MAJOR ARTICLE

Phase 2 Evaluation of Parainfluenza Type 3 Cold Passage Mutant 45 Live Attenuated Vaccine in Healthy Children 6–18 Months Old

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A phase 2 evaluation of live attenuated parainfluenza type 3 (PIV3)–cold passage mutant 45 (cp45) vaccine was conducted in 380 children 6–18 months old; 226 children (59%) were seronegative for PIV3. Of the 226 seronegative children, 114 received PIV3-cp45 vaccine, and 112 received placebo. No significant difference in the occurrence of adverse events (i.e., runny nose, cough, or temperature ⩾38°C) was noted during the 14 days after vaccination. There was no difference between groups in the occurrence of acute otitis media or serous otitis media. Paired serum samples were available for 109 of the seronegative vaccine recipients and for 110 of the seronegative placebo recipients; 84% of seronegative vaccine recipients developed a ⩾4-fold increase in antibody titers. The geometric mean antibody titer after vaccination was 1:25 in the vaccine group and <1:4 in the placebo group. PIV3-cp45 vaccine was safe and immunogenic in seronegative children and should be evaluated for efficacy in a phase 3 field trial.

Human parainfluenza viruses (PIVs) are important causes of serious respiratory tract disease in infants and young children. According to the US Institute of Medicine, 25% of children <5 years old experience a clinically significant PIV infection annually, and ~2% of PIV-infected infants will require hospitalization [1]. Four types of PIV are associated with respiratory illness in young infants and children. Of special significance

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is PIV type 3 (PIV3), which causes pneumonia and bronchiolitis and ranks second only to respiratory syncytial virus (RSV) as a cause of bronchiolitis and pneumonia in infants <6 months old [2–4]. PIV3 can cause severe disease throughout the first 2 years of life, and virtually all children have experienced primary PIV3 infections by age 3–4 years. Overall, PIV3 is considered to be responsible for ~11% of hospitalizations for pediatric respiratory tract disease in the United States [1].

Past attempts to develop inactivated PIV3 vaccines showed that resistance to disease was not induced, despite the development of serum antibodies after vaccination [5]. Protection against PIV3 in humans is most likely to be achieved by the induction of both circulating and mucosal antibodies that are active against the hemagglutinin-neuraminidase (HN) glycoprotein (the attachment protein) and the fusion glycoprotein, 2 surface antigens that induce protective neutralizing antibodies [6, 7]. Such protective mucosal and circulating antibodies should be induced most efficiently by delivery of a live attenuated virus vaccine to the mucosa of the respiratory tract [8].

The live attenuated virus vaccine evaluated in the present study, JS strain, was derived from human wild-type PIV3 that was originally isolated from a 1-year-old child with a febrile respiratory illness [9–11]. Several attenuated mutants of the JS strain were derived by passaging the virus numerous times in primary monkey kidney cells at sequentially lower temperatures that are suboptimal for PIV3 replication. After 12, 18, and 45 cold passages, mutants cp12, cp18, and cp45, respectively, were isolated and characterized. During the process of low-temperature passage, each of the mutants acquired 3 phenotypic markers: cold adaptation (ca; the ability to replicate efficiently in vitro at the suboptimal temperature of 20°C), temperature sensitivity (ts; restricted growth at 39°C in tissue culture), and attenuation, manifested by restricted replication in hamsters and chimpanzees, compared with that of wild-type virus [9–12].

Phase 1 studies of PIV3-cp45 vaccine, which was produced in fetal rhesus monkey lung (FRhL-2) tissue culture cells, have been conducted in seropositive children 6 months to 10 years old, seronegative infants 6–36 months old, and infants 1–2 months old [13, 14]. In general, the vaccine appeared to be satisfactorily attenuated, infectious, immunogenic, and phenotypically stable. However, because PIV3-cp45 replicates to modest titers on monolayer cultures of FRhL-2 cells, production of vaccine virus in this cell line would be inefficient for larger-scale manufacturing; thus, an easily scalable and cost-effective production process for PIV3 that propagates the virus in Vero cells grown on microcarrier beads in a bioreactor was developed by Wyeth Vaccines Research (Pearl River, NY).

In phase 1 studies, PIV3-cp45 grown in Vero tissue culture was generally well tolerated by all cohorts, with the exception that, in the seronegative cohort, otitis media (OM) was observed in 3 of 32 vaccine recipients and in none of 14 placebo recipients [15]. Interpretation of the significance of this finding was uncertain because of the frequent acquisition of other intercurrent viral infections. The overall rates of upper respiratory–tract illness (URI) were very similar in the vaccine and placebo groups, and PIV3 isolates recovered from these children retained the ts phenotype [15]. Thus, as with PIV3-cp45 grown in FRhL, the vaccine virus grown in Vero tissue culture appeared to be satisfactorily attenuated, infectious, immunogenic, and phenotypically stable. We therefore undertook a phase 2 study of PIV3-cp45 vaccine, to compare the safety profile of a dose of 10^5 pfu administered intranasally (inl) to children 6–18 months old with that of placebo and to assess the immunogenicity of the vaccine. The phase 2 study was sufficiently powered to evaluate the frequency of OM in vaccine and placebo groups.

SUBJECTS, MATERIALS, AND METHODS

Study design and vaccine. We enrolled ~400 healthy subjects 6–18 months old in a multicenter, double-blind, placebo-controlled safety and immunogenicity trial. This sample size was chosen to enroll ~200 PIV-seronegative children. Eligible subjects were assigned to receive the investigational study vaccine according to a randomization schedule generated by the sponsor's statistician and provided in sealed randomization envelopes to study personnel at each site responsible for preparing study vaccine for administration. Each subject was randomized to receive either a single dose of PIV3-cp45 at 1×10^5 pfu or placebo (PBS with sucrose, phosphate, and glutamate) inl as nose drops instilled while the subject was supine. Randomization was a 1:1 ratio of vaccine to placebo.

Subjects. Study subjects were healthy children 6–18 months old, whose parents or guardians gave written, informed consent. The human-experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this clinical research. Each subject's history was reviewed, and a physical examination was performed to verify that the health and development of all subjects were normal. Subjects with any of the following conditions or characteristics were excluded from study enrollment or from continued participation: immunosuppression or taking immunosuppressive medication; serious chronic illness; cardiac or respiratory illness, including those with >1 prior episode of wheezing (including illnesses diagnosed as asthma or reactive airway disease) confirmed by a physician or subjects with pressure equalization tubes; members of a household with a pregnant woman, an immunocompromised individual, or an infant <6 months old; or attendance at day care with infants <6 months old. Attendance at a day-care facility in which children were separated by age was acceptable if the vaccine recipient did not spend any time in the area designated for infants <6 months old and if conditions

pertaining to any common area of the facility minimized opportunities for transmission of virus through direct physical contact between children or by the aerosol route. Subjects with self-limiting illnesses were included after the condition resolved and if no other exclusion criteria were met. These exclusion criteria included acute febrile illness (≥38°C), acute OM (AOM), receipt of short-term antibiotic therapy for acute illness, receipt of any vaccine within the previous 2 weeks, receipt of any live vaccine within the previous 4 weeks, or receipt of gamma globulin within the past 3 months. Infants born at <37 weeks of gestation were deferred from study participation until they were at least 1 year old. Children did not receive other vaccines for 42 days after enrollment.

Procedures. Serum samples were obtained before inl vaccination, to determine prevaccination antibody levels. Subjects received either the vaccine or the placebo by nose drops in a volume of 0.25 mL/nostril, for a total dose of 0.5 mL. Vaccinations were administered between 28 October 1998 and 13 November 2000, and vaccinations were not given during winter, to reduce intercurrent wild-type viral infection. The parents were asked to keep track of any illness or symptoms on a parent diary card each day for 14 days after vaccination; electronic thermometers were provided, and parents were asked to obtain daily oral, rectal, or axillary temperatures. Parents were asked to record the child's temperature at bedtime daily for 14 days after vaccination and whenever the child felt warm during the 42 days after vaccination. Children were seen by study personnel twice in the 2 weeks after vaccination (day 7 and 14, ±1 day). These brief visits (20–30 min each) allowed the study staff to examine the child closely for any signs of a runny nose, sore throat, fever (temperature ≥38°C), cough, respiratory illness, or an ear infection. Study staff also contacted the parents by telephone on days 21, 28, and 35 (±2 days), to inquire if the child had experienced any symptoms of illness. Six weeks after the first vaccination visit, all enrolled children returned to the clinic for a brief physical examination, and a blood sample was obtained to measure the child's antibody response to the vaccination.

During the 42 days of the study, a clinician was available 24 h/day to examine ill children. An examination was performed if a child had a rectal temperature ≥38°C (or equivalent if oral or axillary temperatures were taken), respiratory illness, or symptoms suggestive of an ear infection. For all study subjects, fever, URI (rhinorrhea or pharyngitis), cough, and lower respiratory–tract illness (LRI) were defined as described elsewhere [14]. During each illness, the child's ears were examined for signs of an ear infection, and a nasal-wash sample was obtained for viral culture to determine whether there was an intercurrent viral infection. Otoscopy was performed at each clinic visit at which the child was well. AOM was defined as an inflamed, immobile tympanic membrane, with or without bulging, observed by a physician or nurse practitioner and confirmed by tympanometry or a second observer. These findings were noted independently of fever or other respiratory symptoms. An abnormal tympanogram alone was not considered to be diagnostic of AOM. Serous OM was defined as all other cases of OM not fitting the above strict criteria. All children were examined at the end of the study: day 42 (range, 35–66 days) after vaccination. Serum samples from all subjects were tested for antibodies to PIV3 (Washington/57 strain) by the hemagglutination inhibition (HAI) antibody test [13], starting at a serum dilution of 1:4; children with a titer of ≤1:8 were considered to be seronegative. Vaccine virus shedding was not routinely determined in the present study, to avoid any confounding clinical findings that may be caused by the frequent nasal-wash samples that are necessary to obtain samples for viral cultures.

Statistics. The event of primary analysis was AOM in seronegative subjects. It was estimated that ~50% of the enrolled subjects would be seronegative. This sample size was sufficient to reject the hypothesis, with a power of 82%, that the rate of OM in the vaccine group was ≥11% higher (90% confidence interval, upper bound) than the rate of OM in the placebo group, assuming that the placebo rate was 10%. For analysis of possible adverse reactions to vaccination (i.e., runny nose, cough, or fever) Fisher's exact test *P* values were adjusted for each symptom or sign or each day by Bonferroni's method. Fisher's exact test was used to compare the occurrence of OM in vaccine recipients and placebo recipients.

RESULTS

Enrollment. Three hundred eighty children were enrolled in the study and were given either PIV3-cp45 vaccine or placebo inl. Of those 380 children, 226 (59%) were seronegative (antibody to PIV3 ≤1:8 by HAI antibody test). Of the 226 seronegative children, 114 received PIV3-cp45 vaccine, and 112 received placebo. Three hundred seventy-two (97.9%) completed the 42 days of study.

Signs and symptoms of respiratory disease. Figure 1*A*, 1*B*, and 1*C* illustrates, in the seronegative cohort, the daily frequency of runny nose, cough, or fever during the 14 days in which parents recorded symptoms on diary cards. There was no statistically significant difference between groups in the frequency of runny nose, cough, or fever on any day. In both the vaccine and placebo groups, children selected on day 0 at time 0 for the absence of runny nose, cough, or fever experienced an increase in these events during the first days of the study. This is most dramatically seen for the occurrence of runny nose (figure 1*A*). Children with runny nose were excluded at entry, and, therefore, the occurrence of runny nose increased during the study in both the placebo and the vaccine recipients; this

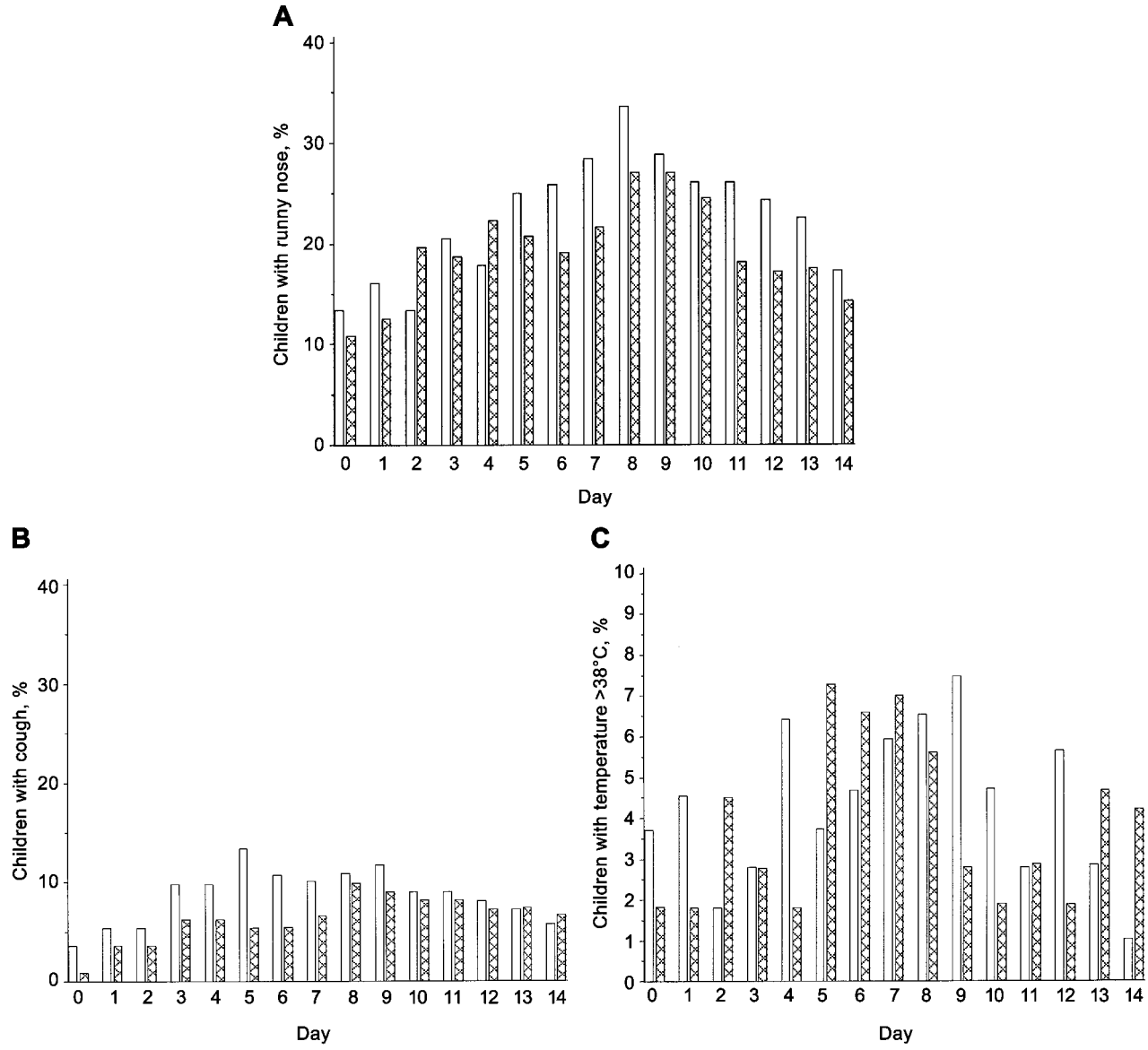

Figure 1. *A,* Percentage of parainfluenza virus 3 (PIV3)–seronegative children with runny nose on days 0–14 after intranasal (inl) vaccination with PIV3–cold passage mutant 45 (cp45) *(hatched bars)* or placebo *(white bars).* No statistically significant differences between vaccine and placebo groups occurred on any day ($P > .05$, Fisher's exact test with Bonferroni's correction). *B,* Percentage of PIV3-seronegative children with cough on days 0–14 after inl vaccination with PIV3-cp45 *(hatched bars)* or placebo *(white bars).* No statistically significant differences between vaccine and placebo groups occurred on any day ($P > .05$, Fisher's exact test with Bonferroni's correction). *C,* Percentage of PIV3-seronegative children with fever (temperature ≥38°C) on days 0–14 after inl vaccination with PIV3-cp45 *(hatched bars)* or placebo *(white bars).* No statistically significant differences between vaccine and placebo groups occurred on any day ($P > .05$, Fisher's exact test with Bonferroni's correction).

illustrates the phenomenon of return to the mean, whereby, on any given day, ~20% of young children have runny nose, as seen in other similar studies [16]. A similar phenomenon was observed for cough and fever, with baseline occurrence of cough in ~10% of the children after day 7, and the baseline occurrence of fever in ~3%–6% of children, depending on the day observed. Among the seropositive children, the frequency of signs and symptoms of respiratory illness (including runny nose, cough, or fever) was not significantly different on any day (data not shown). There was no difference in the frequency of children with findings of LRI (9/189 in the vaccine group vs. 12/191 in the placebo group; $P = .65$, Fisher's exact test).

Isolation of viruses during illness episodes. In the present study, nasal-wash samples were obtained only during illness visits. A total of 19 vaccine virus isolates were recovered from the nasal-wash samples of 17 (9.0%) of 189 vaccine recipients during these unscheduled visits. The majority of the isolates (17/19 [89.5%]) were recovered from seronegative subjects (ta-

Table 1. Isolation of vaccine and wild-type parainfluenza virus 3 (PIV3) from subjects, by prevaccination serostatus and study group.

	Seronegative subjects		All subjects	
Study interval, isolate phenotype	PIV3-cp45 (n = 114)	Placebo (n = 112)	PIV3-cp45 (n = 189)	Placebo (n = 191)
Days 1–11				
Vaccinelike[a]	15	0	17[b]	0
Wild type	0	2	1[b]	2
Days 12–22				
Vaccinelike	1	0	1	0
Wild type	1	1	1	1
Days ⩾23				
Vaccinelike	0	0	0	0
Wild type	2	4	2	5
Any day				
Vaccinelike	16	0	17[b]	0
Wild type	3	7	4[b]	8

NOTE. Data are no. of subjects shedding the indicated virus. cp45, Cold passage mutant 45.

[a] Temperature-sensitive and cold-adapted phenotypes.

[b] Three subjects who shed PIV3 (subject 1024 shed vaccine virus, subject 1829 shed wild-type virus, and subject 1828 shed both strains) were placed in the "all" category because their pretreatment blood samples were either unavailable (1829) or were obtained too many days before vaccination to assure a seronegative status at time of vaccination.

ble 1). All vaccine isolates were recovered within the first 14 days after vaccination. Both vaccine and wild-type PIV3 was detected in the nasal-wash sample of 1 subject during an illness visit. Three subjects who shed PIV3 (1 vaccine, 1 wild type, and 1 both strains) were placed in the "all" category because their pretreatment blood sample was either unavailable (n = 1) or was collected too long before vaccination (n = 2) to assure a seronegative status at time of vaccination.

To determine whether the higher proportion of vaccine isolates recovered from seronegative subjects was due to this cohort's lack of preexisting antibody to PIV3 or to more-frequent sampling of these subjects, nasal-wash sample collection rates among seronegative and seropositive subjects were compared and were found to be statistically similar ($P > .90$). Overall, 137 (60.6%) of 226 seronegative subjects and 95 (61.7%) of 154 seropositive subjects enrolled in the study had at least 1 nasal-wash sample obtained after vaccination. Within the first 11 days after vaccination, the nasal-wash sample collection rates were also similar between cohorts, with 71 (31.4%) of 226 seronegative subjects and 42 (27.3%) of 154 seropositive subjects having at least 1 nasal-wash sample obtained. Wild-type PIV3 was isolated from 4 (2.1%) of 189 vaccine recipients and 8 (4.2%) of 191 placebo recipients in the study (P = .38, Fisher's exact test).

Children enrolled in the present study experienced intercurrent infections with respiratory pathogens other than PIV3. PIV1, PIV2, RSV, adenovirus, cytomegalovirus, enterovirus, and rhinovirus were isolated from subjects enrolled in the present study. With the exception of PIV3, the most commonly recovered pathogens were RSV (2.6% of vaccine recipients and 1.6% of placebo recipients), adenovirus (1.1% of vaccine recipients and 3.7% of placebo recipients), enterovirus (3.2% of vaccine recipients and 2.1% of placebo recipients), and rhinovirus (1.1% of vaccine recipients and 2.1% of placebo recipients). In general, these viruses were isolated from children in both treatment groups throughout the entire study period, with no temporal relationship to vaccination. Among the 9 (4.8%) vaccine recipients and 12 (6.3%) placebo recipients who had evidence of LRI, viruses were recovered from 3 vaccine recipients (1 isolate each of PIV2, RSV, and vaccine virus) and from 5 placebo recipients (RSV, PIV3 [wild type, in 2 subjects], PIV1, and rhinovirus).

AOM. The occurrence of AOM among the seronegative subjects and among all subjects is summarized in table 2. The occurrence of AOM was common in both the vaccine recipients and placebo recipients and was divided into that occurring during the early postvaccination interval (days 1–11, the period of peak vaccine virus replication [13–15]), the interval when most vaccine virus replication had waned to absent or low levels (days 12–22), and the late postvaccination period, when the

Table 2. Subjects experiencing acute otitis media in the seronegative cohort and in all subjects, regardless of antibody status.

	Group		Vaccine group % − placebo group %	90% CI[a]
Cohort, days	PIV3-cp45	Placebo		
Seronegative[b]				
1–11	5 (4.4)	7 (6.3)	−1.9	−9.6 to 5.5
12–22	9 (7.9)	8 (7.1)	0.8	−6.8 to 9.6
23–42	10 (8.8)	8 (7.1)	1.6	−6.0 to 10.7
1–42	20 (17.5)	17 (15.2)	2.4	−6.9 to 13.0
Any	21 (18.4)	18 (16.1)	2.3	−7.1 to 13.1
All[c]				
1–11	10 (5.3)	10 (5.2)	0.1	−5.2 to 5.4
12–22	16 (8.5)	13 (6.8)	1.7	−4.2 to 7.7
23–42	20 (10.6)	12 (6.3)	4.3	−1.7 to 10.5
1–42	38 (20.1)	26 (13.6)	6.5	−0.8 to 14.0
Any	39 (20.6)	27 (14.1)	6.5	−0.9 to 14.1

NOTE. Data are no. (%) of subjects, except where noted. PIV3-cp45, parainfluenza virus 3–cold passage mutant 45.

[a] The 2-sided confidence intervals (CIs) were calculated by use of StatXact (Cytel). 90% CIs represent the 90% CI for the percentage of subjects in the PIV3-cp45 vaccine group with acute otitis media minus the percentage of subjects in the placebo group and, in each case, includes 0%. The denominator is the no. of randomized subjects.

[b] In the seronegative cohort, 114 subjects received PIV3-cp45, and 112 subjects received placebo.

[c] In the "all" cohort, 189 subjects received PIV3-cp45, and 191 subjects received placebo.

viral cultures were generally negative for vaccine virus (days 23–42). There was no statistically significant difference between groups in the frequency of AOM at any of these intervals nor did the overall total number of AOM cases differ between groups. The occurrence of all OM, defined as any evidence of AOM or serous OM, is shown in table 3. There was no significant difference between groups or among all subjects in the occurrence of all OM, regardless of serostatus.

Immunogenicity. Paired serum samples were available for 109 of the seronegative vaccine recipients and for 110 of the seronegative placebo recipients. The antibody response to PIV3 is summarized in figure 2 and table 4. Eighty-six of the seronegative vaccine recipients (79%) developed ⩾4-fold increase in antibody titer or seroconversion from ⩽1:8 to ⩾1:16. After vaccination, the geometric mean antibody titer was 1:25 among the vaccine recipients. In contrast, only 13 (12%) of 110 placebo recipients had an antibody response after vaccination. These 13 children likely had intercurrent natural infection with wild-type PIV3, which was circulating in the community at the time of the study. Postvaccination geometric mean titer was <1:4 in the placebo recipients. Among the seropositive subjects, prevaccine antibody titer was 1:50 and did not increase after vaccination (figure 2*B* and table 4).

Table 3. Subjects experiencing either acute otitis media or serous otitis media in the seronegative cohort and in all subjects, regardless of antibody status.

Cohort, days	Group		Vaccine group % − placebo group %	90% CI[a]
	PIV3-cp45	Placebo		
Seronegative[b]				
1–11	11 (9.6)	12 (10.7)	−1.1	−10.1 to 7.6
12–22	18 (15.8)	20 (17.9)	−2.1	−12.2 to 7.8
23–42	15 (13.2)	15 (13.4)	−0.2	−9.8 to 9.1
1–42	34 (29.8)	33 (29.5)	0.4	−10.1 to 12.2
Any	35 (30.7)	34 (30.4)	0.3	−10.2 to 12.2
All[c]				
1–11	19 (10.1)	20 (10.5)	−0.4	−7.3 to 5.6
12–22	28 (14.8)	32 (16.8)	−1.9	−9.7 to 4.9
23–42	28 (14.8)	27 (14.1)	0.7	−6.4 to 7.9
1–42	56 (29.6)	53 (27.7)	1.9	−6.4 to 10.3
Any	57 (30.2)	54 (28.3)	1.9	−6.5 to 10.4

NOTE. Data are no. (%) of subjects, except where noted. PIV3-cp45, parainfluenza virus 3–cold passage mutant 45.

[a] The 2-sided confidence intervals (CIs) were calculated by use of percentage of subjects in the placebo group and, in each case, includes 0%. The denominator is the no. of randomized subjects.

[b] In the seronegative cohort, 114 subjects received PIV3-cp45, and 112 subjects received placebo.

[c] In the "all" cohort, 189 subjects received PIV3-cp45, and 191 subjects received placebo.

DISCUSSION

Previous clinical trials have confirmed the viral-shedding pattern, genetic stability, and immunogenicity of Vero tissue culture–produced PIV3-cp45 vaccine in adults, children, and infants [15]. A single dose of 10^6 pfu of PIV3-cp45 was evaluated in adults and seropositive children, single doses of 10^4 or 10^5 pfu were evaluated in seronegative children, and 2 doses at 1- or 3-month intervals were evaluated in infants 1–2 months old [15]. Ninety-four percent of seronegative vaccinated children and 94% of vaccinated infants were infected by the vaccine virus after 1 dose. Signs and symptoms of mild respiratory illness were common in both vaccine and placebo groups of seronegative children and infants and occurred in up to one-half of placebo recipients; OM was reported in 3 of 32 vaccinated children. These and other similar phase 1 studies have been effective screening studies to eliminate insufficiently attenuated or overly attenuated live attenuated vaccine candidates, but phase 1 studies are limited in assessing with precision the possible association of OM with vaccine because of study size [15, 17]. The present phase 2 evaluation was specifically undertaken to assess the frequency of common signs and symptoms of AOM and to obtain more-precise estimates of the frequency of signs and symptoms of URI that might be caused by vaccine virus replication.

The occurrence of runny nose and fever increased in both the vaccine and placebo groups during the 7 days after vaccination. Children who were selected for enrollment were afebrile and did not exhibit runny nose or cough. The diary card information recorded by parents noted that, later on the day of vaccination, 11% of children who received vaccine and 13% of children who received placebo exhibited runny nose. Cough and fever were also present in some children on the day of vaccination, but not more frequently in either group. On subsequent days, the frequency of runny nose increased and peaked on day 8 (34% in the placebo group and 28% in the vaccine group) before returning to a level of 16%–18% on day 14, after which data were not collected. We believe that this increase in the frequency of runny nose is a result of the selection of children on day 0 without manifestations of URI and that a return to the mean baseline values of ~20% of children with runny nose accounts for much of this observation. Selection of well children without symptoms of URI may result in the selection of children who are susceptible to prevalent viruses causing URI in the community, and the peak incidence of symptoms of URI on day 8 may represent acquisition of these agents by both the vaccine and placebo groups. In contrast to live attenuated influenza vaccine, which is associated with a slight increase in fever on day 2 after vaccination and runny nose on days 2, 3, 7, and 8 after vaccination [16], an increase in these minor events was not observed in the present study of PIV3-cp45; these findings further indicate the highly atten-

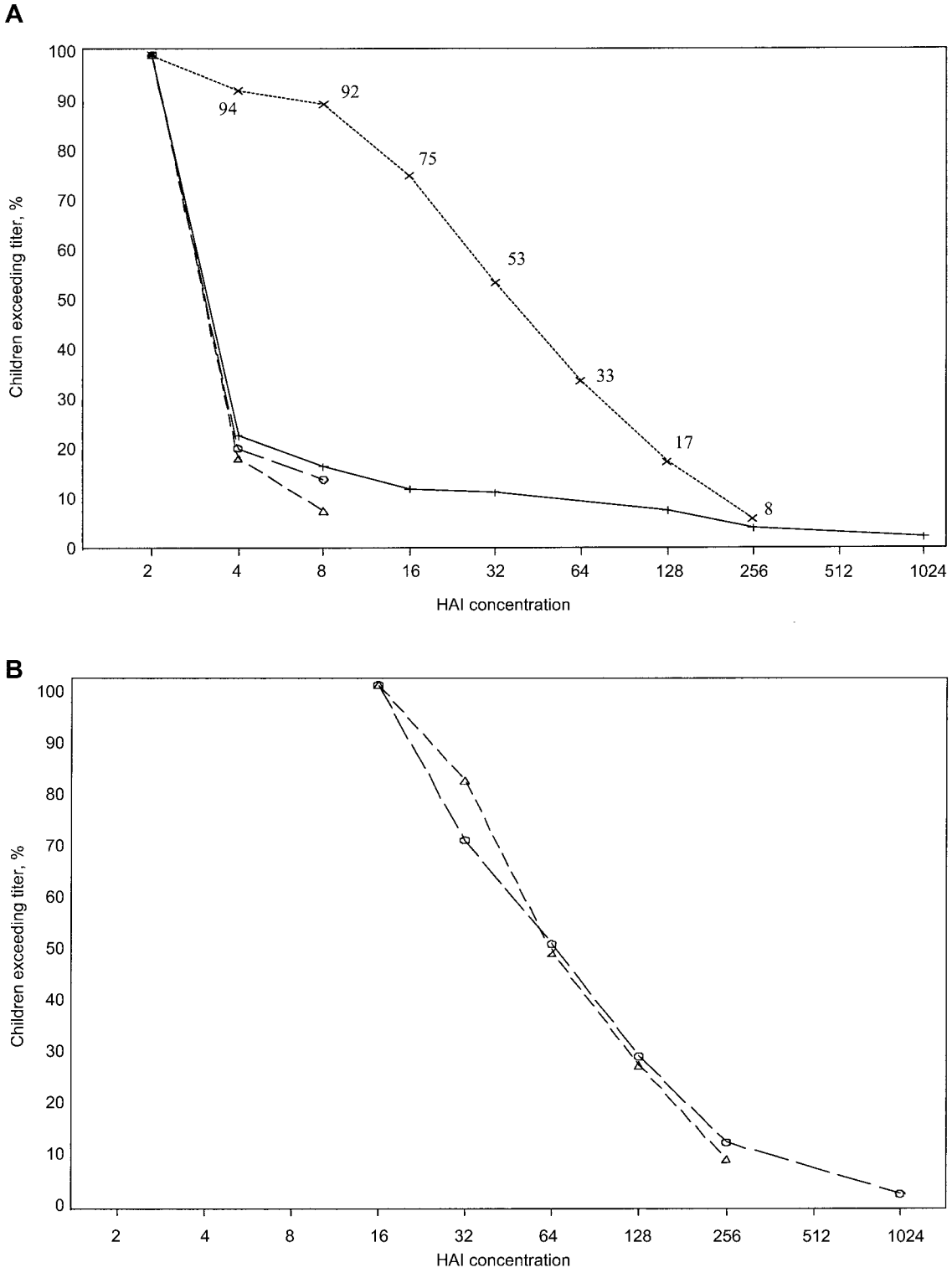

Figure 2. *A,* Reverse cumulative distribution curves of serum hemagglutination inhibition (HAI) antibody titers to parainfluenza virus 3 (PIV3) in seronegative children with paired serum samples available for analysis. Cumulative proportion of children achieving the indicated HAI titer is shown before and after vaccination with PIV3–cold passage mutant 45 (cp45) or placebo. Among 109 seronegative vaccine recipients and 110 placebo recipients, 86 (79%) and 13 (12%), respectively, had a 4-fold antibody increase ($P < .001$, Fisher's exact test). The postvaccine geometric mean antibody titer was 1:25 for vaccine recipients and <1:4 for placebo recipients. ○, Before PIV3-cp45; ×, after PIV3-cp45; △, before placebo; +, after placebo. *B,* Reverse cumulative distribution curves of serum HAI antibody to PIV3 for seropositive subjects. Prevaccination GMT was 1:50 for both vaccine and placebo groups and did not increase after vaccination. ○, PIV3-cp45; △, placebo.

Table 4. Serum hemagglutination inhibition (HAI) antibody response within 42 days (range, 35–56 days) after administration of parainfluenza virus 3 (PIV3)–cold passage mutant 45 (cp45) vaccine or placebo intranasally, by prevaccine antibody status.

	Prevaccine serum antibody status to PIV3							
	Seronegative (HAI ≤1:8)				Seropositive (HAI ≥1:16)			
Study group	No. of subjects tested	No. (%) of subjects with ≥4-fold increase in antibody titer	GMT to PIV3		No. of subjects tested	No. (%) of subjects with ≥4-fold increase in antibody titer	GMT to PIV3	
			Prevaccine	Postvaccine			Prevaccine	Postvaccine
PIV3-cp45	109[a]	86 (79)[b]	<1:4	1:25	60	2 (3)	1:50	1:46
Placebo	110	13 (12)	<1:4	<1:4	60	0 (0)	1:50	1:44

NOTE. GMT, geometric mean titer.

[a] Nos. of subjects with paired serum samples available are reported.

[b] $P < .001$, vs. placebo (Fisher's exact test).

uated nature of this PIV3 vaccine. Vaccination did not appear to confer significant protection against the causes of runny nose, cough, or fever during the 14 days after vaccination. Vaccination did not cause or protect against AOM or serous OM occurring within 42 days of vaccination. This result does not mean that the vaccine will not protect against PIV3-associated URI, LRI, or OM; rather, the occurrence of these illnesses caused by wild-type PIV3 during this short study was too infrequent to measure. Phase 3 studies are needed to measure these potential vaccine benefits.

PIV3-cp45 vaccine was immunogenic, and a single dose induced HAI antibody in 79% of seronegative (HAI titer ≤1:8) children. A single dose of vaccine increased serum PIV3 antibody to within 2-fold of preexisting, naturally acquired antibody titers observed in the seropositive subjects. In a previous study of PIV3-seronegative children, 18 (90%) of 20 children tested had ≥4-fold increases in HAI antibody titers, and 21 (100%) of 21 tested shed vaccine virus [15]. In contrast, seropositive children (titer ≥1:16) or adults shed vaccine virus less frequently than did seronegative children (2/16 [12%] seropositive children and 2/20 [10%] adults shed virus) and did not boost serum antibody titers (0/12 seropositive children and 0/10 adults had increases in antibody titers) [15]. Seropositive children 6–18 months old did not have increases in serum antibody titers in the present study. However, 2 doses of vaccine might improve the proportion of seronegative children who develop antibody after vaccination with PIV3-cp45.

In a previous study of this vaccine in infants 4–12 weeks old [15], 31 (94%) of 33 infants shed vaccine virus after dose 1 of PIV3-cp45 vaccine, but serum HAI antibody titers increased in the presence of maternal antibody (prevaccine geometric mean titer, 5.3 $\log_2$) in only 4 (13%) of 31 infants tested. A second dose of PIV3-cp45 vaccine given 1 or 3 months later resulted in vaccine virus shedding in 47% and 77% of infants, respectively, and only 1 infant (3%) had an HAI antibody response [15]. In contrast, IgA antibody to HN protein developed in more than one-half of the vaccinated infants after dose 1, and IgA levels were boosted in 47% and 66% of infants after dose 2 at 1 or 3 month intervals, respectively. These results are similar to the low frequency of serum antibody response observed in infants who are vaccinated with live attenuated RSV vaccines; multiple doses of live attenuated vaccine appear to be needed when infants are vaccinated in the face of maternal antibody and an immature immune system [17].

PIV3-cp45 vaccine is expected to prevent several significant clinical syndromes commonly caused by PIV3, including AOM, LRI, and febrile URI, but determination of efficacy will require an extended period of surveillance because PIV3 infections occur throughout the year [2–4]. An inl vaccine schedule to prevent LRI in infants, with vaccination at 1 month and again at 4 months, followed by inl boosting at 1 year, would be an attractive vaccine schedule to evaluate for efficacy, considering the results of the present phase 2 safety trial and those of the previous phase 1 trial in infants [15]. The addition of live attenuated RSV vaccine and, possibly, other live attenuated vaccines (PIV1, PIV2, and human metapneumovirus) would be a significant advance in controlling viral respiratory disease in young children, but these vaccines may not be available for several years. The safety and immunogenicity of PIV3-cp45 indicate that this vaccine should be evaluated for efficacy and effectiveness to help control this common cause of significant respiratory disease and AOM in infants and young children.

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