Efficacy of Intranasal Virosomal Influenza Vaccine in the Prevention of Recurrent Acute Otitis Media in Children

Paola Marchisio,[1] Roberta Cavagna,[1] Barbara Maspes,[2] Stefania Gironi,[1] Susanna Esposito,[1] Lara Lambertini,[1] Alessandra Massimini,[1] Christian Herzog,[1] and Nicola Principi[3]

[1]Pediatric Department 1, University of Milan, Milan, and [2]Istituto Sieroterapico Berna, Como, Italy; and [3]Berna Biotech, Berne, Switzerland

To evaluate the efficacy of an intranasal, inactivated, virosomal subunit influenza vaccine for prevention of new episodes of acute otitis media (AOM) in children with recurrent AOM, 133 children aged 1–5 years were randomized to receive the vaccine ($n = 67$) or no vaccination ($n = 66$). During a 6-month period, 24 (35.8%) vaccine recipients had 32 episodes of AOM; 42 (63.6%) control subjects had 64 episodes. The overall efficacy of vaccination in preventing AOM was 43.7% (95% confidence interval, 18.6–61.1; $P = .002$). Children vaccinated before influenza season had a significantly better outcome than did those vaccinated after the onset of influenza season. The cumulative duration of middle ear effusion was significantly less in vaccinated children than in control subjects. Data suggest that the intranasal virosomal influenza vaccine might be considered among the options for the prevention of AOM in children <5 years old with recurrent AOM.

Recurrent acute otitis media (AOM) is common in infants and children, and its possible sequelae make prevention desirable [1]. Chemoprophylaxis, immunoprophylaxis, surgery, and the control of environmental risk factors have been proposed as preventive measures, but the first of these has long been considered the best option because of its ease and effectiveness in reducing the incidence of AOM [2]. However, its use has been questioned because of the risk of nasopharyngeal colonization by drug-resistant bacteria [3–5].

The emergence of drug-resistant bacteria has increased the importance of immunoprophylaxis, the aim of which is to provide protection against the major pathogens known to be direct or indirect causal agents of AOM [6, 7]. The evidence that viral infections are associated with many, if not most, episodes of AOM [8] has caused immunoprophylaxis against respiratory viruses to receive growing attention.

It has been shown that administration of standard parenteral inactivated influenza vaccine decreased the incidence of AOM by approximately one-third in children attending day care centers during a community outbreak of influenza [9–11]. Live, attenuated, cold-adapted intranasal influenza vaccine was shown to be effective in reducing the number of episodes of febrile otitis media by 30% among healthy children without a history of ear disease [12, 13]. However, although live attenuated influenza vaccine is effective and readily acceptable, some researchers have expressed concerns about the use of living influenza particles in humans [14–16].

Virosomes are small, suspended spheres with a lipid bilayer that can serve as a vehicle for solubilized viral proteins and that may improve antigen immunogenicity without causing toxicity [17]. An influenza vaccine has been developed for intranasal administration, which

is formed by inserting purified hemagglutinin from influenza strains into virosomes and is adjuvanted with *Escherichia coli* heat-labile toxin [18, 19]. In adults, it is immunogenic and is as safe as the classical inactivated vaccines, and it may be an alternative to live attenuated vaccine [20]. In the present study, we evaluated the efficacy of this intranasal virosomal influenza vaccine for the prevention of new episodes of AOM in children with recurrent AOM.

METHODS

Vaccine. The intranasal, inactivated, virosomal subunit influenza vaccine (Nasalflu) was supplied by Berna Biotech at the beginning of November 1999 and stored at 2°C–8°C. Each dose consisted of 7.5 μg of purified hemagglutinin from each of the three 1999–2000 World Health Organization–recommended influenza strains (A/Beijing/262/95-like, H1N1; A/Sydney/5/97-like, H3N2; and B/Beijing/184/93-like), coupled with a reconstituted immunopotentiating influenza virosome and adjuvanted with 2 μg of *E. coli* heat-labile toxin. The special delivery spray consisted of a 2-shot large-particle aerosol designed to deliver one 0.1-mL aliquot of vaccine per nostril, for a total volume of 0.2 mL.

Population and eligibility criteria. Children aged 1–5 years with a history of recurrent AOM, defined as ≥3 episodes in the preceding 6 months or ≥4 episodes in the preceding 12 months, with the most recent episode of AOM in the previous 2–8 weeks, were included in the study. The episodes were documented by medical records, with ≥2 episodes documented by symptoms and otoscopy and tympanometry findings. The children had to be free of AOM, but they could have otitis media with effusion (OME). The exclusion criteria were acute febrile illness (rectal temperature, ≥38.1°C), severe atopy, any previous influenza vaccination, acquired or congenital immunodeficiency, recent administration of blood products, cleft palate, chronically ruptured eardrum, obstructive adenoids, sleep apnea syndrome, and placement of tympanostomy tubes.

Study design. This single-center, prospective, randomized, single-blind study was conducted in Italy during the 1999–2000 influenza season. The study protocol was approved by the ethics committee of the University of Milan, the research was conducted in accordance with the guidelines for human experimentation specified by the authors' institutions, and a parent or legal guardian was required to provide written informed consent for each child. The single-blind design was chosen because, for technical reasons, the preparation of a placebo containing all the components of the formulation except influenza antigens was impossible.

Intervention. Children were assigned randomly 1:1 to the vaccine group or to the control group. The vaccine was administered in 2 doses on days 1 and 8 (±1 day). In order to ensure investigator blinding, the assignment and vaccine administration were performed by 2 investigators (R.C. and S.G.), and the parents were instructed not to discuss group assignment with the investigator responsible for the clinical and otological follow-up (P.M.), who remained blinded to group assignment until the end of the follow-up period.

Safety. The parents were asked to record daily on a diary card, for the 4 days after the administration of each dose of vaccine, the child's temperature and the occurrence of systemic symptoms (rectal temperature of ≥38.1°C, shivering, irritability, earache, cough, nausea, and diarrhea) and nasal symptoms (itchy, stuffy, or runny nose and sneezing). Parents rated their satisfaction with the safety and tolerability of the vaccine as "bad," "good," or "very good."

Study procedures. Children were examined at study entry and every 4–6 weeks for 25 weeks. To overcome the possible underreporting of disease in vaccine recipients related to a parent's feeling that the vaccine was giving protection, all the families were called twice per week by telephone to inquire about day-to-day status and to remind parents of the possibility of freely contacting an investigator at any time of day to arrange an extra visit within 24 h whenever the child developed symptoms of respiratory tract illness. At each visit, a history of infection between the 2 visits was obtained, and pneumatic otoscopy (Welch Allyn, model 20200) and tympanometry (Amplaid 770; Amplifon) were performed, together with a complete physical examination [21, 22]. The procedures were always carried out by the same investigator (P.M.), who was validated in the use of the instruments.

The diagnosis of AOM was made on the basis of the presence of any combination of the following findings: fever, earache, irritability, and hyperemia or opacity accompanied by bulging or immobility of the tympanic membrane; tympanometry assisted in establishing the presence of effusion in doubtful cases. The diagnosis of OME was made on the basis of impaired mobility, opacification, fullness or retraction of the eardrum associated with a tympanogram with a flat tracing, and the absence of signs and symptoms of acute infection.

Whenever AOM was diagnosed, amoxicillin plus clavulanic acid (50 mg/kg per day of amoxicillin) was given for 10 days. No other treatments were allowed for AOM, except for acetaminophen in the case of fever. Bilateral ear involvement was considered a single episode. Relapse and recurrence were defined as reappearance of signs and symptoms of AOM ≤4 days after the end of therapy or 5–14 days after the end of therapy, respectively. If 2 episodes of AOM occurred within a 6-week period, chemoprophylaxis with amoxicillin (20 mg/kg per day) was administered.

Analysis. The primary outcome measure was the occur-

rence of AOM within the 6-month period. Secondary outcome measures were the occurrence of febrile respiratory illnesses, the use of antibiotics, and the estimated proportion of time with bilateral OME. The analyses were conducted for the intent-to-treat population. The sample-size determination was based on data from the literature [9]. On the basis of those data, the percentage of control subjects who did not respond to the vaccine (i.e., those with AOM) was estimated to be 29.4% (55 of 187 subjects). Assuming that the expected percentage of vaccine recipients with no response to the vaccine would have been ∼12% (i.e., ∼40% of the percentage among control subjects) and assuming the use of a 1-tailed test with $\alpha = 0.05$ and $\beta = 0.20$, we sought a sample size of 66 children in each group.

To evaluate the efficacy of the vaccine in relation to the influenza season, the vaccine recipients were stratified into 2 groups: those who had completed vaccination at least 1 week before the start of influenza season and those who were incompletely or not vaccinated when the season began. The influenza season was defined as the period from the date of the first isolation of influenza virus through the date of the last isolation of influenza virus in Italy, as determined by the surveillance system that collected clinical reports from sentinel physicians, integrated with virological surveillance (information is available at http://www.influnet.it). The occurrence of AOM was also analyzed with respect to the possible influence of epidemiological variables. A finding of OME in the same ear on

2 consecutive occasions was considered to indicate its persistence during the interval; when OME was present at one examination and absent in the other, it was considered to have been present for half of the intervening period.

Statistical analysis. The statistical comparisons were made by nonparametric or exact tests with SAS software, version 8 (SAS Institute). $P < .05$ was considered statistically significant. The categorical data were summarized by counts and percentages and compared by the χ^2 test with Yates' correction for 4-fold tables; if the sample was too small, Fisher's exact t test was used. The risk of the appearance of AOM was compared between groups by calculating protective efficacy and its 95% confidence interval. A logistic multivariate model was used to investigate whether, in addition to being influenced by the method of treatment, outcome was influenced by age, sex, day care attendance, or passive exposure to smoking. A forward stepwise selection was used with a value of $P = .10$. The adverse event data are presented in terms of the rates of reporting frequency on a per-patient basis.

RESULTS

Population. Table 1 summarizes the characteristics of the 133 consecutive children enrolled from 9 November 1999 through 18 January 2000; of these 133, a total of 67 were vaccinated, and 66 were assigned to the control group. The groups were similar, without significant statistical differences,

Table 1. Characteristics of 133 consecutive children who received intranasal virosomal influenza vaccine to prevent acute otitis media.

Characteristic	Vaccine recipients ($n = 67$)	Control subjects ($n = 66$)
Male sex	38 (56.7)	42 (63.6)
Age		
Mean months ± SD	32.6 ± 14.6	36.2 ± 15.9
≤24 months	27 (40.3)	22 (33.3)
>24 months	40 (59.7)	44 (66.7)
Enrolled before influenza period	32 (47.8)	33 (50.0)
Breast-feeding	59 (88.1)	58 (87.9)
Duration of breast-feeding, mean months ± SD	5.7 ± 3.4	5.4 ± 3.1
Prolonged use of pacifier	13 (19.4)	13 (19.7)
≥1 older sibling	23 (34.3)	22 (33.3)
No. of cohabiting family members, mean ± SD	3.6 ± 0.7	3.5 ± 0.5
Passive exposure to smoking	10 (14.9)	9 (13.6)
Day care attendance	50 (74.6)	54 (81.8)
Hospitalization in the previous 3 months	1 (1.5)	3 (4.5)
Time since last episode of AOM, median days (range)	21 (14–45)	30 (15–60)
Presence of otitis media with effusion	59 (88.0)	59 (89.4)
Previous adenoidectomy	0	2 (3.0)

NOTE. Data are no. (%) of patients, unless otherwise specified. Differences were NS. AOM, acute otitis media.

Table 2. Effectiveness of influenza vaccine, as indicated by the occurrence of febrile respiratory illness and acute otitis media (AOM) and the receipt of antibiotic treatment in children during the 6-month period after the administration of vaccine.

Variable	Vaccine recipients ($n = 67$)	Control subjects ($n = 66$)	Vaccine efficacy, %	P
Febrile respiratory illness[a]	55 (82.1)	63 (95.5)	13.2	.03
Receipt of ≥1 course of antibiotics	26 (38.8)	42 (63.6)	38.9	.007
≥1 episode of AOM	24 (35.8)	42 (63.6)	43.7	.002
1 episode of AOM	18 (26.9)	26 (39.4)	31.8	.21
≥2 episodes of AOM	6 (9.0)	16 (24.2)	63.1	.03

NOTE. Data are no. (%) of patients, unless otherwise specified.

[a] Temperature ≥38.1°C.

with respect to the epidemiological variables likely to influence the recurrence of AOM.

All of the vaccine recipients received the 2 doses at the scheduled times. Sixty-five (97%) of 67 vaccine recipients completed the study; 2 (3.0%) were given chemoprophylaxis after 6 and 19 weeks. The study was completed by 61 (92.4%) of 66 control subjects; 5 (7.6%) required chemoprophylaxis after 8, 9, 10, 11, and 16 weeks. The overall follow-up period, therefore, was 1637 patient-weeks for the vaccine recipients and 1567 patient-weeks for the control subjects.

Rates of febrile respiratory illness, AOM, and antibiotic use. Table 2 lists the occurrence of febrile respiratory illnesses, AOM episodes, and antibiotic administration during the study period; the rates for all these events were significantly lower for the group of vaccine recipients. Twenty-four vaccine recipients had 32 episodes of AOM, whereas 42 control subjects had 64 episodes. No patient experienced a relapse. The mean number of episodes per patient was twice as high in the control group as in the vaccine recipient group (0.97 vs. 0.48 episodes per patient). In comparison with the 6 months preceding enrollment, there was a substantial reduction in the mean rate of

occurrence of AOM in both groups: from 0.53 to 0.08 episodes per patient among vaccine recipients and from 0.55 to 0.17 episodes per patient among control subjects.

The overall efficacy of vaccination for prevention of AOM was 43.7% (95% CI, 18.6–61.1). Its efficacy for prevention of 1 episode during the 6-month study was 31.8% (95% CI, 11.9–58.45) and reached 63.1% (95% CI, 11.43–84.59) for prevention of ≥2 episodes during the study period.

Occurrence of AOM in relation to the influenza season. Table 3 shows data on the occurrence of AOM in relation to the time of enrollment and the influenza season, which lasted from 10 December 1999 until 28 February 2000. The predominant influenza virus was A/H3N2, with very few H1N1 or type B isolates [23]. Sixty-five (48.9%) of 133 children (32 vaccine recipients and 33 control subjects) were enrolled before the influenza season and 68 children (51.1%; 35 vaccine recipients and 33 control subjects) after the onset of influenza season. The rate of AOM was always lower among vaccine recipients than among control subjects, regardless of the time of enrollment, but this difference was statistically significant only if the period before influenza season was considered. Among the vac-

Table 3. Occurrence of acute otitis media (AOM), by timing of vaccination and influenza season, among children who received influenza vaccine to prevent AOM.

Time of enrollment, patient group	Children with ≥1 episode of AOM			
	Total	Before influenza season	During influenza season	After influenza season
Before influenza season				
Vaccine recipients ($n = 32$)	6 (18.8)[a]	2 (6.3)	2 (6.3)[b]	2 (6.3)
Control subjects ($n = 33$)	17 (51.5)	2 (6.0)	10 (30.3)	5 (15.2)
Reduction, % (P)	63.6 (.01)	—	79.5 (.02)	58.9 (.42)
During influenza season				
Vaccine recipients ($n = 35$)	18 (51.4)[a]	—	11 (31.4)[b]	7 (20.0)
Control subjects ($n = 33$)	25 (75.8)	—	18 (54.5)	7 (21.2)
Reduction, % (P)	32.1 (.06)	—	42.4 (.09)	5.6 (.85)

NOTE. Data are no. (%) of patients, unless otherwise specified.

[a] Comparison of groups vaccinated before and during influenza season, $P = .01$.

[b] Comparison of groups vaccinated before and during influenza season, $P = .02$.

Table 4. Occurrence of acute otitis media in relation to epidemiological factors among children who received influenza vaccine to prevent acute otitis media.

Factor	Vaccine recipients ($n = 67$)	Control subjects ($n = 66$)	P
Age			
12–24 months	8/27 (29.6)	13/22 (59.1)	.07
25–60 months	16/40 (40.0)	29/44 (65.9)	.03
Sex			
Male	12/38 (31.6)	23/42 (54.8)	.06
Female	12/29 (41.4)	19/24 (79.2)	.01
Day care attendance			
Yes	19/50 (38.0)	34/54 (63.0)	.01
No	5/17 (29.4)	8/12 (66.7)	.10
Passive exposure to smoking			
Yes	7/10 (70.0)	6/9 (66.7)	1.00
No	17/57 (29.8)	36/57 (63.2)	.0007

NOTE. Data are no. of patients with factor/no. in the subgroup (%). P value is for the comparison of vaccine recipients and control subjects for each subgroup.

cine recipients, AOM was less frequent among those vaccinated before than among those vaccinated after the beginning of the influenza season; this difference was statistically significant for the follow-up period as a whole (6 [18.8%] of 32 patients vs. 18 [51.4%] of 35; $P = .01$), and for the period during the influenza epidemic (2 [6.3%] of 32 patients vs. 11 [31.4%] of 35; $P = .02$), but not for the period after the influenza season (2 [6.3%] of 32 patients vs. 7 [20%] of 35; $P = .15$).

Occurrence of AOM in the epidemiological subgroups. As shown in table 4, the rate of occurrence of AOM in the epidemiological subgroups was always lower among the vaccine recipients, but this difference was significant only for the subgroups of children older than 24 months, girls, children attending day care centers, and children not passively exposed to smoking. The stepwise logistic analysis demonstrated that there were no statistically significant interactions between any of the subject characteristics and the method of treatment. In addition to the method of treatment, only passive exposure to smoking influenced outcome (OR, 3.0; 95% CI, 1.01–9.0).

Persistence of OME. By the end of the 6-month period, the proportion of children with OME had declined in both groups: 26 (40.0%) of 65 vaccine recipients had bilateral OME, 11 (16.9%) had unilateral OME, and 28 (43.1%) were free of effusion. Among control subjects, 35 (57.4%) of 61 had bilateral OME, 15 (24.6%) had unilateral OME, and 11 (18.0%) were free of effusion. The proportion of children free of effusion is statistically significant ($P = .004$). The cumulative duration of bilateral effusion, expressed as a percentage of the total number of patient-weeks, was significantly different in the 2 groups: 58.0% (949 of 1637 patient-weeks) for the vaccine recipient group and 74.5% (1168 of 1567) for the control group ($P < .0001$).

Safety. Table 5 summarizes data on adverse events that occurred in the 4 days after administration of each dose and that were judged to be possibly (even if remotely) related to the vaccine, which was generally well tolerated by the children,

Table 5. Occurrence of clinical adverse events during the 4 days after administration of each dose of influenza vaccine to children.

Adverse event	No. (%) of patients	
	After first dose ($n = 67$)	After second dose ($n = 67$)
Systemic		
Rectal temperature $\geqslant$38.1°C	6 (9.0)	2 (3.0)
Shivering	4 (6.0)	5 (7.5)
Irritability	12 (17.9)	17 (25.4)
Earache	5 (7.5)	7 (10.4)
Nausea	1 (1.5)	2 (3.0)
Diarrhea	2 (3.0)	3 (4.5)
Coughing	29 (43.3)	19 (28.4)
$\geqslant$1 systemic event	36 (53.7)	33 (49.3)
Local events		
Irritation	7 (10.4)	7 (10.4)
Sneezing	20 (29.9)	19 (28.4)
Stuffy nose	29 (43.3)	22 (32.8)
Runny nose	29 (43.3)	27 (40.3)
$\geqslant$1 local event	43 (64.2)	33 (49.3)

NOTE. Differences were NS.

regardless of their age. No serious adverse events were reported. After administration of the first dose, two-thirds of the children experienced ⩾1 local symptoms, and ~50% experienced ⩾1 systemic symptom. The majority of the adverse events were transient (lasting 1–2 days) and mild to moderate in severity. Cough was the most common systemic event, followed by irritability. A runny or stuffy nose was the most frequently reported local symptom. The proportion of children with symptoms was lower after the second dose, but not significantly so. The parents of 66 (98.5%) of 67 children were satisfied with the vaccine (13 [19.4%] of 67 rated it "very good" and 53 [79.1%] of 67 rated it "good").

DISCUSSION

Our results indicate that in children with recurrent AOM, the intranasal, inactivated, virosomal subunit influenza vaccine enhances the natural time-linked decline in the occurrence of new episodes of AOM. In fact, the vaccine was associated with a further 43.7% reduction in the number of episodes of AOM during the 6-month follow-up period. The efficacy of the vaccine was greater than that reported in earlier studies of parenteral inactivated [9, 10] and intranasal live-attenuated [12, 13] influenza vaccines. One possible reason is that our study involved selected children with a recent history of recurrent AOM, in whom, as has been demonstrated for pneumococcal vaccine [24], vaccine efficacy may be greater than in children not prone to otitis. It is worth noting that the vaccine interrupted long-term recurrences of AOM: its efficacy in reducing the number of episodes was greatest after the occurrence of the first episode.

As reported by Belshe et al. [12, 13], we also observed a reduction in the number of febrile respiratory illnesses and in the amount of antibiotic consumption in the vaccinated group. Even in the absence of virological diagnoses, but considering the prevalent circulation of type A strain, these findings suggest that the vaccine-induced prevention of influenza also leads to the prevention of both AOM and other respiratory infections, in accordance with the finding that influenza A virus infection in the nasopharynx is associated with enhanced bacterial colonization and infection [25, 26].

Our study could be criticized because it was not a double-blind, placebo-controlled study. However, we think that the absence of a placebo was compensated for by 2 facts: first, all the parents were contacted twice per week to inquire about day-to-day status and to remind them of the possibility of freely contacting an investigator; and second, they were instructed at study entry and reminded at each visit not to discuss group assignment with the single investigator responsible for otological follow-up.

The timing of the administration of influenza vaccine has

an important effect on its efficacy against AOM. Although all of the vaccine recipients experienced fewer AOM episodes than did the control subjects, the children vaccinated before the start of influenza season had significantly better outcomes throughout the follow-up period than did children vaccinated during the influenza season. Therefore, the vaccination of children with recurrent AOM should be carefully planned to complete the administration of vaccine doses before the estimated onset of the influenza season.

To our knowledge, this is the first study to evaluate the influence of epidemiological variables on the efficacy of influenza vaccine against recurrent AOM. The intranasal virosomal influenza vaccine was more effective in children aged 2–5 years than in younger children, and although the lack of significance for the findings in the latter group may be related to the small size of the sample, the results for the former group are of particular interest in clinical practice, because antibiotic prophylaxis was shown to be less effective in this age group [27, 28]. The finding that influenza vaccine was more effective in children attending day care centers is in agreement with our previous studies of chemoprophylaxis [27, 28] and supports the hypothesis that prevention is more effective for children with ⩾1 of the known risk factors for recurrent AOM. However, the fact that the vaccine was of no relevant benefit to children passively exposed to smoking suggests that its advantages are at least neutralized in the presence of such a strong risk factor [29]. Finally, we are unable to interpret the fact that the vaccine was more effective in girls.

The intranasal virosomal influenza vaccine substantially reduced the persistence of middle ear effusion. The proportion of children without OME at the end of the study period and the cumulative duration of bilateral OME were significantly less for the vaccine recipient group. This findings is in agreement with the results of Clements et al. [10], who found a 28% reduction in the incidence of OME during the influenza season among children who were given inactivated influenza vaccine, but it contrasts with our own findings concerning chemoprophylaxis [27, 28], which showed that long-term low-dosage antibiotic administration had no significant effect on the natural history of OME. Our findings for the vaccine recipients can be explained by the reduction in the number of episodes of AOM and of febrile respiratory illnesses, both of which are known to be strictly related to eustachian tube dysfunction and, therefore, to the persistence of OME [30].

Although approximately one-half of the vaccine recipients experienced at least 1 adverse event, the vaccine was generally well tolerated. The adverse events were predominantly mild and transient; they usually resolved within 24–48 h. Because a placebo was not used in the study, the parents' knowledge that the vaccine was administered may have led to some excess reporting, but the prevalences of both local and systemic re-

actions were quite similar to those reported previously for the same vaccine in adults [20]. Moreover, the scanty clinical relevance of the vaccine-related adverse effects is supported by the parents' overall satisfaction with the vaccine.

In conclusion, provided that timing is appropriate, our data suggest that the intranasal inactivated virosomal influenza vaccine might be considered among the options for the prevention of new AOM episodes in children <5 years old with recurrent AOM.

References

1. Berman S. Otitis media in children. N Engl J Med **1995**;332:1560–5.
2. Klein JO. Nonimmune strategies for prevention of otitis media. Pediatr Infect Dis J **2000**;19(Suppl):S89–92.
3. Williams RL, Chalmer TC, Stange KC, Chalmers FT, Bowlin SJ. Use of antibiotics in preventing recurrent acute otitis media and in treating otitis media with effusion: a meta-analytic attempt to resolve the brouhaha. JAMA **1993**;270:1344–51.
4. Block SL, Harrison CJ, Hedrick JA et al. Penicillin-resistant *Streptococcus pneumoniae* in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. Pediatr Infect Dis J **1995**;14:751–9.
5. Brook I, Gober AE. Prophylaxis with amoxicillin or sulfisoxazole for otitis media: effect on the recovery of penicillin-resistant bacteria from children. Clin Infect Dis **1996**;22:143–5.
6. Karma PH, Bakaletz LO, Giebink GS, Mogi G, Rynnel-Dagöö B. Immunological aspects of otitis media: present views on possibilities of immunoprophylaxis of acute otitis media in infants and children. Int J Pediatr Otorhinolaryngol **1995**;32(Suppl):S127–34.
7. Pelton SI, Klein JO. The promise of immunoprophylaxis for prevention of acute otitis media. Pediatr Infect Dis J **1999**;18:926–35.
8. Heikkinen T. Role of viruses in the pathogenesis of acute otitis media. Pediatr Infect Dis J **2000**;19:S17–23.
9. Heikkinen T, Ruuskanen O, Waris M, Ziegler T, Arola M, Halonen P. Influenza vaccination in the prevention of acute otitis media in children. Am J Dis Child **1991**;145:445–8.
10. Clements DA, Langdon L, Bland C, Walter E. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. Arch Pediatr Adolesc Med **1995**;149:1113–7.
11. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep **2000**;49(RR03):1–38.
12. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. N Engl J Med **1998**;338:1405–12.
13. Belshe RB, Gruber WC. Prevention of otitis media in children with live attenuated influenza vaccine given intranasally. Pediatr Infect Dis J **2000**;19:S66–71.
14. Scholtissek C. Potential hazards associated with influenza virus vaccine. Dev Biol Stand **1995**;84:55–8.
15. Beyer WEP. Routine influenza vaccination for healthy children—old concept, new technologies. Arch Dis Child **2000**;83:461–2.
16. US panel rejects Aviron's flu vaccine. Scrip No. 2665, 1 August **2001**: 24. Available at: http://www.pjbpubs.com/scrip. Accessed September 9, 2001.
17. Holm KJ, Goa KL. Liposomal influenza vaccine. BioDrugs **1999**;11: 137–44.
18. Glück U, Gebbers J-O, Glück R. Phase 1 evaluation of intranasal virosomal influenza vaccine with and without *Escherichia coli* heat-labile toxin in adult volunteers. J Virol **1999**;73:7780–6.
19. Cusi MG, Lomagistro MM, Valassina M, Valensin PE, Glück R. Immunopotentiating of mucosal and systemic antibody responses in mice by intranasal immunization with HLT-combined influenza virosomal vaccine. Vaccine **2000**;18:2838–42.
20. Glück R, Mischler R, Durrer P, et al. Safety and immunogenicity of intranasally administered inactivated trivalent virosome-formulated influenza vaccine containing *Escherichia coli* heat-labile toxin as a mucosal adjuvant. J Infect Dis **2000**;181:1129–32.
21. Pelton SI. Otoscopy for the diagnosis of otitis media. Pediatr Infect Dis J **1998**;17:540–3.
22. Brookhouser PE. Use of tympanometry in office practice for diagnosis of otitis media. Pediatr Infect Dis J **1998**;17:544–51.
23. Crovari P, Salmaso S, Donatelli I, Pregliasco F, Brignoli O. Influnet, the new epidemiological and virological influenza surveillance network in Italy. In: Osterhaus ADME, Cox N, Hampson AW, eds. Proceedings of the World Congress on Options for the Control of Influenza IV (Crete, Greece), 23–28 September 2000. Amsterdam: Elsevier, **2001**:90.
24. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr Infect Dis J **2000**;19:187–95.
25. Julkunen I, Melén K, Nyqvist M, Pirhonen J, Sareneva T, Matikainen S. Inflammatory responses in influenza A virus infection. Vaccine **2000**;19(Suppl 1):S32–7
26. Tong HH, Wieser JN, James MA, DeMaria TF. Effect of influenza A virus infection on nasopharyngeal colonization and otitis media induced by transparent or opaque phenotype variants of *Streptococcus pneumoniae* in the chinchilla model. Infect Immun **2001**;69:602–6.
27. Principi N, Marchisio P, Massironi E, Grasso RM, Filiberti G. Prophylaxis of recurrent acute otitis media and middle-ear effusion. Arch Dis Adolesc Med **1989**;143:1414–8.
28. Marchisio P, Principi N, Sala E, Lanzoni L, Sorella S, Massimini A. Comparative study of once-weekly azithromycin and once-daily amoxicillin treatments in prevention of recurrent acute otitis media in children. Antimicrob Agents Chemother **1996**;40:2732–6.
29. Uhari M, Mantysaari K, Niemela M. A meta-analytic review of the risk factors for acute otitis media. Clin Infect Dis **1996**;22:1079–83.
30. Heikkinen T. Temporal development of acute otitis media during upper respiratory tract infection. Pediatr Infect Dis J **1994**;13:659–61.