

Research Paper

# Phase I, Randomized, Controlled Trial to Study the Reactogenicity and Immunogenicity of a Nasal, Inactivated Trivalent Influenza Virus Vaccine in Healthy Adults

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## ABSTRACT

We performed a randomized, placebo-controlled, dose-escalating clinical trial to evaluate the safety and immunogenicity of an inactivated, split virion, trivalent, nasal influenza vaccine using lipid/polysaccharide molecules as carriers. A total of 64 adults (mean age 29; range 19–69 years) were randomly allocated to receive a mixture of lipid/polysaccharide carrier molecules and 7.5, 15, or 30 µg hemagglutinin antigen of each of the three influenza strains (A/Johannesburg/82/96 [H1N1], A/Nanchang/933/95 [H3N2], B/Harbin/07/94) or placebo via nasal spray on two occasions separated by 28 days. Adverse events were assessed immediately after immunization and for 14 days after each dose. Nasal and serum antibodies were measured before and two weeks after each dose. All but three participants completed the study; no withdrawals were because of adverse events. Adverse events were similar immediately after immunization except for anterior nasal dripping after the first dose which was more common in the combined vaccine groups (64.4%) than in the placebo group (31.3%;  $p < 0.05$ ). A similar trend was observed after the second dose. Nasal dripping was also more common in the first two days after immunization in the vaccine groups than the placebo group (31.3%–50% vs. 0%) with no difference with increasing vaccine dose. The vaccine elicited a modest serum antibody response against all three viruses, with the highest dose eliciting the highest serum antibody levels. In contrast, significant nasal antibody rises were observed for all three viruses; again, the 30 µg group achieved the highest mucosal antibody levels at the earliest time points. We conclude that this trivalent, split virion, inactivated nasal influenza vaccine formulated with lipid/polysaccharide molecule carriers is well tolerated and modestly immunogenic in healthy adults.

## INTRODUCTION

Influenza, caused by influenza A or B virus, is an acute viral respiratory illness manifested by fever, cough, coryza, headache, myalgia, and fatigue. Although usually a self-limited disease in healthy individuals, influenza and its complications including secondary bacterial infections can be life-threatening in the elderly and those with underlying medical conditions. Children younger than 2 years of age are also at risk of severe disease; other than the elderly, they are at the highest risk of hospitalization.<sup>1</sup> Influenza occurs in annual epidemics during the winter season in the temperate zones; in addition to increases in hospitalizations and excess respiratory deaths in the elderly, influenza accounts for massive school and industry absenteeism, affecting productivity and imposing a burden on the health care system.<sup>2–4</sup>

Influenza vaccine has been used for over 50 years as the primary measure of protection against influenza. Influenza vaccine is recommended for the elderly, residents of long-term care facilities, and persons with underlying health conditions including chronic respiratory or cardiac conditions, metabolic diseases, and renal diseases. Recently, in Canada<sup>5</sup> and the United States,<sup>3,6</sup> recommendations for annual influenza vaccine have been extended to all children 6 to 23 months of age and all household contacts of these individuals at high risk for influenza.

Currently available influenza vaccines are produced from three strains (two A and one B) of influenza virus harvested from chick embryo allantoic fluids. These viruses are inactivated in formalin and supplied either as whole virus vaccines or detergent-treated (split) virus vaccines. The latter have been shown to be less reactogenic than the whole virus formulations and are exclusively recommended for children younger than 13 years of age.<sup>3,5</sup> Recently, a live, cold-adapted, nasal trivalent influenza vaccine was licensed for use in individuals 5 to 49 years of age.<sup>7–8</sup> Nasally administered influenza vaccines have the

advantage of inducing both humoral and mucosal antibody responses, ease of administration, and avoiding annual injections, which might lead to better public acceptance, particularly in healthy young children.<sup>9</sup> In theory, inactivated influenza viral nasal vaccines might be advantageous because there is no risk the vaccine virus will revert to virulence. Although delivery of inactivated virus alone to the nasal mucosa does not induce substantial antibody responses, inclusion of a mucosal adjuvant such as the heat-labile toxin of *Escherichia coli* enhances the mucosal response, resulting in a highly efficacious vaccine.<sup>10,11</sup> One such vaccine was licensed in Switzerland; however, it was withdrawn from the market after an increased incidence of Bell's palsy was associated with its use.<sup>12</sup> It has been hypothesized that the Bell's palsy and other neurological toxicities may be a result of the known neurotropism of the *E. coli* heat-labile toxin and other similar toxins such as cholera toxin.<sup>13,14</sup>

Other systems have been shown to deliver influenza antigens to mucosal sites and elicit mucosal antibody responses.<sup>15</sup> Lipid/polysaccharide carrier molecules (SMBV, Biovector Therapeutics, S.A.) are spherical polysaccharidic nanoparticles surrounded by a lipid bilayer;<sup>16</sup> they are made of cross-linked maltodextrin with glycerol ether bonds and functionalized with cationic moieties (methoxycholine). The lipid bilayer surrounding these particles retains most of the qualities of a liposome bilayer, making the particles good candidates to mimic a synthetic virus. We tested the reactogenicity and immunogenicity of an inactivated, split virion, trivalent nasal influenza vaccine using the lipid/polysaccharide molecules as carrier macromolecules.

## MATERIALS AND METHODS

**Study population.** Healthy adults between the ages of 18 and 50 years of age were recruited into the study in 1998 after obtaining written, informed consent. Individuals were not eligible to participate if they had received an influenza vaccine in the preceding 8 months or if they had an influenza-like illness in the preceding 6 months, had received blood products or immunoglobulin in the preceding 3 months, were allergic to hen's eggs or any other vaccine constituent, used immunosuppressive medications in the previous 12 months, had chronic liver, renal, or inflammatory bowel disease or collagen vascular disease, had an immunodeficiency or immunocompromised health state, or had received any experimental medication in the previous 3 months. The protocol was approved by the Research Ethics Board of the IWK Health Centre.

**Vaccines.** A single nasal vaccine formulation consisting of a mixture of SMBV lipid/polysaccharide carrier molecules (Biovector Therapeutics, S.A., Toulouse, France) and split virion of each of three influenza viruses recommended for the 1997–1998 season (A/Johannesburg/82/96 [H1N1], A/Nanchang/933/95 [H3N2], B/Harbin/07/94) (BioChem Pharma, Inc., Laval, Quebec, Canada) was used. Split virion were obtained by cultivating the virus on hen's eggs, using the same methods as used in commercial production of injectable influenza vaccine. In the final split virion/SMBV mixture, the hemagglutinin (HA) antigen concentration for each virus strain was 7.5 µg/100 µL. The mixture was a sterile, aqueous, slightly opalescent liquid contained in an amber sealed vial. The formulation could contain trace residual amounts of the detergent deoxycholate and triton X-100 used in the manufacture of split vaccines. Each vial contained approximately 1 mL of the vaccine formulation. Participants received two doses of the assigned vaccine formulation 28 days apart. Participants allocated to receive 7.5 µg of each strain HA were administered 100 µL of the vaccine at each of the two vaccine visits. Participants allocated to receive 15 µg of each strain HA were administered 200 µL, and those allocated to receive 30 µg of each strain HA were administered 400 µL of the vaccine formulation at each visit.

**Randomization, blinding and immunization procedures.** Participants were randomized in equal numbers to one of the three vaccine dose formulations

or placebo in a balanced block format using a computer-generated list of random numbers. Vaccine was administered using a nasal spray device that was screwed to the uncapped vaccine vial. Depending on the group to which the subject was assigned, the provided spray device delivered a fixed volume of 50 µL or 100 µL. After priming the pump, the study nurse sprayed the fixed volume of the formulation once in each nostril, with the participant's head tilted backwards.

In order to avoid anterior and posterior dripping of the vaccine formulation in the nose, the maximum volume of liquid that was sprayed in each nostril did not exceed 100 µL. Participants in the 7.5 µg group received 50 µL of placebo/nostril at 0 minutes and 50 µL of vaccine/nostril at 30 minutes; participants in the 15 µg group received 100 µL placebo/nostril at 0 minutes and 100 µL of vaccine/nostril at 30 minutes; and participants at the highest dose (30 µg) received 100 µL of vaccine in each nostril at both 0 and 30 minutes. Subjects allocated to placebo received 50 µL or 100 µL of the saline spray at both the 0 and 30 minute time points. Because of the remote possibility that study staff might detect a difference in the volume administered by the allocated spray device, study staff who administered the vaccine were not involved in collection of any clinical data.

**Clinical and serological monitoring.** Blood and nasal wash samples were collected immediately before each of the two immunizations and approximately 14 days following each immunization. Adverse events were recorded daily in a diary and collected via structured questionnaire 30 minutes after immunization, 2 days after immunization, and 14 days after immunization. Solicited adverse events included anterior and posterior nasal dripping, nasal irritation, nasal stuffiness or congestion, sneezing, nasal burning, nasal itching, and nasal bleeding. Solicited systemic events included fever, chills, headache, muscle aches, decreased appetite, nausea, vomiting, diarrhea, and rash. Any other symptoms, the use of analgesics, non-planned medical consultations or doctor's visits, and hospitalizations were also ascertained. Adverse events were categorized as absent, mild (symptom present but not bothersome, a nuisance at most), moderate (symptom bothersome; frequent and annoying; somewhat distressing; may require self-medication), or severe (symptoms very distressing; interference with normal functioning, may require medical attention). In addition to self-reporting of nasal adverse reactions, a single otolaryngologist performed a clinical examination of the nasal mucosa before, immediately after, and 14 days after the nasal administration of each dose of the vaccine. Mucosal irritation was evaluated by examining the anterior nasal septum for signs ranging from simple hyperemia to frank bleeding and scored semi-quantitatively as 1+ to 4+. Mucosal inflammation was evaluated by examining the area of the inferior turbinate and graded in the same fashion.

**Laboratory analysis.** Serum antibody against each of the three vaccine strains was measured by microtiter hemagglutination inhibition assay (HI) as previously described.<sup>17</sup> Sera were treated with receptor-destroying enzyme (RDE, Senka, Japan) prior to use and tested at an initial dilution of 1:4. Antigens were egg-grown pools of the test viruses, and the B antigen was ether-extracted.

Enzyme immunoassays were performed as described.<sup>18</sup> Briefly, 96 well plates (Nunc Immulon) were coated with sucrose gradient purified hemagglutinin of the test viruses<sup>19</sup> at a concentration of 10 ng/well, followed by the nasal wash sample to be tested in serial two-fold dilutions, and alkaline phosphatase-conjugated goat anti-human IgA (TAGO, Burlingame, CA). The endpoint titer of the sample was the highest dilution resulting in an optical density of 0.20 units or greater, and twice the background in wells with no antigen. Four-fold or greater changes in titer were considered significant. Nasal wash samples were tested for HA specific IgA after approximately 10-fold concentration by evaporation in dialysis tubing, and the titer adjusted for the total concentration of IgA as determined by enzyme immunoassay using human colostrum IgA (DAKO, Carpinteria, CA) as standard control. All serological assays were performed without knowledge of the vaccine assignment.

In addition to the clinical examination of the nasal mucosa, further evaluation of nasal inflammation and irritation was performed by counting neutrophils and red blood cells in nasal wash fluid with a hemocytometer.

Table 1 Adverse events reported in the first two days after immunization with nasal influenza vaccine in healthy adults

Adverse Event	Dose	Percent (95% Confidence Interval) in Each Group Reporting the Adverse Event			
		placebo	7.5 µg	15 µg	30 µg
Nasal dripping	1	0 (0.0–20.6)	31.3 (11.0–58.7) <sup>1</sup>	50.0 (24.7–75.3) <sup>2</sup>	31.3 (11.0–58.7) <sup>1</sup>
	2	6.7 (0.2–31.9)	12.5 (1.6–38.3)	0 (0.0–21.8)	26.7 (7.8–55.1)
Nasal stuffiness	1	18.8 (4.0–45.6)	31.3 (11.0–58.7)	50.0 (24.7–75.3)	37.5 (15.2–64.6)
	2	26.7 (7.8–55.1)	25.0 (7.3–52.4)	20.0 (4.3–48.1)	40.0 (16.3–67.7)
Nasal burning	1	0 (0.0–20.6)	0 (0.0–20.6)	6.3 (0.2–30.2)	6.3 (0.2–30.2)
	2	0 (0.0–21.8)	0 (0.0–20.6)	0 (0.0–21.8)	6.7 (0.2–31.9)
Nasal itching	1	6.3 (0.2–30.2)	18.8 (4.0–45.6)	6.3 (0.2–30.2)	12.5 (1.6–38.3)
	2	6.7 (0.2–31.9)	6.3 (0.2–30.2)	0 (0.0–21.8)	13.3 (1.7–40.5)
Nasal bleeding	1	0 (0.0–20.6)	0 (0.0–20.6)	0 (0.0–20.6)	6.3 (0.2–30.2)
	2	0 (0.0–21.8)	0 (0.0–20.6)	0 (0.0–21.8)	6.7 (0.2–31.9)
Sneezing	1	25.0 (7.3–52.4)	37.5 (15.2–64.6)	37.5 (15.2–64.6)	25.0 (7.3–52.4)
	2	26.7 (7.8–55.1)	18.8 (4.0–45.6)	26.7 (7.8–55.1)	46.7 (21.3–73.4)
Fever	1	0 (0.0–20.6)	6.3 (0.2–30.2)	0 (0.0–20.6)	12.5 (1.6–38.3)
	2	26.7 (7.8–55.1)	0 (0.0–20.6) <sup>1</sup>	0 (0.0–21.8)	0 (0.0–21.8)
Chills	1	6.3 (0.2–30.2)	0 (0.0–20.6)	0 (0.0–20.6)	31.3 (11.0–58.7) <sup>1</sup>
	2	0 (0.0–21.8)	6.3 (0.2–30.2)	0 (0.0–21.8)	0 (0.0–21.8)
Headache	1	12.5 (1.6–38.3)	37.5 (15.2–64.6)	18.8 (4.0–45.6)	25.0 (7.3–52.4)
	2	13.3 (1.7–40.5)	18.8 (4.0–45.6)	13.3 (1.7–40.5)	20.0 (4.3–48.1)
Muscle ache	1	0 (0.0–20.6)	12.5 (1.6–38.3)	6.3 (0.2–30.2)	6.3 (0.2–30.2)
	2	6.7 (0.2–31.9)	6.3 (0.2–30.2)	6.7 (0.2–31.9)	6.7 (0.2–31.9)
Decreased appetite	1	0 (0.0–20.6)	6.3 (0.2–30.2)	18.8 (4.0–45.6)	6.3 (0.2–30.2)
	2	0 (0.0–21.8)	6.3 (0.2–30.2)	0 (0.0–21.8)	6.7 (0.2–31.9)
Nausea	1	0 (0.0–20.6)	6.3 (0.2–30.2)	12.5 (1.6–38.3)	6.3 (0.2–30.2)
	2	0 (0.0–21.8)	0 (0.0–20.6)	0 (0.0–21.8)	6.7 (0.2–31.9)
Vomiting	1	0 (0.0–20.6)	0 (0.0–20.6)	0 (0.0–20.6)	6.3 (0.2–30.2)
	2	0 (0.0–21.8)	0 (0.0–20.6)	0 (0.0–21.8)	6.7 (0.2–31.9)
Diarrhea	1	0 (0.0–20.6)	12.5 (1.6–38.3)	12.5 (1.6–38.3)	6.3 (0.2–30.2)
	2	6.7 (0.2–31.9)	0 (0.0–20.6)	6.7 (0.2–31.9)	0 (0.0–21.8)

<sup>1</sup>p < 0.05 compared to placebo. <sup>2</sup>p < 0.01 compared to placebo.

**Statistical analysis.** Baseline comparability of the groups was assessed by Fisher's exact test for proportions and t-tests for continuous variables. Adverse events were analyzed at each contact time at each visit. Adverse events were also analyzed after grouping for severity. Clinically significant events were defined as oral temperature  $\geq 38.0^\circ\text{C}$  and any other symptom graded as moderate or severe. Severe reactions were defined as oral temperature  $\geq 39.0^\circ\text{C}$  and any symptom graded as severe. Rates of each adverse event were estimated using binomial distribution point estimates and exact 95% confidence intervals and the rates compared between groups using two-sided Fisher's exact tests. No adjustments were made for multiple comparisons. The degree of nasal mucosal irritation and inflammation were compared across study groups by Wilcoxon tests, and the differences between the pre- and post-immunization examination measurements at each visit were compared using McNemar's test. Neutrophil and red blood cell counts in the nasal wash fluid samples were analyzed as continuous variables and compared between study groups and within subjects, pre- and post-immunization, using profile analysis of variance.

Geometric mean hemagglutination inhibition titers and adjusted mucosal IgA antibody titers and 95% confidence intervals were calculated for each of the two vaccination visits (day 0 and day 28) and each of the two recall visits (day 14 and day 42). Fold-antibody rises were determined by comparing titers at day 14 vs. day 0, day 28 vs. day 0, the maximum of the day 14 and day 28 vs. day 0, and day 42 vs. day 0. Comparisons between groups were made using analysis of variance and t-tests. Within-subject variation was assessed using profile analysis. Means were analyzed after log transformation.

Antibody levels below the lowest dilution tested (1:10) were assigned a level of half the lowest value tested (1:5). The proportion of subjects achieving four-fold antibody rises in pre- and post-vaccination samples and the proportions achieving predefined threshold levels were estimated by vaccine group and compared between groups using the same methods as for adverse events. A supplementary analysis was performed on the subset of participants who had pre-immunization titers  $< 40$  (presumed "non-immune").

As a phase 1 study, no formal sample size was calculated. Sufficient numbers of participants were enrolled (a total of 45 comprising 15 at each dosage level) to detect the most significant adverse events that occurred at a frequency of 8% or more, assuming that such reactions were not dose dependent.

## RESULTS

**Demographics.** A total of 64 healthy adults entered the study; 61 of the 64 received both doses of the vaccine and had all nasal wash and blood specimens collected. One participant withdrew voluntarily from the study prior to the second immunization. Two participants were withdrawn from the study when it was discovered that the vaccine designated for one participant had been given to another and a replacement dose for the second participant was not available. Females comprised 45 (70%) of the 64 participants; the mean age was 29 (range 19–49). There were no differences between the groups for nasal septal deviation (25%–50% of participants), history of tonsillectomy or adenoidectomy, nasal or sinus surgery, or other medical

Table 2 Serum hemagglutination inhibition and nasal IgA geometric mean antibody titers before and 14 days after each dose of nasal influenza vaccine in healthy adults

Virus	Day <sup>1</sup>	Geometric Mean Antibody Titer (95% confidence interval)			
		placebo	7.5 µg	15 µg	30 µg
<i>Serum HAI antibody</i>					
A/Johannesburg/H1N1	0	64.4 (27.5–151)	67.3 (33.6–135)	91.9 (48.7–174)	52.8 (19.8–141)
	14	76.6 (41.0–143)	104 (54.4–198)	111 (57.9–211)	87.7 (36.4–212)
	28	73.4 (38.5–140)	95.1 (50.9–178)	96.2 (50.7–183)	106 (50.5–221)
	42	72.9 (37.0–144)	93.8 (51.1–172)	133 (73.8–240)	116 (57.0–235)
A/Nanchang/H3N2	0	19.2 (10.1–36.3)	22.8 (11.9–43.6)	23.0 (14.1–37.3)	13.8 (7.62–25.1)
	14	24.8 (13.6–45.2)	24.8 (13.4–45.9)	21.9 (14.2–33.8)	16.6 (9.07–30.5)
	28	24.8 (15.7–39.4)	29.5 (15.7–55.6)	25.2 (16.9–37.7)	16.6 (9.76–28.3)
	42	20.0 (11.6–34.4)	21.8 (13.7–34.7)	24.1 (15.0–38.5)	20.9 (11.2–39.1)
B/Harbin	0	24.8 (12.4–49.9)	28.3 (13.3–59.9)	40.0 (16.4–97.8)	20.0 (9.28–43.1)
	14	32.2 (16.0–64.7)	38.3 (20.5–71.6)	43.9 (19.9–96.9)	43.9 (24.2–79.6)
	28	29.5 (15.1–57.9)	40.0 (22.9–69.8)	48.1 (23.8–97.2)	48.1 (29.4–78.6)
	42	30.3 (12.6–73.1)	43.6 (25.1–75.9)	57.9 (25.5–131)	60.6 (41.5–88.5)
<i>Nasal IgA antibody</i>					
A/Johannesburg/H1N1	0	3.39 (2.17–5.28)	3.09 (2.05–4.67)	3.50 (2.29–5.36)	3.02 (1.85–4.92)
	14	2.84 (1.64–4.92)	5.85 (3.60–9.51)	5.95 (3.32–10.7)	6.93 (3.22–14.9) <sup>2</sup>
	28	3.61 (2.61–5.01)	7.22 (3.70–14.1)	8.54 (4.47–16.3) <sup>2</sup>	8.25 (4.47–15.3) <sup>2</sup>
	42	3.80 (2.53–5.70)	10.5 (6.12–21.6) <sup>2</sup>	16.2 (7.70–33.9) <sup>3</sup>	19.2 (9.21–40.0) <sup>4</sup>
A/Nanchang/H3N2	0	11.4 (7.57–17.1)	12.9 (7.44–22.5)	8.13 (3.90–16.9)	7.60 (4.50–12.8)
	14	11.9 (8.28–17.0)	15.2 (8.58–26.8)	15.7 (9.24–26.7)	17.5 (9.34–32.7)
	28	13.3 (7.82–22.5)	16.4 (10.3–26.2)	19.6 (10.9–35.4)	25.0 (15.6–40.3)
	42	16.0 (9.93–25.6)	23.4 (11.1–49.2)	24.5 (13.3–45.0)	40.2 (26.0–62.1) <sup>2</sup>
B/Harbin	0	10.9 (5.82–20.4)	5.93 (3.46–10.2)	8.13 (4.89–13.5)	8.33 (5.79–12.0)
	14	10.9 (6.18–19.1)	6.66 (3.94–11.2)	10.9 (6.39–18.4)	14.5 (10.7–19.7)
	28	9.79 (5.26–18.2)	8.96 (5.70–14.1)	12.9 (7.07–23.7)	16.5 (11.3–24.2)
	42	10.7 (6.36–18.1)	18.9 (9.35–38.2)	21.3 (12.1–37.6)	35.0 (20.9–58.7) <sup>3</sup>

<sup>1</sup>Day 0: pre-dose 1; Day 14: 14 days post-dose 1; Day 28: 28 days post-dose 1, pre-dose 2; Day 42: 42 days post-dose 1, 14 days post-dose 2. <sup>2</sup>p < 0.05 compared to placebo. <sup>3</sup>p < 0.01 compared to placebo. <sup>4</sup>p < 0.001 compared to placebo.

conditions. Between 25% and 56% of participants of each group had received an influenza vaccine in prior years.

**Adverse events.** Adverse events within the first 30 minutes after administration of the nasal vaccine were similar in recipients of the influenza vaccine and placebo except for anterior nasal dripping which was observed in 64.6% of the combined nasal influenza vaccine recipients compared to 31.3% of the placebo recipients ( $p < 0.05$ ) after dose 1. A similar trend was observed after dose 2 (23.9% compared to 0%) but did not reach statistical significance. There was a trend toward more sneezing in the first 30 minutes after vaccine compared to placebo after dose 1 (8.3% vs. 0%) and dose 2 (13.0% vs. 0%) but neither reached statistical significance. Nasal symptoms were also similar in the 2 days after administration of the nasal spray (Table 1). Nasal dripping was more commonly reported by recipients of all three doses of the vaccine compared to placebo (31.3–50% vs. 0%;  $p < 0.05$ ) with no increase in nasal dripping with increasing vaccine dose. Reports of systemic adverse events were similar amongst all groups except for chills which were reported by 31.3% of first dose 30 µg recipients compared to 6.3% of placebo recipients ( $p < 0.05$ ) and fever which was reported by 26.7% of second dose placebo recipients and 0% of 7.5 µg recipients ( $P < 0.05$ ). Chills were not reported by any of the 30 µg recipients after the second dose and fever was not reported by any of the placebo recipients after the first dose, suggesting that these reports may not have been vaccine-related. Most of the adverse events reported were described as mild; moderate and severe adverse events were restricted to headache which were reported with similar frequency in both vaccine and placebo recipients. Unsolicited adverse events were reported in similar frequency between vaccine and placebo recipients and mostly related to intercurrent viral infections, trauma, or physiological events (such

as menorrhagia). There were no serious adverse events reported by study participants.

Mucosal irritation was present in 12.5% of placebo recipients and 16.7% of vaccine recipients prior to the first immunization. Similar degrees of mucosal irritation were observed 30 minutes post-immunization (12.5% vs. 22.9%) and 14 days after the first immunization (6.3% vs. 10.4%) and pre- (0% vs 6.7%), immediately after (0% vs. 4.3%), and 14 days after (0% vs. 4.3%) the second immunization. Mucosal inflammation was observed in 12.5% of placebo recipients and 10.4% of vaccine recipients before the first dose. Similar frequencies of inflammation were observed immediately after the first dose and at the subsequent observations (data not shown). There was no bleeding detected by direct examination or by nasal wash red blood cell counts; there were no differences between the groups in nasal white blood cell counts.

**Serum antibody response.** The nasal influenza vaccine only elicited a limited augmentation of serum HAI antibody response (Table 2). Although geometric mean serum antibody titers tended to increase with time after the highest vaccine doses, these increases did not achieve statistical significance. The mean fold serum HAI antibody rises (post-immunization/pre-immunization) were significantly higher in the 30 µg group than the placebo group for A/Johannesburg (2.19 fold rise vs. 1.15 fold rise,  $p < 0.05$ ), A/Nanchang (1.52 fold rise vs. 0.95 fold rise,  $p < 0.05$ ), and B/Harbin (3.03 fold rise vs. 1.20 fold rise,  $p < 0.01$ ). In the 30 µg group, 40% of participants had a four-fold or greater increase in serum HAI titer against A/Johannesburg compared to 13.3% of placebo recipients, and 20% had a four-fold antibody rise against A/Nanchang compared to none of the placebo recipients ( $p$  not significant). A total of 46.7% of the 30 µg recipients had

a four-fold or greater HAI antibody rise against B/Harbin compared to 6.7% of placebo recipients ( $p < 0.05$ ). In general, when seronegative (pre-immunization titers  $<40$ ) participants were evaluated separately, the proportions with a four-fold or greater antibody rise increased; as an example, in the 30  $\mu\text{g}$  group, 75% of seronegative participants had a four-fold or greater increase in serum HAI titer against A/Johannesburg compared to 14.3% of the seronegative placebo recipients ( $p < 0.05$ ).

**Mucosal antibody response.** The nasal vaccine induced a significant nasal IgA antibody response against all three viruses (Table 2). For A/Johannesburg, a significant antibody rise was observed in the 7.5  $\mu\text{g}$  group 14 days after dose 2 and in the 15  $\mu\text{g}$  group 28 days after dose 1 and 14 days after dose 2. In the 30  $\mu\text{g}$  group, a significant antibody rise was observed 14 and 28 days after dose 1 and 14 days after dose 2. Against A/Nanchang and B/Harbin, nasal antibody titers increased for all dose levels but only reached statistical significance for the 30  $\mu\text{g}$  group 14 days post-dose 2.

Geometric mean fold antibody rises between pre- and post-dose 2 immunization were significantly higher in the vaccine groups than the placebo groups. The combined vaccine group had a 4.81 mean fold rise in nasal antibody against A/Johannesburg compared to 1.04 fold rise in the placebo group ( $p < 0.001$ ), a 3.27 mean fold rise against A/Nanchang compared to 1.34 fold rise in the placebo group ( $p < 0.01$ ), and a 3.15 mean fold rise against B/Harbin compared to 0.95 in the placebo group ( $p < 0.001$ ). A dose response was observed with the 30  $\mu\text{g}$  group mean fold rises ranging from 4.20 against B/Harbin, and 5.29 against A/Nanchang, to 6.37 against A/Johannesburg; however, the differences between doses did not achieve statistical significance.

The proportion of participants achieving four-fold or greater nasal IgA antibody responses was also higher in the vaccine recipients than in placebo recipients. In the combined vaccine groups, 61.9% of vaccine recipients and no placebo recipients had a four-fold response against A/Johannesburg ( $p < 0.001$ ); 40.5% of vaccine recipients and 7.1% of placebo and 40.5% of vaccine recipients and no placebo recipients had four-fold or greater responses against A/Nanchang ( $p < 0.05$ ) and B/Harbin ( $p < 0.01$ ) respectively. The highest proportions (80% for A/Johannesburg, 66.7% for A/Nanchang, and 60% for B/Harbin) were achieved after the 30  $\mu\text{g}$  dose.

Although it is not known what role mucosal IgA has in the protection against influenza virus infections, the proportion of participants achieving a threshold response (a nasal IgA antibody response  $>16$ ) was significantly higher in the vaccine recipients than placebo recipients. A total of 51.2% of combined vaccine recipients had a titer  $\geq 16$  against A/Johannesburg 14 days after dose 2 compared to 7.1% of placebo recipients ( $p < 0.01$ ) and 72.1% vs. 50% respectively against A/Nanchang ( $p$  not significant). Against B/Harbin, 67.4% of vaccine recipients and 14.3% of placebo recipients exceeded a titer of 16 after dose 2 ( $p < 0.001$ ).

## DISCUSSION

The results of this study demonstrate that this inactivated split virion nasal influenza vaccine formulated with lipid/polysaccharide carriers was well tolerated and elicited both a mucosal and limited systemic immune response. Nasal dripping was the adverse event seen most frequently in the influenza vaccinated groups immediately after immunization, with approximately 25%–30% excess over the frequency in the placebo group. This excess (attributable) increase of 33%–50% of participants reporting nasal dripping persisted in the influenza vaccinated groups in the two-day post-immunization observation period after the first dose (but not the second dose). There was no consistent increase in systemic adverse events in the influenza immunized groups compared to the placebo groups, and no subjects were withdrawn from the study because of an adverse event. There were also no clinically apparent abnormalities visualized in vaccine recipients by the otolaryngologist.

Significant antibody rises to all three viral strains were demonstrated by profile analysis in both serum and nasal secretions. Although the study did not have sufficient power to answer the question of the optimum dose of the influenza antigens, the 30  $\mu\text{g}$  dose produced the highest serum and nasal antibody levels and was frequently the only dose that was demonstrated to be statistically significantly different from placebo. The increased immunogenicity was not accompanied by increased adverse effects.

Several aspects of the study design limit the conclusions that can be made from this study. Although the study demonstrates that the nasal influenza vaccine formulated with the lipid/polysaccharide carrier was well tolerated and modestly immunogenic, the incremental benefit of the formulation with the carrier cannot be assessed. In pre-clinical mouse studies, the formulation with the lipid/polysaccharide carrier was more immunogenic for mucosal antibody than either nasally administered influenza vaccine alone or parenterally administered influenza vaccine. Whether the enhancement observed and the requirement for the carrier demonstrated in the animal studies is relevant to the clinical situation could only be confirmed by additional study arms using the influenza antigens administered alone nasally and parenterally. However, others have observed that inactivated influenza antigens administered intranasally are poorly immunogenic without some mucosal carrier or adjuvant.<sup>10</sup>

Despite these limitations, the formulation tested was well tolerated and induced serum and mucosal antibodies against the vaccine antigens. Rates of adverse events were not substantially different than those reported in children given the cold-adapted live nasal influenza vaccine now licensed in the United States.<sup>7,20,21</sup> Although one must be cautious in comparing anti-influenza antibody data from different laboratories, levels of serum antibodies achieved with this vaccine were of similar magnitude to levels achieved by the cold-adapted influenza vaccine in children (nasal antibody levels were not reported in those studies)<sup>7,22</sup> and in healthy adults.<sup>23</sup>

Given the recent expansion of recommendations for the routine administration of influenza vaccine to normal children between 6 and 23 months of age and to household contacts (including children) of children younger than 2 years of age,<sup>3,5,6</sup> there will be an increasing desire for non-injectable influenza vaccines. Inactivated alternatives to the cold-adapted live virus influenza vaccine will be useful for individuals in whom live virus vaccines are contraindicated and should concerns arise about its ability to revert to virulence. However, given recent reports of an increased incidence of Bell's palsy after administration of an inactivated nasal influenza vaccine that uses the heat labile toxin of *E. coli* as a mucosal adjuvant (although the effect was thought to be related to the adjuvant rather than the influenza antigen),<sup>12,13,14</sup> large safety studies will likely be required prior to licensure of inactivated nasal influenza vaccines.

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