Comparison of New Triton X-100- and Tween-Ether-Treated Split-Product Vaccines in Children

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Split-product vaccines (SPVs) combine the desirable properties of no systemic reactogenicity and adequate immunogenicity when two doses are given. We compared a new Triton X-100 SPV (Connaught Laboratories, Inc.) with the commercially available Tween-ether SPV (Parke-Davis & Co.) in 76 children and young adults 2 to 25 years old; there were 39 and 37, respectively, in each vaccine group. Both vaccines contained influenza A/Brazil/78, A/Texas/77, and B/Hong Kong/72 (7 μ g of hemagglutinin for each strain); two doses were administered 1 month apart. Among persons seronegative by the hemagglutination inhibition test, the geometric mean antibody titers rose to approximately 100 after the first vaccination for influenza A/Brazil/78 and A/Texas/77. For B/Hong Kong/72, however, seronegative recipients developed lower geometric mean titers of approximately 32 after one immunization. Against the new B/Singapore/79 strain neither SPV stimulated adequate cross-reacting hemagglutination inhibition antibody (geometric mean titers of approximately 10). In conclusion, the new Triton X-100 SPV appears to be comparable to the ether-treated SPV in primed subjects. Further studies in unprimed children should be done to confirm this impression. In addition, it would be advisable to study other dosage regimens in unprimed children with these SPVs.

In recent years, split-product vaccines (SPVs) have been shown to be adequately immunogenic and less reactogenic than whole-virus vaccines (4, 11). A Tween-ether vaccine (manufactured by Parke-Davis & Co.) and a tributyl phosphate vaccine (manufactured by Wyeth Laboratories) are currently the only licensed SPVs used in the United States. Other detergents have been used to split influenza virus to avoid the problems of working with ether; for example, Triton X-100, a nonionic detergent (Connaught Laboratories), and cetyl-trimethylammonium bromide, a cationic detergent (Sandoz Pharmaceuticals), are being used to produce SPVs in Europe, where they have been shown to be safe and effective in primed adults studied (1; D. Metzgar, Connaught Laboratories, personal communication). Ammonium deoxycholate and Nonidet are additional detergents used elsewhere (1, 10). These newer unlicensed vaccines, however, have not been extensively studied in unprimed high-risk children.

In the fall of 1979 we performed a clinical

study to compare standard doses of a Triton X-100 SPV to the licensed Tween-ether SPV in high-risk children. Both vaccines proved to be non-reactogenic and equally immunogenic. This is the first published study demonstrating the immunogenicity of Triton X-100 vaccine.

MATERIALS AND METHODS

In the fall of 1979, we vaccinated children with moderate and severe asthma at Babies Hospital and children with cystic fibrosis at St. Vincent's Hospital and Medical Centers of New York. Vaccines were coded at the Bureau of Biologics, Food and Drug Administration, Bethesda, Md., and given by the double-blind method. Informed consent was obtained from the parent or, where appropriate, from the patient.

The vaccines were obtained from a commercial lot of vaccine from Parke-Davis & Co., Detroit, Mich. (no. YH 604) and from an experimental lot produced by Connaught Laboratories, Swiftwater, Pa. (no. 2934 from D. Metzgar). Each 0.5-ml dose contained 7 μ g of hemagglutinin (HA) from A/Brazil/11/78 (H1N1), 7 μ g of HA from influenza A/Texas/1/77 (H3N2), and 7 μ g of HA from influenza B/Hong Kong/5/72. Chil-

dren between 6 months and 3 years of age received two 0.25-ml intramuscular injections 1 month apart. Those between 3 and 26 years received two 0.5-ml intramuscular injections 1 month apart. If an individual in either age group had received at least one dose of the 1978-1979 influenza vaccine, only one dose of the current vaccine was administered.

Clinical observation. Local and systemic reactions were monitored for 48 h after priming and booster vaccinations as done in the 1976 national influenza immunization trials (4). Temperatures were recorded at 0, 6, 9, and 24 h and later if indicated. The symptom index was defined as a point per degree (or fraction of degree) of fever starting with 100°F (ca. 37.8°C) plus the sum of scores for malaise, headache, and nausea (scale of 0 to 3). The total for each person was divided by the number of persons vaccinated. Neurological symptoms within 10 weeks after immunization were also monitored.

Serological studies. Blood for serological testing was collected before and 1 month after each vaccination.

Hemagglutination inhibition (HAI) tests were done by the microtiter method with an initial test serum dilution of 1:10 as previously described (6). The test antigens were A/Brazil/11/78, A/Texas/1/77, and B/Singapore/222/79 (ether treated, Parke-Davis & Co.), supplied by the Respiratory Branch, Centers for Disease Control, Atlanta, Ga., and a high-passage influenza B/Hong Kong/72 strain (BX-1 recombinant) supplied by the Bureau of Biologics, Food and Drug Administration. Individuals without detectable HAI antibody at the initial test dilution (1:10) were considered seronegative.

Neutralization tests were done in monolayer roller-tube cultures of cynomolgus monkey kidney cells. Virus strains used were identical to those used in the HAI test. The challenge virus dose was 50 50% tissue culture infective doses (3). The initial serum concentration tested was undilute. Complement fixation tests for influenza B were done by a modified Kolmer microtechnique (8, 9). All serological titers are expressed as the reciprocal of the serum dilution. Comparison of geometric mean antibody titers (GMTs) of HAI and neutralization antibodies employed Student's *t* test.

Neutralization tests and ether-treated antigens were used for influenza B only to further explore the unprimed state. In the age groups studied, most persons were primed for the influenza A strains (5).

RESULTS

Seventy-six children and young adults were vaccinated. After the reactogenicity and immunogenicity results were recorded, the vaccine code was broken. There were 39 recipients of the Triton X-100 vaccine and 37 recipients of the Tween-ether vaccine. No febrile reactions or other systemic reactions were noted within 24 h postvaccination; hence the reaction index was zero for both groups. Local arm tenderness was noted in six recipients of the Tween-ether vaccine and in seven who received the Triton X-100 vaccine.

Antibody responses were similar for the two vaccines. Adequate numbers of patients for statistical analysis were present in all age groups except the "after second vaccination" group, where many of the patients did not need a second immunization because of prior immunization with related strains. Only three failed to return when asked to do so.

The antibody responses to the B/Hong Kong/72 strain are shown in Table 1. Children without detectable HAI antibodies developed a GMT of about 30, whereas those with preexisting HAI antibody developed a GMT more than three times as high after the first immunization. By 6 months postvaccination, the GMTs were below what is considered a protective level, though they were higher than before vaccination in the initially seronegative group. In the initially seropositive group, the antibody titer had returned almost to the pre-immunization level. Twenty-four children without detectable HAI antibody had sufficient sera to analyze for neutralizing antibody. Those with no pre-immunization neutralizing antibody had significantly lower antibody response to the first immunization than did those with detectable neutralizing antibody (Table 2).

The antibody response to the B/Hong Kong antigen in the vaccine was evaluated with the recently detected heterologous B/Singapore/79. Cross-reacting antibody to the newly emerged B strain was poor in those initially seronegative as

TABLE 1. HAI GMTs to influenza B/Hong Kong/5/72 after vaccination with 7 µg of HA

Initial HAI status	Vaccine	Mean age (yrs)	GMT		
			Before vaccination	After first vaccination	After second vaccination
Antibody negative	Triton X-100	8	<10 (15) ^a	30 (15)	24 (7)
	Tween-ether	9	<10 (13)	34 (13)	49 (8)
Antibody positive	Triton X-100	15	34 (24)	97 (24)	160 (4)
	Tween-ether	13	28 (24)	119 (24)	69 (5)

^a Parentheses indicate number of vaccinees.

well as those initially seropositive (Table 3). Most patients were initially seronegative for B/Singapore in contrast to B/Hong Kong (Table 4). If an ether-treated B/Singapore/79 antigen was used in the test, fewer vaccinees were initially seronegative, and the GMT after the first vaccination was higher and comparable to that observed with the homologous non-ether-treated B/Hong Kong/72 strain. Using the complement fixation test for influenza B, we found

TABLE 2. *Pre-immunization serum neutralizing antibody and post-immunization HAI antibody titers for influenza B/Hong Kong/72 after one vaccination dose in persons without detectable prevaccination HAI antibody*

Neutralizing antibody level before immunization	No. of patients	HAIGMT ^a
0	8	14
2	6	57
4	6	48
16	4	40

^a Dose: 7 μ g of HA from B/Hong Kong/72.

TABLE 3. *HAI GMTs to influenza B/Singapore/79 after vaccination with 7 μ g of HA from influenza B/Hong Kong/72*

Initial HAI status	Vaccine	Mean age (yrs)	GMT		
			Before vaccination	After first vaccination	After second vaccination
Antibody negative	Triton X-100	12	<10 (32) ^a	10 (32)	21 (10)
	Tween-ether	12	<10 (25)	13 (25)	15 (13)
Antibody positive	Triton X-100	15	28 (7)	45 (7)	40 (1)
	Tween-ether	13	14 (12)	28 (12)	— (0)

^a Parentheses indicate number of vaccinees.

TABLE 4. *HAI and complement fixation GMTs to influenza B strains after vaccination with B/Hong Kong/5/72 in patients without detectable antibody before vaccination*

Strain	Serological test	Vaccine	No. (%) of vaccines without detectable antibody prevaccination ^a	GMT after first vaccination
B/Hong Kong/72	HAI	Triton X-100	15 (38%)	30
		Tween-ether	13 (35%)	34
B/Singapore/79	Standard HAI antigen	Triton X-100	32 (82%)	10
		Tween-ether	25 (68%)	13
	Ether-treated antigen	Triton X-100	6 (15%)	23
		Tween-ether	8 (22%)	40
Type B	CF ^b	Triton X-100	29 (78%)	10
		Tween-ether	27 (77%)	6

^a (Number of initially seronegative patients/total number of initially seronegative and seropositive patients vaccinated) $\times$ 100.

^b CF, Complement fixation.

that the serological results were more comparable to those seen with the standard HAI test for B/Singapore.

The initially seronegative individuals developed GMTs to the influenza A/Brazil strain of almost 100 after one dose (Table 5). Individuals with antibody developed a GMT two to three times that of the initially seronegative group; a second dose of vaccine did not boost antibody levels in this group. However, the number who received the second dose is small because most patients had been vaccinated the previous year. Individuals without detectable HAI antibody developed a GMT to the influenza A/Texas strain of about 100, whereas the GMT in initially seropositive individuals was somewhat higher (Table 6).

The HAI titers for all vaccine strains fell $\geq$ 2-fold 6 months postvaccination, yet titers remained higher than those of the prevaccination specimens.

DISCUSSION

The clinical reactions and antibody responses to the new Triton X-100 SPV appear to be

TABLE 5. HAI GMTs to influenza A/Brazil/11/78 after vaccination with 7 µg of HA

Initial HAI status	Vaccine	Mean age (yrs)	GMT		
			Before vaccination	After first vaccination	After second vaccination
Antibody negative	Triton X-100	12	<10 (19) ^a	84 (19)	181 (5)
	Tween-ether	11	<10 (17)	97 (17)	169 (9)
Antibody positive	Triton X-100	13	34 (20)	181 (20)	207 (6)
	Tween-ether	12	37 (20)	294 (20)	320 (4)

^a Parentheses indicate number of vaccinees.

TABLE 6. HAI GMTs to influenza A/Texas/77 after vaccination with 7 µg of HA

Initial HAI status	Vaccine	Mean age (yrs)	GMT		
			Before vaccination	After first vaccination	After second vaccination
Antibody negative	Triton X-100	12	<10 (11) ^a	111 (11)	320 (6)
	Tween-ether	10	<10 (11)	69 (11)	128 (7)
Antibody positive	Triton X-100	13	52 (28)	147 (28)	119 (5)
	Tween-ether	12	56 (26)	208 (26)	320 (6)

^a Parentheses indicate number of vaccinees.

comparable to responses to the licensed Tween-ether SPV in young high-risk individuals. Neither vaccine was associated with fever or other systemic reactions. Sore arms occurred in a similarly small percentage with each vaccine. Analysis of the antibody responses also showed the two vaccines to be comparable. The influenza A/Brazil and A/Texas vaccine strains stimulated presumably protective levels of HAI antibody after one immunization in the majority of antibody-negative recipients. This, however, did not occur with the influenza B/Hong Kong strain in initially seronegative recipients. Either the B/Hong Kong strain is a poorer antigen or the vaccine recipients were less well primed to this strain than to the two A strains (5). In an earlier study the HAI antibody responses in antibody-negative vaccine recipients were distinguished by the presence or absence of low levels of neutralizing antibody to the same strain used in the HAI test. Individuals with very little or no neutralizing antibody responded poorly to one dose of a whole vaccine or SPV (5). These results also demonstrate that persons initially seronegative by the neutralization test responded significantly less well to immunization with the B/Hong Kong strain than did those with some pre-immunization neutralizing antibody.

Immunization with influenza B/Hong Kong antigen is an inefficient method of immunizing against influenza B/Singapore. The demonstration of an immunologically significant drift of B/Singapore is confirmed by these vaccination

studies (2). After one dose of B/Hong Kong vaccine, a barely onefold increase of antibody to B/Singapore occurred. Higher doses of SPV should be investigated in an attempt to improve immunogenicity for the influenza B component. Such an approach has been successful in a limited study with an A strain (7).

The historic variability in influenza immunogens, the use of single vaccine lots, and the use of relatively small numbers of patients indicates that similar studies should be done with other influenza vaccine strains.

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