (6.2 ^b)	21	81	33	2.7	76	7.5	9.6
	<1:8 (2.0 ^b)	27	71	46	3.9	74	6.7	9.5
H1N1	H3N2 vaccine status ^c							
	Infected	17	88	59	2.0	82	8.5	11.8
	Not infected	22	91	41	1.9	91	7.6	10.9

^a The differences in means and proportions between vaccinees with and without heterosubtypic immunity do not reach statistical significance.

^b Mean of reciprocal log₂ antibody titers.

^c Infected subjects are those who demonstrated H3N2 vaccine virus shedding or seroconversion among children who participated in H3N2 vaccine studies; uninfected subjects were not infected in H3N2 vaccine studies and had H3N2 HAI titers of <1:8.

ysis because sufficient pre- and postimmunization sera were available to permit the appropriate retesting of specimens needed for this analysis.

Both HAI and enzyme-linked immunosorbent assay (ELISA) serology were performed at the time of immunization of the children in these evaluations. Preimmunization HAI screening for both H1N1 and H3N2 subtypes was performed for 39 H1N1 vaccinees and 48 H3N2 vaccine recipients. Serum immunoglobulin G antibodies in preimmunization and 4-week postimmunization sera were measured by ELISA using purified homologous whole vaccine virus as an antigen (5). The effect of preexisting heterosubtypic immunity on infection with vaccine virus was evaluated by measuring (i) the rate of seroconversion (an at least fourfold rise in serum immunoglobulin G or nasal wash immunoglobulin A antibody) and the mean pre- and postimmunization ELISA serum antibody titers, (ii) the frequency and magnitude of virus shedding (quantitative recovery of vaccine virus from nasal secretions during a 7- to 10-day period of observation after inoculation), and (iii) the rate of infection with vaccine virus (the occurrence of either seroconversion or virus shedding). The magnitude of virus shedding was compared between those with and without heterosubtypic antibody by using a virus shedding score. This score was derived by adding the titers of virus shed on each day for 7 days after inoculation. For example, if a subject shed vaccine virus for 2 days at log₁₀ titers of 0.75 and 1.50 50% tissue culture infective doses per ml, respectively, the shedding score would be 2.25.

For H3N2 vaccinees, we defined heterosubtypic immunity as a titer of HAI antibody to H1N1 virus of ≥1:8. Very few of the 39 H1N1 vaccinees had H3N2 HAI antibody titers of ≥1:8, indicating that few of the vaccinees had been infected previously with wild-type H3N2 virus. Therefore, for H1N1 vaccinees we defined heterosubtypic immunity as documented prior infection with H3N2 vaccine virus (H3N2 virus shedding or seroconversion in children who participated in an H3N2 vaccine study). Seventeen of the 39 H1N1 vaccinees had been previously infected with an H3N2 vaccine.

The means of titers of heterosubtypic HAI antibody titer or of virus shedding scores were compared by analysis of variance tests, and the rates of infection, seroconversion, and virus shedding were assessed by two-tailed chi-square tests.

RESULTS

The effects of previous heterosubtypic influenza virus infection on infection with influenza A vaccine virus are shown in Table 1. For H3N2 vaccinees, there were no significant differences in the rates of infection, seroconversion, or virus shedding or in the mean shedding scores between vaccinees with high and low heterosubtypic (H1N1) HAI antibody titers. There were no differences in these outcomes between those who had previously received *ca* or *ah* H1N1 vaccine. Similarly, for H1N1 vaccinees, there were no significant differences in rates of infection, seroconversion, or H1N1 vaccine virus shedding or in mean shedding scores between those who were previously infected with H3N2 virus and those who were not. For both H3N2 and H1N1 vaccinees neither the mean pre- and postimmunization ELISA antibody titers nor the mean rises in titer were significantly different between children with and without heterosubtypic immunity. None of the rates or means in Table 1 were statistically significantly different between vaccinees with and without heterotypic immunity ($P > 0.40$ for all analysis of variance and chi-square tests).

DISCUSSION

Our data indicate that heterosubtypic influenza A virus antibodies do not appear to influence immunogenicity, infectivity, or the magnitude of virus shedding in young children who are inoculated with a live attenuated influenza A H1N1 or H3N2 virus. A subgroup of our subjects had been immunized and infected with attenuated H3N2 vaccine virus before receiving H1N1 vaccine virus. Despite documentation of heterosubtypic H3N2 virus infection, these children were as likely to be infected with H1N1 vaccine virus as were subjects who had not been previously immunized.

Wright et al. have also shown little impact of heterologous influenza virus antibodies on viral shedding of *ca* influenza A virus vaccines (7). Our data are also in accordance with two prior reports that show little heterosubtypic immunity in subjects sequentially infected with different influenza A virus subtypes in a single winter (1, 8). In contrast, a single study of immunized Japanese schoolchildren reported evidence of protection against H1N1 infection after infection with H3N2 virus documented by HAI serology (4). Our data for young children seronegative to vaccine virus fail to provide evidence of protection associated with heterosub-

typic antibody, although our previous studies showed that these vaccines clearly induced protection against reinfection with homologous vaccine virus (5, 6). In fact, the observation that proven infection with an attenuated H3N2 virus did not influence infection with an H1N1 virus suggests that neither serum or nasal antibody nor nonantibody mechanisms, including the cumulative effect of cellular responses to influenza virus antigens, appear to provide substantial heterosubtypic protection in young children. These experimental data derived from the study of live attenuated vaccine viruses are consistent with the epidemiologic observations in 1957 and 1968 when a newly introduced influenza A virus subtype caused widespread infection in a population with extensive preexisting heterosubtypic immunity. Our data and the existing epidemiologic data further suggest that immunity to influenza virus surface glycoproteins (hemagglutinin and neuraminidase), rather than immunity to cross-reactive internal proteins, is required to prevent or significantly modify influenza virus infection.

These observations suggest that heterosubtypic immunity does not present an obstacle to the immunogenicity of live attenuated influenza A viruses in young children. Accordingly, neither a history of infection by wild-type heterosubtypic virus nor prior immunization with live heterosubtypic virus should limit the utility of live attenuated influenza virus vaccines in this age group.

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