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Cross-Reactive Antibodies Induced by a Monovalent Influenza B Virus Vaccine

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Influenza viruses related to the markedly antigenically divergent strains B/Yamagata/16/88 and B/Victoria/2/87 are circulating in human populations. Adults develop cross-reacting antibodies against recent and earlier influenza B virus strains after vaccination with B/Yamagata/16/88, probably because of previous influenza B virus infections or immunizations. Vaccines containing B/Yamagata/16/88 should adequately protect adults against B/Victoria/2/87 infections.

Influenza viruses cause illness in human populations during the winters of most years (1–3, 9, 18, 19). While two types of influenza A virus (H3N2 and H1N1) have been in circulation since 1977, two distinct evolutionary lines of influenza B virus represented in human populations by the reference strains B/Victoria/2/87 and B/Yamagata/16/88 have only recently gained attention (1, 15). The occurrence of infections caused by both lines of influenza B virus during the same season in many parts of the world including the United States is a cause for concern, since modern trivalent influenza virus vaccines contain a single influenza B virus component (1–3, 9, 18, 19). Although protection against infection may be achieved with a vaccine using inactivated influenza virus, reimmunizations are generally required when antigenically altered strains appear. The replacement of B/Victoria/2/87 with B/Yamagata/16/88 as the strain for the recommended trivalent influenza vaccine for the 1989–1990 influenza season resulted from epidemiologic and immunologic data, including the geographic advance of infections caused by B/Yamagata/16/88-like viruses (18, 19) and the relatively poor stimulation of antibodies against B/Yamagata/16/88 in people vaccinated with B/Victoria/2/87 (7).

As part of strain characterization and standardization procedures, antisera were produced in mature animals. Sheep antisera (comparable to ferret sera in specificity of reactivity [17]) were obtained after intramuscular injection of purified hemagglutinins (Connaught Laboratories, Swiftwater, Pa.) in complete Freund adjuvant for the primary dose and one to three booster doses in incomplete Freund adjuvant. Ferret antisera were produced by infection of ferrets with influenza virus given as nose drops.

Antibody titers were determined by standard hemagglutination inhibition (HI) techniques (13), except that the test antigens were gradient-purified, detergent-disrupted monovalent vaccine pools (Connaught Laboratories). The strains for the studies included influenza viruses B/Yamagata/16/88, B/Victoria/2/87, B/Ann Arbor/1/86, B/USSR/100/83, and B/Singapore/222/79, which represent the commercial vaccines used in the last decade. As determined by hemagglutinin sequence analysis, influenza virus B/Singapore/222/79 represents the ancestor of contemporary strains which have diverged with B/Ann Arbor/1/86 and B/Victoria/2/87 on one path and B/USSR/100/83 and B/Yamagata/16/88 on a second path (15).

Unlike the antisera produced by immunization of animals with other influenza B virus strains, the antiserum produced by immunization with influenza B/Yamagata/16/88 was highly monospecific and showed little heterotypic reactivity (Table 1). The absence of antibodies that are cross-reactive with influenza virus B/Victoria/2/87 in serum from the sheep immunized with influenza virus B/Yamagata/16/88 was confirmed by identical results from ferrets infected with B/Yamagata/16/88 (3, 14).

In order to determine whether people would produce antibodies with cross-reactivity against B/Victoria/2/87, healthy volunteers received a monovalent, inactivated, detergent-disrupted subunit influenza B/Yamagata/16/88 vaccine (Connaught Laboratories). A group of ambulatory elderly persons who had all been immunized in the previous year and most of whom had been immunized in other preceding years was enrolled to reflect immunization of individuals in high-risk groups for whom annual vaccination against influenza viruses is recommended (9). A group of young adults with histories of infrequent immunizations (less than 15% had been immunized with an influenza virus vaccine within the preceding year and 50% had never been immunized) was chosen to reflect vaccination of unselected persons of unimpaired immune responses.

After informed consent was obtained, a preimmunization blood sample was collected, and the vaccine was then administered by intramuscular injection into the deltoid muscle (15 μg of viral hemagglutinin per 0.5-ml dose). The postimmunization blood sample was collected approximately 3 weeks later. Pre- and postimmunization serum samples from the same individual were tested simultaneously for each influenza B virus antigen.

Fifty-four volunteers were immunized with the monovalent vaccine: 24 elderly (age range, 69 to 83; mean, 74) and 30 young adults (age range, 23 to 45; mean, 35). Sixty-three percent of each group were males. The percentage of the elderly with preimmunization HI titers that were ≥1:32 was less than 50% for B/Yamagata/16/88 but more than 60% for other strains (Table 2). The percentage of preimmunization HI titers that were ≥1:32 among the young adults varied from 13 for B/Yamagata/16/88 to almost 40 for other strains.

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TABLE 1. HI antibody titers for sera from immunized animals

Vaccine[a]	HI antibody titers for[b]:				
	Yam	Vic	Ann	USSR	Sing
B/Yamagata/16/88	640	80	20	20	160
B/Victoria/2/87	<20	320	80	160	320
B/Ann Arbor/1/86	<20	80	320	160	320
B/USSR/100/83	<20	80	80	320	320
B/Singapore/222/79	<20	80	160	160	640

[a] Detergent-split subunit vaccine.
[b] Values shown are the reciprocals of the titer. Yam, sheep antiserum to B/Yamagata/16/88; Vic, sheep antiserum to B/Victoria/2/87; Ann, sheep antiserum to B/Ann Arbor/1/86; USSR, ferret antiserum to B/USSR/100/83; Sing, sheep antiserum to B/Singapore/222/79.

The findings of increased frequency of preimmunization titers that were ≥1:32 and the preimmunization geometric mean titers (GMTs) of HI antibodies being two to three times higher among the elderly than among young are consistent with the fact that the elderly received the influenza vaccine with greater frequency before the study, and it indicates the noncomparability of the two populations.

After immunization there were increases in HI titers in serum against all of the influenza B virus antigens tested. Seventy-five percent or more of the elderly had postimmunization HI titers of ≥1:32 against each of the influenza B virus strains. The magnitude of the increases in HI titers among the elderly was somewhat less than a doubling of the GMT for each antigen. The relatively modest increase in HI titers was reflected by the observation that fourfold or greater increases in HI titers were identified for the test antigens in 8% or fewer of the serum pairs from the elderly.

The most marked increase in HI titers seen after immunization among the young adults was a more than sixfold increase in GMT for the vaccine strain between the pre- and postimmunization sera. All but one of the young adults had postimmunization titers of ≥1:32 against the vaccine strain. The percentages of postimmunization titers that were ≥1:32 against the other influenza B antigens were lower, with the lowest result against B/Victoria (63%). However, the GMTs for all strains were increased by more than twofold. HI titers against the vaccine strain increased fourfold or greater for 87% of the young adults, and HI titers against the other influenza B antigens increased fourfold or greater for 33 to 43%.

These studies indicate that immunization with a vaccine derived from influenza B/Yamagata/16/88 produces increased amounts of HI antibodies in young and elderly adults against a broad range of influenza B viruses, including influenza virus B/Victoria/2/87. In this study, immunization represents the first exposure of the volunteers to B/Yamagata/16/88-like viruses, since only strains related to B/Victoria/2/87 were identified in the United States before the completion of the study (1, 2, 15, 18, 19). Immunization and natural exposure to strains with shared epitopes (such as B/USSR/100/83 and B/Singapore/222/79) are indicated by preimmunization HI antibody levels for B/Yamagata/16/88. However, the increases in amounts of antibodies to strains involved in previous exposures after immunization with the B/Yamagata/16/88 vaccine are in concert with "original antigenic sin" (4, 5), particularly for the elderly, whose postimmunization GMT for B/Yamagata/16/88 was lower than those for other influenza B antigens.

Unlike previous influenza B virus strains, the hemagglutinin of influenza virus B/Yamagata/16/88 produces a narrowly reactive antibody response against influenza B viruses in animals. The closer relation of B/Yamagata/16/88 to B/Singapore/222/79 and B/USSR/100/83 than to B/Ann Arbor/1/86 and B/Victoria/2/87, as determined by hemagglutinin sequence data (15), is not reflected in the HI results presented here for the animal sera. However, the lack of heterologous HI antibodies after immunization with B/Yamagata/16/88 results mainly from immunization with a single strain for primary and subsequent exposures. The use of purified hemagglutinin (rather than vaccine containing additional viral peptides) for immunization of the sheep probably does not account for the effect, since ferrets infected with influenza B/Yamagata/16/88 and other recent related variants also produce narrowly defined antibody responses (3, 14) and since the experience is reproducible in immunologically unprimed children, who developed no detectable neutralizing or HI antibodies against B/Victoria/2/87 after immunization with a commercial subunit vaccine containing B/Yamagata/16/88 (11).

Influenza B virus infections and immunizations often produce relatively low HI titers (12), an effect which is the result of not only variable strain immunogenicity but also the method of measuring HI antibodies (10, 12). Antigens from influenza B viruses disrupted ("split") by treatment with an organic solvent such as ether provide a more sensitive but less specific assay (8, 10, 12, 16). However, the HI titers obtained with antigens after ether or detergent disruption correlate extremely well with neutralization antibody titers, and extrapolation from published studies of protection from infection and illness suggests that HI titers at dilutions in

TABLE 2. Increases in HI antibody titers[a]

Virus strain	Elderly (n = 24)					Young (n = 30)				
	% ≥32[b]		GMT[c]		Fourfold[d]	% ≥32[b]		GMT[c]		Fourfold[d]
	Pre	Post	Pre	Post		Pre	Post	Pre	Post	
B/Yamagata/16/88	46	75	26	44	8	13	97	13	84	87
B/Victoria/2/87	62	75	35	60	4	17	63	14	36	33
B/Ann Arbor/1/86	83	92	44	62	0	37	77	18	43	37
B/USSR/100/83	71	83	41	53	0	27	83	16	53	40
B/Singapore/222/79	67	88	33	62	8	20	67	16	46	43

[a] Increases in HI antibody titers to various influenza virus strains in sera of elderly and young adult volunteers immunized with a monovalent influenza virus B/Yamagata/16/88 vaccine.
[b] Percentage of pre- and postimmunization serum samples with antibody to indicated virus strains at dilutions greater than or equal to 1:32.
[c] Reciprocal of GMT for pre- and postimmunization serum samples.
[d] Percentage of persons with fourfold or greater increases in titer between pre- and postimmunization serum samples.

excess of 1:32 after immunization with detergent-disrupted antigens could be associated with protection against illness and infection (6, 10, 11, 16). Since the presence of neutralizing antibody is the best surrogate measure of protection, which is the desired outcome of immunization, the antibody responses detected by HI with detergent-disrupted antigens suggest that protection against many influenza B virus strains including those related to B/Victoria/2/87 should be expected in adults after immunization with a vaccine containing B/Yamagata/16/88.

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