JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1992, p. 2230–2234
0095-1137/92/092230-05$02.00/0

Vol. 30, No. 9

Evaluation of a Cold-Adapted Influenza B/Texas/84 Reassortant Virus (CRB-87) Vaccine in Young Children

EDWIN L. ANDERSON,[1,2]* FRANCES K. NEWMAN,[1,2] H. F. MAASSAB,[3] AND ROBERT B. BELSHE[1,2]

Center for Vaccine Development, Departments of Internal Medicine and Pediatrics, St. Louis University School of Medicine, St. Louis, Missouri 63104[1]; Research Service, Huntington Veterans Administration Medical Center, Huntington, West Virginia 25755-9410[2]; and Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109[3]

Received 4 December 1991/Accepted 21 May 1992

A cold-adapted (*ca*) influenza B reassortant virus vaccine that contained the six internal RNA segments from influenza B/Ann Arbor/1/66 *ca* virus and the neuraminidase and hemagglutinin genes from wild-type influenza B/Texas/1/84 virus was evaluated in children ranging in age from 8 months to 14 years. The children were vaccinated intranasally with doses ranging from $10^{3.2}$ to $10^{6.2}$ 50% tissue culture infective doses ($TCID_{50}$). Thirty children were seropositive, and 26 were seronegative. Thirty-three children participated as unvaccinated controls. The vaccine was well tolerated by both seronegative and seropositive children. The amount of virus required to infect 50% of seronegative children was approximately $10^{4.5}$ $TCID_{50}$. Vaccine viruses recovered from airway secretions retained temperature-sensitive and cold-adapted characteristics. The results of this study indicate that the vaccine virus, influenza B/Texas/84 *ca* reassortant virus, is attenuated, immunogenic, and phenotypically stable when given to young seronegative children.

Significant morbidity can occur among children during outbreaks of influenza B virus infection (14, 24). Community epidemics of influenza B have resulted in high rates of school absenteeism (10). Infants also may develop severe infection, leading to extensive evaluation to rule out bacterial sepsis (9, 10, 24). Influenza B virus infections in young children may be complicated by gastrointestinal complaints, otitis media, high fever, acute myositis, and Reye syndrome (2, 7, 10, 12, 15, 17, 23, 24). Severe illness and death have occurred among persons of all ages infected with influenza B virus (2, 19, 22).

Inactivated influenza B virus vaccines have not provided the same level of immunity as have inactivated or live attenuated influenza A virus vaccines (4, 15). Recombinant strains of cold-adapted (*ca*) live influenza A and B virus vaccine candidates for intranasal administration have been developed as a possible alternative to parenteral inactivated vaccine. Several studies with live attenuated reassortant *ca* influenza A virus vaccine and one study with *ca* influenza B virus vaccine have shown them to be safe and antigenic in infants and young children (1, 3, 8, 20, 21, 25). The significant morbidity associated with outbreaks of influenza B virus infection confirms the need for a safe, antigenic live attenuated *ca* influenza B virus vaccine (18, 25). Influenza B/Texas/84 *ca* reassortant virus was derived by crossing influenza B/Ann Arbor/1/66 *ca* virus with influenza B/Texas/84 wild-type virus. The *ca* vaccine contained the six internal genes of influenza B/Ann Arbor/1/66 virus and the hemagglutinin and neuraminidase genes of influenza B/Texas/84 virus. The present study was done to evaluate the safety and antigenicity of influenza B/Texas/84 *ca* reassortant virus among young seropositive and seronegative children and to determine the quantity of vaccine virus required to infect seronegative children. Also tested was the ability of the influenza B/Ann Arbor/1/66 virus donor to reproducibly attenuate different wild-type influenza B viruses.

MATERIALS AND METHODS

Vaccine. The vaccine was derived by H. F. Maassab at the University of Michigan by crossing influenza B/Ann Arbor/1/66 *ca* virus with wild-type influenza B/Texas/84 virus. The clone which was selected exhibited the *ca* property and contained the six internal genes of influenza B/Ann Arbor/1/66 virus but possessed the hemagglutinin and neuraminidase genes of influenza B/Texas/84 virus. The resulting vaccine virus was designated influenza B/Texas/84 *ca* reassortant virus or CRB-87.

Volunteers. Parents who were interested in having their child participate in the cold-adapted reassortant influenza B virus vaccine study were given information about the vaccine and encouraged to ask questions and to discuss the study with a vaccine center nurse or physician before giving their written informed consent. Fifty-six healthy children between the ages of 8 months and 14 years participated as vaccinees in the study; an additional 33 children were enrolled as unvaccinated controls. The study was approved by the St. Louis University Institutional Review Board.

Clinical design. On the day of vaccination, each child was examined by a pediatrician and had blood and nasal secretions collected before receiving 0.5 ml of influenza B/Texas/84 *ca* reassortant virus. The first group of vaccinees received $10^{5.2}$ 50% tissue culture infective doses ($TCID_{50}$) of vaccine diluted in veal infusion broth. Children in subsequent groups received titers of vaccine ranging from $10^{3.2}$ to $10^{6.2}$ $TCID_{50}$ in 10-fold increments. Volunteers were inoculated intranasally while in a supine position by alternating drops of inoculum into the right and left nares. Daily for 11 days following vaccination, nasopharyngeal swab samples were collected during a home visit by one of the study nurses for isolation and quantitation of influenza B virus. Control volunteers did not receive a placebo inoculation but were

* Corresponding author.

TABLE 1. Clinical responses of children vaccinated with influenza B/Texas/84/*ca* Reassortant Virus

Prevaccination antibody status	Mean age (range) in mo	No. of children in group	No. of children with indicated finding[a]				
			Fever	Upper respiratory tract disease symptoms		Lower respiratory tract illness	Otitis media
				Rhinorrhea	Cough		
Seropositive (HAI ≥ 4)	53 (8–177)						
Infected with vaccine virus[b]		16	0	1	2	0	1
Not infected		14	0	3	1[c]	0	0
Seronegative (HAI < 4)	19 (8–41)						
Infected with vaccine virus[b]		14	0	3	0	1	2[d]
Not infected		12	1	2[e]	2	1	2
Control	32 (8–120)	33	1	5	2	1	6

[a] Clinical definitions: fever, rectal temperature ≥ 101°F (38.3°C); rhinorrhea, rhinorrhea on 2 or more consecutive days; cough, cough on 2 or more consecutive days; lower respiratory illness, wheezing or pneumonia. Otitis media was diagnosed by a pediatrician.
[b] Infected with vaccine virus as indicated by viral shedding and/or HAI or ELISA antibody response (see text and Table 2).
[c] Parainfluenza virus type 3 recovered from this child.
[d] Parainfluenza virus type 1 recovered from one child; parainfluenza virus type 2 recovered from the other.
[e] Enterovirus (not poliovirus) isolated from respiratory secretions from one of two children.

included in the daily home surveillance to assess the presence of other viruses in the community. Because of concern about a relationship between influenza B virus infection and Reye syndrome, serum transaminase levels (aspartate aminotransferase and alanine aminotransferase) in 39 vaccinees were determined during the initial screening process and on day 14 of the study.

Virus isolation and serologic tests. Nasopharyngeal swab samples were inoculated onto primary rhesus monkey kidney (RMK) cells in duplicate and incubated at 32°C for 14 days. RMK cell layers were hemadsorbed with 0.4% guinea pig erythrocytes on days 5, 9, and 14 after inoculation for isolation of influenza B/Texas/84 *ca* reassortant virus. Each of the positive specimens was identified by a hemagglutinin inhibition (HAI) test and quantitated by $TCID_{50}$ in RMK cells (1, 3).

Serum samples were collected from each vaccinee before and 28 days after vaccination to evaluate their HAI and enzyme-linked immunosorbent assay (ELISA) antibody responses to influenza B virus infection by previously described methods (1, 3). HAI tests were done with homologous influenza B/Texas/*ca* reassortant virus which had been passaged once in MDCK tissue culture cells. Purified hemagglutinin from influenza B/Yamagata virus (a gift from Connaught Laboratories, Inc., Swiftwater, Pa.) was used to coat the microtiter plates for the ELISA. Children who had HAI titers of <1:4 were considered seronegative.

Genetic stability of vaccine virus. Vaccine virus recovered from children who shed virus for prolonged periods was tested for the temperature-sensitive (*ts*) phenotype by previously described methods (5, 16). To assess the genetic stability of the influenza B virus isolates recovered from the vaccinees, specimens were assayed at 39°C to determine the presence of revertant virus, i.e., virus that had regained the ability to grow at the restrictive temperature of 39°C. Also, viruses were tested for the *ca* phenotype by plaque assay at 25°C. Vaccine virus with the *ca* phenotype forms plaques at 25°C, but wild-type influenza virus does not.

Statistics. The chi-square test was used to test for differences in adverse reactions among the vaccine groups. The *t* test was used to compare geometric mean titers between groups.

RESULTS

Seropositive children. Thirty seropositive children with a mean age of 53 months (range, 8 to 177 months) were vaccinated intranasally with doses of $10^{4.2}$, $10^{5.2}$, or $10^{6.2}$ $TCID_{50}$ of influenza B/Texas/84 *ca* reassortant vaccine virus. Respiratory symptoms following vaccination were uncommon and were not more frequent among vaccinees, whether or not they were infected by vaccine virus, than among control children (Table 1).

Shedding of vaccine virus was detected among 42, 8, and 40% of children vaccinated with $10^{4.2}$, $10^{5.2}$, or $10^{6.2}$ $TCID_{50}$, respectively. Overall, 8 (27%) of the 30 seropositive children shed vaccine virus. For each group, the mean duration of viral shedding was short (8 days or fewer), and the mean peak titer among children shedding was low ($\leq 10^{3.0}$ $TCID_{50}$/ml of nasal swab specimen [Table 2 and Fig. 1]). Seven of the 30 seropositive children developed fourfold HAI increases in antibody (0 of 5, 4 of 13, and 3 of 12 of the seropositive children vaccinated with $10^{4.2}$, $10^{5.2}$, or $10^{6.2}$ $TCID_{50}$ of vaccine virus, respectively). Nine of the seropositive children (0 of 5, 4 of 13, and 6 of 12 given $10^{4.2}$, $10^{5.2}$, or $10^{6.2}$ $TCID_{50}$ vaccine virus, respectively) had fourfold rises in antibody titer in the ELISA. As determined by either shedding of vaccine virus, a fourfold or greater rise in HAI or ELISA antibody titer, or both, 16 of 30 seropositive children were infected by vaccine virus.

Seronegative children. Twenty-six seronegative children participated in the study (mean age, 19 months; range 8 to 41 months). The seronegative children were significantly younger than seropositive children (mean ages of 19 and 53 months, respectively; $P = 0.01$). Symptoms of respiratory disease were not more common among the 26 seronegative children who received inocula of $10^{3.2}$, $10^{4.2}$, $10^{5.2}$, or $10^{6.2}$ $TCID_{50}$ of vaccine virus compared with control children or seropositive children (Table 1). Also, the occurrence of symptoms of respiratory disease among the 14 seronegative children who were infected with vaccine virus was not more frequent than that among the seronegative children who were not infected by vaccine virus. Intercurrent viral respiratory disease was common. Two children who developed otitis media shed parainfluenza virus type 1 or 2, and one

TABLE 2. Infectivity of influenza B/Texas/84 *ca* reassortant virus as indicated by shedding of vaccine virus or antibody increase

Prevaccination antibody status	Mean age (mo)	Dose ($TCID_{50}$)	No. of children vaccinated	Virus shedding			Serum antibody response[a]						No. (%) of children infected[b]
							HAI			ELISA			
				No. (%) of children shedding	Mean duration (days)[c]	Mean peak titer (log_{10} $TCID_{50}$)	GM log_2 reciprocal titer		No. of children with 4-fold increase/no. tested	GM log_{10} reciprocal IgG titer		No. of children with 4-fold increase/no. tested	
							Prevaccination	Postvaccination		Prevaccination	Postvaccination		
Seropositive (HAI ≥ 4)	35	6.2	12	5 (42)	5.5	1.7	3.2	3.4	3/12	1.3	1.8[d]	6/12	9 (75)
	79	5.2	13	1 (8)	8.0	3.0	3.7	4.7	4/13	1.9	2.3	4/13	5 (38)
	30	4.2	5	2 (40)	7.0	1.8	2.8	3.0	0/5	1.7	1.7	0/5	2 (40)
Seronegative (HAI < 4)	23	6.2	5	3 (60)	2.0	1.2	<2	2.8[d]	3/5	1.0	1.5	2/5	4 (80)
	24	5.2	8	4 (50)	6.0	1.4	<2	2.9[d]	5/8	1.0	1.6[d]	4/8	6 (75)
	16	4.2	7	2 (29)	5.0	1.5	<2	2.6	3/7	1.0	1.5	3/7	4 (57)
	14	3.2	6	0 (0)	0	0	<2	<2	0/6	1.0	1.0	0/6	0 (0)

[a] GM, geometric mean; IgG, immunoglobulin G.
[b] As indicated by viral shedding and/or HAI or ELISA antibody response.
[c] The last day postvaccination on which shedding of virus was detected was taken as the duration of viral shedding; mean duration and peak titers were calculated for children who shed virus.
[d] Significantly higher than prevaccination antibody titer ($P < 0.05$, Student's t test).

child with rhinorrhea also shed a nonpoliovirus enterovirus. Two children manifested wheezing as the only sign of lower respiratory illness; one was infected by vaccine virus, and one was not infected by vaccine virus. Five of 33 controls had rhinorrhea, and six developed otitis media.

The proportion of seronegative children who shed vaccine virus was directly related to the inoculum: among infants given the lowest dose, $10^{3.2}$ $TCID_{50}$, no shedding was detected; the middle doses, $10^{4.2}$ and $10^{5.2}$ $TCID_{50}$, resulted in shedding among 29 and 50% of vaccinees, respectively; and at the highest dose, $10^{6.2}$ $TCID_{50}$, 60% of children shed vaccine virus (Table 2). The mean duration of viral shedding for all groups was short (6 days or fewer), and mean peak titers were low among children who shed vaccine virus ($\leq 10^{1.5}$ $TCID_{50}$/ml of nasal swab specimen).

HAI antibody response to vaccine among the seronegative children occurred in 0, 43, 63, and 60% of the four vaccine groups inoculated with $10^{3.2}$, $10^{4.2}$, $10^{5.2}$, and $10^{6.2}$ $TCID_{50}$ of vaccine, respectively (Table 2). No additional vaccine virus infections were detected by ELISA antibody rise among the seronegative children. In two instances in which HAI antibody titer increased, the ELISA did not detect a rise in antibody titer after vaccination. As indicated by both antibody response and vaccine virus shedding, there was a direct relationship between dose and response: $10^{3.2}$ $TCID_{50}$ of vaccine was not infectious, while $10^{6.2}$ $TCID_{50}$ of vaccine infected 80% of seronegative vaccinees. The amount of vaccine virus required to infect 50% of seronegative children (HID_{50}) was $10^{4.5}$ $TCID_{50}$ as calculated by the Reed-Muench method.

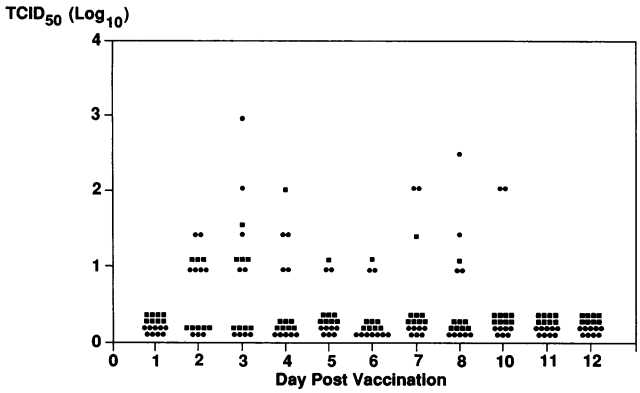

FIG. 1. Shedding of influenza B/Texas/84 *ca* reassortant virus after vaccination. Symbols: ■, seropositive child, ●, seronegative child.

Sera for measurement of aspartate aminotransferase and alanine aminotransferase were obtained from 28 seropositive children and 16 seronegative children before vaccination and 14 days after vaccination. No elevations in aspartate aminotransferase or alanine aminotransferase levels occurred among the 13 seropositive children who were infected with vaccine virus or among the 10 seronegative children who were infected by vaccine virus.

Phenotypic stability of vaccine virus. In order to evaluate the phenotypic stability of vaccine virus shed for prolonged intervals by young children, isolates obtained from 10 vaccinees 2 to 8 days postvaccination were tested for genotype and phenotype (Table 3). All isolates tested retained the *ts* and *ca* phenotypes. Five isolates tested for genotype were confirmed to be reassortant vaccine virus, with six *ca* genes (derived from influenza B/Ann Arbor/1/66 virus) and hemagglutinin and neuraminidase genes from influenza B/Texas/84 virus.

DISCUSSION

Previous studies of adults with other *ca* recombinant influenza B virus vaccines have confirmed the safety, genetic stability, and antigenicity of these live attenuated vaccines (4, 6, 13, 18). The influenza B/Texas/84 *ca* reassortant virus derived from influenza B/Ann Arbor/1/66 *ca* virus and influenza B/Texas/84 wild-type virus was found to be safe in infants and young seronegative children in doses ranging from $10^{3.2}$ to $10^{6.2}$ $TCID_{50}$, and it was immunogenic at the higher doses. The HID_{50} was $10^{4.5}$ $TCID_{50}$. A dose of $10^{6.2}$

TABLE 3. Evaluation of phenotype and genotype of isolates of cold-adapted influenza B virus vaccine from young children

Volunteer no. or virus	Day postvaccination	Virus genotype[a]	$\log_{10}$ PFU/ml in PCK cells at indicated temp (°C)		
			25	33	37
1	2	6/2	4.3	4.9	<3
	3	NT	3.5	3.5	<2
	4	6/2	4.0	4.7	<3
2	8	NT	3.7	3.3	<1
3	8	NT	3.6	2.6	<1
4	3	6/2	5.7	5.0	<2
5	4	NT	5.0	3.9	<1
6	7	NT	3.5	2.5	<1
	8	NT	4.3	3.9	<1
7	2	NT	5.6	5.0	<1
8	4	6/2	5.3	4.7	<1
9	3	NT	6.0	5.5	<1
10	8	6/2	5.6	4.5	<1
Vaccine virus		6/2	6.0	6.0	<1
Wild-type virus			<3	6.0	6.0

[a] 6/2 refers to gene profile on selected samples determined by polyacrylamide gel electrophoresis, where 6 indicates 6 cold-adapted genes and 2 indicates hemagglutinin and neuraminidase genes from wild-type influenza B/Texas/84 virus. NT, not tested for genotype.

$TCID_{50}$ infected 46% of the seropositive children. When influenza B/Texas/84 *ca* reassortant and wild-type influenza B/Texas/1/84 viruses were evaluated in a comparative study of adults, the *ca* vaccine was safe and the HID_{50} was $10^{5.4}$ $TCID_{50}$. In contrast, the wild-type virus, influenza B/Texas/1/84 virus, infected all volunteers at all doses ranging from $10^{3.9}$ to $10^{7.1}$ $TCID_{50}$. There was significantly more illness among the volunteers given wild-type virus than among influenza B/Texas/84 *ca* reassortant virus vaccinees (13).

One other *ca* influenza B virus derived from the parent *ca* influenza B/Ann Arbor/1/66 virus has been evaluated in adults: influenza B/Ann Arbor/1/86 *ca* reassortant virus (CRB-117) has the hemagglutinin and neuraminidase genes from the influenza B/Ann Arbor/1/86 virus. In studies of adults vaccinated with influenza B/Ann Arbor/1/86 *ca* reassortant virus, the HID_{50} ranged from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ (4, 8). Influenza B/Ann Arbor/1/86 *ca* reassortant virus was safe in children, but the infectivity of this virus was greater than the infectivity we observed for influenza B/Texas/84 *ca* reassortant virus. In contrast to a HID_{50} of $10^{4.5}$ $TCID_{50}$ for seronegative children in the present study, the HID_{50} for influenza B/Ann Arbor/1/86 *ca* reassortant virus was $10^{2.5}$ $TCID_{50}$ (8). The reason for the higher infectivity of the influenza B/Ann Arbor/1/86 *ca* reassortant virus among children in comparison with the influenza B/Texas/84 *ca* reassortant virus is not known, since influenza B/Ann Arbor/1/86 *ca* reassortant and influenza B/Texas/84 *ca* reassortant viruses are derived from the same parent *ca* strain and differ only in the hemagglutinin and neuraminidase genes. Apparently, the hemagglutinin or neuraminidase influences the infectivity in seronegative children.

The safety of influenza B/Texas/84 *ca* reassortant virus was demonstrated in this study. The occurrence of respiratory symptoms was no more frequent among either seropositive or seronegative vaccinees than among unvaccinated controls. Similar safety data on other cold-adapted reassortant influenza A or B virus vaccines have been reported elsewhere (1, 3, 4, 8, 13). In the present study, the quantity and duration of shedding of vaccine virus were markedly less than those reported for wild-type influenza B virus (4, 11). The amount of vaccine virus shed was low in our study ($\leq 10^3$ $TCID_{50}$). This low-level shedding should reduce the chance of transmission of vaccine to other children, since it was below the HID_{50} for the influenza B/Texas/84 *ca* reassortant virus.

Influenza B/Texas/84 *ca* reassortant virus was found to be stable for temperature sensitivity after up to 8 days of replication in seronegative children. No reversion of either the *ts* or *ca* phenotype to the wild-type phenotype was detected among the isolates tested. The reassortant virus was safe and immunogenic in seronegative children when given in doses of up to $10^{6.2}$ $TCID_{50}$. This vaccine should be considered for combination with cold-adapted influenza A virus vaccine to develop intranasal trivalent influenza A and B virus vaccines.

ACKNOWLEDGMENTS

We thank the parents of the children involved in this study for their interest and cooperation. Joan Cannon and Sharon Nugent provided study coordination and technical support. Kathy Feurer and Julia Harrison prepared the manuscript.

This work was supported by contracts N01-AI-52575 and N01-AI-05051 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. **Anderson, E. L., R. B. Belshe, B. Burk, J. Bartram, and H. F. Maassab.** 1989. Evaluation of cold-recombinant A/Korea (CR-59) virus vaccine in infants. J. Clin. Microbiol. **27**:909–914.
2. **Baine, W. B., J. P. Luby, and S. M. Martin.** 1980. Severe illness with influenza B. Am. J. Med. **68**:181–189.
3. **Belshe, R. B., and L. P. Van Voris.** 1984. Cold-recombinant influenza A/California/10/78 (H1N1) virus vaccine (CR-37) in seronegative children: infectivity and efficacy against investigational challenge. J. Infect. Dis. **149**:735–740.
4. **Clements, M. L., M. H. Snyder, S. D. Sears, H. F. Maassab, and B. R. Murphy.** 1990. Evaluation of the infectivity, immunogenicity, and efficacy of live cold-adapted influenza B/Ann Arbor/1/86 reassortant virus vaccine in adult volunteers. J. Infect. Dis. **161**:869–877.
5. **Cox, N. J., H. F. Maassab, and A. P. Kendall.** 1979. Comparative studies of wild-type and cold-mutant (temperature-sensitive) influenza viruses: nonrandom reassortment of genes during preparation of live virus vaccine candidates by recombination at 25 between recent H3N2 and H1N1 epidemic strains and cold-adapted A/Ann Arbor/6/60. Virology **97**:190–194.
6. **Davenport, F. M., A. V. Hennessy, H. F. Maassab, E. Minuse, L. C. Clark, G. D. Abrams, and J. R. Mitchell.** 1977. Pilot studies on recombinant cold-adapted live type A and B influenza virus vaccines. J. Infect. Dis. **136**:17–25.
7. **Dietzman, D. E., J. G. Schaller, C. G. Ray, and M. E. Reed.** 1976. Acute myositis associated with influenza B infection. Pediatrics **57**:255–258.
8. **Edwards, K. M., J. C. King, M. C. Steinhoff, J. Thompson, M. L. Clements, P. F. Wright, and B. R. Murphy.** 1991. Safety and immunogenicity of live attenuated cold-adapted influenza B/Ann Arbor/1/86 reassortant virus vaccine in infants and children. J. Infect. Dis. **163**:740–745.
9. **Foy, H. M., M. K. Cooney, and I. Allen.** 1976. Longitudinal studies of types A and B influenza among Seattle school children and families. 1968–1974. J. Infect. Dis. **134**:362–369.
10. **Glezen, W. P., R. B. Couch, L. H. Taber, A. Paredes, J. E. Allison, A. L. Frank, and C. Aldridge.** 1980. Epidemiologic observations of influenza B virus infections in Houston, Texas, 1976–1977. Am. J. Epidemiol. **111**:13–22.
11. **Hall, C. B., R. G. Douglas, J. M. Geiman, and M. P. Meagher.** 1979. Viral shedding patterns of children with influenza B infection. J. Infect. Dis. **140**:610–613.
12. **Hurwitz, E. S., M. J. Barrett, D. Bregman, W. J. Gunn, P. Pinsky, L. B. Schonberger, J. S. Drage, R. A. Kaslow, B.**

Burlington, G. V. Quinnan, J. R. LaMontagne, W. R. Fairweather, D. Dayton, and W. R. Dowdle. 1987. Public health service study of Reye's Syndrome and medications. Report of the main study. JAMA **257:**1905–1911.

13. **Keitel, W. A., R. B. Couch, T. R. Cote, H. R. Six, and B. D. Baxter.** 1990. Cold recombinant influenza B/Texas/1/84 vaccine virus (CRB87): attenuation, immunogenicity, and efficacy against homotypic challenge. J. Infect. Dis. **161:**22–26.
14. **Klein, J. D., A. M. Collier, and W. P. Glezen.** 1976. An influenza B epidemic among children in day-care. Pediatrics **58:**340–345.
15. **LaMontagne, J. R.** 1980. Summary of a workshop in influenza B viruses and Reye's Syndrome. J. Infect. Dis. **142:**452–465.
16. **Maassab, H. F., and D. C. DeBorde.** 1985. Development and characterization of cold-adapted viruses for use as live virus vaccines. Vaccine **3:**355–369.
17. **Marchant, C. D., P. A. Shurin, V. A. Turczyk, D. E. Wasikowski, M. A. Tutihasi, and S. E. Kinney.** 1984. Course and outcome of otitis media in early infancy: a prospective study. J. Pediatr. **104:**826–831.
18. **Monto, A. S., F. D. Miller, and H. F. Maassab.** 1982. Evaluation of an attenuated, cold-recombinant influenza B virus vaccine. J. Infect. Dis. **145:**57–64.
19. **Serwint, J. R., R. M. Miller, and B. M. Korsch.** 1991. Influenza type A and B infections in hospitalized pediatric patients. Am. J. Dis. Child. **145:**623–626.
20. **Steinhoff, M. C., N. A. Halsey, L. F. Fries, M. H. Wilson, J. King, B. A. Burns, R. K. Samarodin, V. Perkis, B. R. Murphy, and M. L. Clements.** 1991. The A/Mallard/6750/78 avian/human, but not the A/Ann Arbor/6/60 cold-adapted, influenza A/Kawasaki/86 (H1N1) reassortant virus vaccine retains partial virulence for infants and children. J. Infect. Dis. **163:**1023–1028.
21. **Steinhoff, M. C., N. A. Halsey, M. H. Wilson, B. A. Burns, R. K. Samarodin, L. F. Fries, B. R. Murphy, and M. L. Clements.** 1990. Comparison of live attenuated cold-adapted and avian-human influenza A/Bethesda/85 (H3N2) reassortant virus vaccines in infants and children. J. Infect. Dis. **162:**394–401.
22. **Van Voris, L. P., R. B. Belshe, and J. L. Schaffer.** 1982. Nosocomial influenza B virus infections in the elderly. Ann. Intern. Med. **96:**153–156.
23. **Wald, E. R., N. Guerra, and C. B. Byers.** 1991. Upper respiratory tract infections in young children: duration of and frequency of complications. Pediatrics **87:**129–133.
24. **Wright, P. F., J. B. Bryant, and D. T. Karzon.** 1980. Comparison of influenza B/Hong Kong virus infections among infants, children, and young adults. J. Infect. Dis. **141:**430–435.
25. **Wright, P. F., and D. T. Karzon.** 1987. Live attenuated influenza vaccines. Prog. Med. Virol. **34:**70–88.