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Different Virulence of Influenza A Virus Strains and Susceptibility to Pneumococcal Otitis Media in Chinchillas

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We have previously shown that chinchillas infected with a multiply passaged laboratory strain of influenza A/NWS/33 (H1N1) develop negative middle-ear pressure; polymorphonuclear leukocyte oxidative, bactericidal, and chemotactic dysfunction; and increased susceptibility to pneumococcal otitis media. Because influenza A virus strains show different virulence in humans, three such strains were compared in the chinchilla model. Negative middle-ear pressure and tympanic membrane inflammation developed significantly more often in chinchillas infected with wild-type H3N2 virus than with either wild-type H1N1 virus or an attenuated, cold-adapted H3N2 vaccine strain, CR29. Marked depression in polymorphonuclear leukocyte chemiluminescent activity also developed significantly more often in H3N2 infected animals than in H1N1- or CR29-infected animals. Intranasal challenge of influenza virus-infected animals with type 7 *Streptococcus pneumoniae* resulted in a significantly greater occurrence of pneumococcal otitis media in H3N2-infected animals than in H1N1-, CR29-, or non-influenza-infected control animals. Clearance of pneumococci from nasal washings of animals infected with wild-type H3N2 was significantly delayed in comparison with the other groups. Thus, the previously demonstrated increased susceptibility to otitis media among children infected with H3N2 influenza virus may relate to the capacity of this strain to induce negative middle-ear pressure, polymorphonuclear leukocyte dysfunction, and alteration in the mucosal clearance of pneumococci.

Influenza A virus infection in humans has been associated with an increased prevalence of secondary bacterial disease principally due to *Streptococcus pneumoniae* and *Staphylococcus aureus*. Enhanced susceptibility to bacterial disease in patients with influenza virus infection may be due to phagocytic cell dysfunction, damaged ciliated epithelium, tissue edema, or other, less-well-defined disturbances of normal host defense mechanisms, as previously reviewed (1, 2, 12).

A role for certain respiratory viruses in the pathogenesis of otitis media has been suggested by the association of viral upper respiratory tract infection and an increased risk of otitis media in children (19). Using the chinchilla model of experimental otitis media, we have shown enhanced susceptibility of chinchillas infected with influenza A/NWS/33 (H1N1), a multiply passaged laboratory strain, to pneumococcal otitis media (2, 12). Development of pneumococcal otitis media was temporally associated with depressed polymorphonuclear leukocyte (PMN) chemiluminescence (CL), chemotactic and bactericidal activity, and negative middle-ear (ME) pressure (12). The greatest incidence of pneumococcal otitis media occurred when pneumococci were inoculated just before the time of influenza-induced negative ME pressure and PMN dysfunction.

Others have shown that the virulence of influenza A virus varies from year to year, with some epidemics described as unusually virulent, such as the 1918 epidemic, and others as surprisingly mild, such as the introduction of the H1N1 subtype in 1947 (5). A study of sequential epidemics due to influenza A/Texas/77 (H3N2) and A/USSR/77 (H1N1) in Nashville, Tenn., during the winter of 1977 to 1978 revealed that equivalent amounts of typical influenzal disease were documented with both strains in young adults. The A/USSR strain, however, was less virulent, particularly in young seronegative children in whom it caused no recognizable illness, whereas the A/Texas strain caused typical febrile respiratory disease (35). Even more striking was the difference in prevalence of otitis media, which was diagnosed in 41% of children with A/Texas

infection, in only 10% of children with A/USSR infection, and in 18% of children without A/Texas or A/USSR infection.

These observations of different virulence among influenza A virus strains provided an incentive to examine the relative influence of different strains on host PMN function, ME pressure, and susceptibility to pneumococcal otitis media in the chinchilla model. A cold-adapted H3N2 vaccine strain which appears to be attenuated in seronegative adults and young children and causes little or no inflammation in the nasopharynxes of ferrets was also evaluated in chinchillas for comparison with the wild-type H3N2 and H1N1 strains.

MATERIALS AND METHODS

Healthy, young adult chinchillas, 1 to 2 years of age, were used. Each animal was used in only one experimental protocol. Animals were housed singly in large, clear plastic rodent boxes fitted with micropore bonnets.

Three influenza A virus strains were used. An influenza A/Alaska/6/77 (H3N2) strain of demonstrated virulence in adult volunteers (35) was passaged once in MDCK, a continuous canine kidney cell line, for use in these experiments. An influenza A/Alaska vaccine strain (CR29), which is a cold-adapted strain attenuated by recombination of the above wild-type strain with influenza A/Ann Arbor/6/60 by the technique of Maassab, was also used (25). This vaccine appears to be attenuated in seronegative adults and young children and has been evaluated in a ferret model in which it causes little or no inflammatory change in the nasopharynx (6). The attenuated CR29 strain was passaged once in MDCK cells for use in these experiments. An influenza A/Hong Kong/123/77 (H1N1) strain of demonstrated virulence in adult volunteers (27) was also passaged once in MDCK cells before use in these experiments.

S. pneumoniae type 7F (type 51 by American nomenclature) was prepared as previously described (16), and an infecting dose of 10^5 CFU/ml was prepared by diluting a previously frozen pneumococcus with phosphate-buffered saline.

Chinchillas were inoculated intranasally with 0.2 ml of virus suspension or 0.5 ml of pneumococcal suspension or both by passive inhalation of droplets placed on the surface of the anterior nares. Intramuscular ketamine-hydrochloride anesthesia was used for all animal inoculation and sampling procedures.

The tympanic membranes (TMs) of each chinchilla were examined with an otoscope, and tympanometry was performed with model 1722 ME analyzer (Grason-Stadler, Inc., Littleton, Mass.) before and at 3- to 7-day intervals after microbial inoculation. TM color and opacity were recorded and graded, in increasing order of inflammation and increasing correlation with the presence of ME effusion, as follows: 0, gray and translucent; 1, gray and opaque; 2, red and translucent; 3, red and opaque; 4, yellow and translucent; 5, yellow and opaque (14). We have previously shown that ears with a yellow TM (score, 4 or 5) and flat (type B) tympanogram yield effusion in 96% of cases with 89% specificity for detecting effusion (12). TM inflammation was defined in this study as a color-opacity score of 2 or greater. ME pressure was measured by tympanometry and recorded as the pressure in mm H_2O giving peak TM conductance and as the type of configuration (13).

Nasal washings for pneumococcal culture and for attempts at influenza virus isolation were obtained at 3- to 7-day intervals after inoculation of the microbe with 0.5 ml of sterile 0.85% NaCl. Saline flowing out of the contralateral nares was collected and inoculated in serial dilutions on 5% sheep blood agar for quantitation of viable pneumococci. A sample of the saline wash was also placed immediately into virus transport medium consisting of Hanks balanced salt solution supplemented with 0.5% gelatin and antibiotics, quick-frozen in dry ice, and stored at −70°C until an attempt at virus isolation was made. Viruses were isolated and titered in MDCK cells as previously described (23).

Serum was obtained by cardiac puncture after microbial inoculation and at 7- to 14-day intervals thereafter and was stored at −70°C for determination of hemagglutination-inhibiting antibody. A titer of less than 1:8 was considered negative. Passage of the H3N2 virus in the presence of chinchilla serum was necessary to derive an inhibitor-resistant antigen.

Aspiration of the ME fossa by the transbullar approach (11) was performed 10 days after pneumococcal inoculation on all animals inoculated with pneumococcus. ME effusion or the needle tip, in the case of dry aspirations, was inoculated on 5% sheep blood agar for culture. All animals were sacrificed at the termination of the study, and bacterial cultures of blood, ME fossa, and lung tissue were performed on 5% sheep blood agar (15).

Heparinized blood (25 U of heparin in 2.5 ml of blood) was obtained by cardiac puncture for determination of PMN CL activity three to five times before microbial inoculation and at 3- to 7-day intervals thereafter. PMN CL activity was measured on duplicate samples with 3×10^4 PMNs per ml, zymosan preopsonized in 50% pooled normal chinchilla serum, and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St. Louis, Mo.) amplification. CL activity was expressed as the mean peak activity in counts per minute (1). Peak PMN CL activity in healthy and infected chinchillas occurred after 3 to 9 min of incubation of the phagocytic mixture. Student's *t* test for paired data was used to analyze the significance of the post-inoculation change in PMN CL activity within a group of animals by comparing the paired post-inoculation and mean preinoculation CL values.

The Student's *t* test for non-paired data with equal variance was used to compare mean TM color-opacity scores, mean ME pressure, mean PMN CL activity, geometric mean (GM) viral PFU, and GM serum hemagglutination-inhibiting antibody titers between the various groups. The chi-square test was used to evaluate the incidence of otitis media, labyrinthitis, and mortality in pneumococcus-inoculated animals and to compare the incidence of TM inflammation and negative ME pressure in virus-inoculated animals.

RESULTS

H3N2 infection. The nares of 20 chinchillas were inoculated with 0.2 ml of the A/Alaska

TABLE 1. Replication of influenza viruses in chinchillas

Influenza virus	Mean ± SE virus titer (log_{10} PFU/ml) in nasal washings on day:				
	3	4	5	7	10
H3N2	2.9 ± 0.5	2.8 ± 0.5	1.9 ± 0.8	<1.0	<1.0
H1N1	0.7 ± 0.7	1.4 ± 0.6	1.3 ± 0.8	0.5 ± 0.4	<1.0
CR29	1.1 ± 0.6	1.2 ± 0.7	1.0 ± 0.6	0.7 ± 0.6	<1.0

H3N2 wild-type virus suspension containing $10^{6.3}$ PFU. Four days later, the nares of 10 chinchillas were inoculated with $10^{4.7}$ CFU of type 7 pneumococcus. All 20 animals became infected, as evidenced by virus shedding in nasal washings on day 3 post-inoculation, and virus titers in nasal washings were significantly greater in this group than in either the H1N1- or the CR29-infected group on day 3 ($P = 0.002$) or day 4 ($P = 0.021$) (Table 1). Two animals shed virus through day 7. All animals were seronegative before inoculation, and all showed at least an eightfold titer rise on day 14 post-inoculation. The GM reciprocal serum hemagglutination-inhibiting antibody titer of the 10 animals not receiving pneumococcus on days 14 and 21 was 64.0 and 137.2, respectively; the GM titer in the pneumococcus-inoculated animals did not differ significantly.

All animals had normal TMs and normal, type A tympanograms before inoculation. Among animals inoculated with virus alone, TM inflammation was observed in three animals (4 ears) on day 3, in nine animals (15 ears) on day 5, in nine animals (18 ears) on day 10, and in five animals (8 ears) on day 21 at the time of sacrifice and ME fossa examinations. None of the ears contained effusion on day 21. The mean TM inflammation score peaked at 3.9 on day 10 and fell rapidly over the next 4 days (Fig. 1). Bilateral negative ME pressure was observed in all 10 animals during the study period (Table 2). ME pressure reached a nadir on day 5, but only three ears (three animals) had persistent negative ME pressure by day 14, and all ears had normal tympanograms by day 21 (Fig. 1). Arithmetic mean PMN CL activity of these animals before H3N2 inoculation was 205.3 × 10^3 cpm, dropping significantly on day 7 post-inoculation and returning to normal by day 10 (Fig. 2).

Of the 10 animals inoculated with H3N2 virus and pneumococcus, 9 (17 of 18 tested ears) had negative ME pressure and 5 (7 of 19 tested ears) showed TM inflammation at the time of pneumococcal inoculation. The average degree of TM inflammation in virus-infected animals with and without pneumococcus was similar through day 10 after virus inoculation but was significantly higher in pneumococcus-inoculated animals on day 14 (Fig. 3). Average ME pressure was significantly lower in the pneumococcus-inoculated group on days 10 and 14 (Fig. 3).

Of the 10 animals, 9 developed pneumococcal otitis media with effusion (Table 3). Four animals died with pneumococcal otitis media and labyrinthitis; in three, pneumococcemia was demonstrated postmortem. Two of the surviving animals also developed labyrinthitis on days 14 and 28, respectively. The MEs of seven animals were aspirated on day 14, and six animals (10 ears) had pneumococcal otitis media. Persistent effusion was present in five of six animals surviving to day 28; five pneumococcal effusions remained culture positive, and three became sterile.

H1N1 infection. The nares of 21 chinchillas were inoculated with 0.2 ml of wild-type H1N1 virus suspension containing $10^{5.8}$ PFU. Four days later, the nares of 10 chinchillas were inoculated with 5 × $10^{4.7}$ CFU of type 7 pneumococcus. All 21 animals became infected, as judged by virus shedding (12 animals) (Table 1) or by a serological response (19 animals).

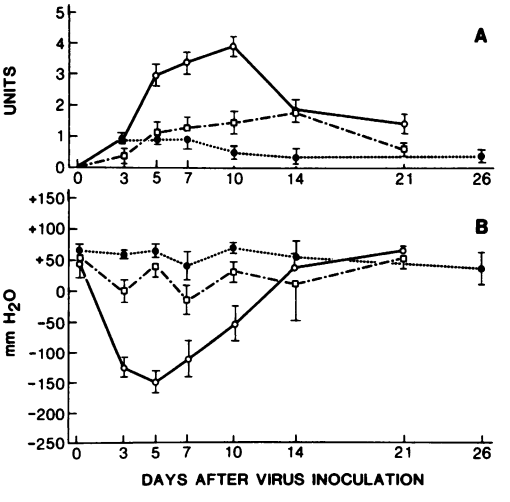

FIG. 1. Mean (± standard error) TM color-opacity score (see text for grading) after intranasal inoculation of influenza A viruses in chinchillas (A) and mean (± standard error) ME pressure measured by tympanometry (B). O——O, H3N2; □- · - · -□, H1N1; ● · · · · · ●, CR-29.

TABLE 2. Results of tympanometry and otoscopy during infection with influenza A viruses

Influenza virus (no. of animals inoculated)	Number of animals (ears) developing:		
	−ME pressure (<0 mm of H_2O)	TM inflammation (score of ≥2)	ME effusion[a]
H3N2 (10)	10 (20)	9 (18)	0
H1N1 (11)	10 (17)	4 (8)	1 (1)
CR29 (9)	4 (5)	1 (1)	0

[a] ME effusion was defined as a TM score of ≥4 and a flat tympanogram, or presence of effusion at sacrifice.

Among the 11 animals inoculated with virus alone, the serum GM titers on days 14 and 21 were 32.0 and 112.8, respectively. The GM titers in pneumococcus-inoculated animals did not differ significantly. Five animals inoculated with virus alone were seropositive, with hemagglutination-inhibiting antibody titers of 32 to 64 before inoculation; however, virus shedding was observed in four of these five seropositive animals. None of the pneumococcus-challenged animals were antibody positive at inoculation.

TM inflammation was observed in only four of the 11 animals (eight ears) infected with virus alone during the study period (Table 2). Negative ME pressure was demonstrated in 17 of the 22 ears; two animals developed unilateral type B tympanograms, and the ME fossa of one animal contained effusion (Fig. 1). None of the remaining ears contained effusion on day 21. Inflammation and negative pressure were seen as often in animals that were initially seropositive as in those that were initially seronegative. Mean PMN CL activity did not change significantly over the course of the experiment, despite some variation in individual values (Fig. 2).

No enhancement of TM inflammation was seen in the 10 animals inoculated with H1N1 virus and pneumococcus. ME pressures were similar in animals with and without pneumococcus. One animal inoculated with virus and pneumococcus had a unilateral sterile ME effusion at the time of transbullar aspiration on day 14 which resolved by day 28 (Table 1). None of the animals died during the 24 days after pneumococcal inoculation, and none had ME effusion at sacrifice on day 28. One animal had a positive ME culture for pneumococcus on day 28 without grossly visible effusion.

CR29 infection. The nares of 19 chinchillas were inoculated with 0.2 ml of the attenuated vaccine containing $10^{6.1}$ PFU. Four days later, the nares of 10 chinchillas were inoculated with $10^{4.7}$ CFU of type 7 pneumococcus. All 19 animals became infected, as judged by virus shedding (14 animals) or serological response (17 animals). Eighteen animals were seronegative before inoculation, and one animal inoculated with virus alone had antibody detectable at a 1:8 dilution. The pattern of virus shedding and antibody response demonstrated the more limited replication and serological response characteristic of an attenuated virus when compared with its parental wild-type strain, with GM titers of 34.6 and 21.8 on days 14 and 21, respectively. The GM titers in pneumococcus-inoculated animals did not differ significantly.

All animals had normal otoscopic examinations and tympanograms before inoculation. Only one animal inoculated with virus alone developed TM inflammation during the study period. The incidence of TM inflammation in CR29-infected animals (1 of 18 ears) was significantly less than that in H1N1-infected animals (8 of 22 ears; $P = 0.020$) or in H3N2-infected animals (18 of 18 ears; $P < 0.0001$) (Table 2). Four animals inoculated with virus alone (five ears) developed type B tympanograms during the study, but mean ME pressure for the group did not change during the trial (Fig. 1), and no ears contained effusion on day 26. Mean PMN CL activity did not change significantly during the study period, despite some variation in individual values (Fig. 2).

No enhancement of TM inflammation or ME pressure was seen in the 10 animals inoculated with CR29 virus and pneumococcus. Three animals (4 ears) had pneumococcal otitis media with effusion when aspirated on day 14, and two animals had unilateral sterile effusions (Table 3).

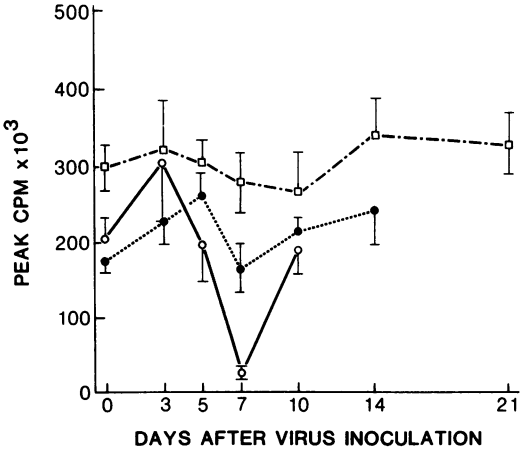

FIG. 2. Mean (± standard error) peak CL activity of peripheral blood PMNs obtained at intervals from influenza virus-infected chinchillas during phagocytosis of opsonized zymosan. □-·-·-□, H1N1; ●·····●, CR-29; ○——○, H3N2.

The frequency of pneumococcal or sterile otitis media with effusion was not significantly different for CR29-infected and saline control animals (Table 3). The single animal with bilateral pneumococcal otitis media with effusion died on day 19 with persistent positive ME cultures and pneumococcemia. Persistent effusion was present in three of four animals surviving to day 28; both sterile effusions remained sterile, one pneumococcal effusion became sterile, and one pneumococcal effusion resolved. None of the animals showed signs of labyrinthitis.

Pneumococcal challenge of non-influenza-infected animals. The nares of 10 chinchillas were inoculated with 0.5 ml of sterile 0.85% saline, followed 4 days later by intranasal inoculation of 5×10^4 CFU of type 7 pneumococcus. All animals were seronegative for H1N1 and H3N2 antigens before and 14 and 28 days after saline inoculation. Average ME pressure among these animals did not change from the level obtained before inoculation through day 14 after saline inoculation (Fig. 3).

Two animals developed pneumococcal otitis media with effusion (Table 3). One animal developed unilateral TM inflammation on day 3 after pneumococcal inoculation, and effusion from this ear obtained on day 10 cultured pneumococcus. Another animal without prior signs of TM inflammation had a unilateral sterile ME effusion on day 10. A third animal showed unilateral TM inflammation on day 24, and effusion from this ear yielded pneumococcus.

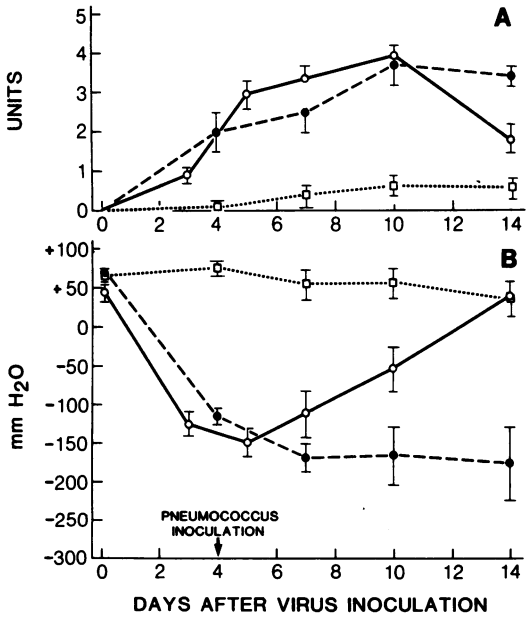

FIG. 3. Mean (± standard error) TM (A) coloropacity score (see text for grading) and mean (± standard error) ME (B) pressure in chinchillas after H3N2 virus inoculation with (● – – – ●) and without (○——○) inoculation of pneumococcus on day 4 post-virus inoculation. Also shown is a group of non-influenza-infected saline control animals challenged with pneumococcus (□ · · · · · □).

TABLE 3. Results of intranasal type 7 *S. pneumoniae* challenge of influenza A virus-infected chinchillas

Influenza virus[a]	No. of animals (ears) developing[b]: Pneumococcal otitis media with effusion	Sterile ME effusion	Labyrinthitis	Mortality
H3N2	9* (17**)	0	6***	4****
H1N1	1 (1)	1 (1)	2	0
CR29	3 (4)	2 (2)	0	1
Saline	2 (2)	1 (1)	1	0

[a] Each group comprised 10 animals.
[b] Chi-square comparison of virus-infected animals with saline control group: *, $P = 0.002$, **, $P < 0.001$; ***, $P = 0.019$; ****, $P = 0.025$.

ME pressure and susceptibility to otitis media. A strong correlation was observed between the presence of negative ME pressure on days 3 through 7 after virus inoculation and the subsequent development of pneumococcal otitis media when data from the three influenza virus-pneumococcus challenge experiments was pooled. Of 22 ears, 18 (82%) with negative pressure on days 3 through 7 developed pneumococcal otitis media, whereas only 5 of 36 ears (14%) with positive pressure on days 3 through 7 developed otitis media ($P = 0.0001$). Three of the five ears with positive pressure developed sterile effusion.

Nasopharyngeal clearance of pneumococci. Nasal washings were obtained on days 3, 6, 10, 17, and 24 after pneumococcal inoculation in influenza-infected and saline control animals for titration of pneumococcal CFU per milliliter of washing. Animals infected with wild-type H3N2 virus had significantly higher concentrations of pneumococci in nasal washings on days 3, 10, and 17 as compared with those in saline controls (Fig. 4). Animals infected with the attenuated CR29 virus had significantly higher concentrations of pneumococci only on day 3, and H1N1-infected animals had pneumococcal concentrations similar to those in controls throughout the study period.

DISCUSSION

Influenza A viruses have a profound effect on host defense mechanisms, causing cytological changes in ciliated columnar epithelial cells and necrosis of bronchial and bronchiolar epithelium (34); impaired transport of bacteria via the mu-

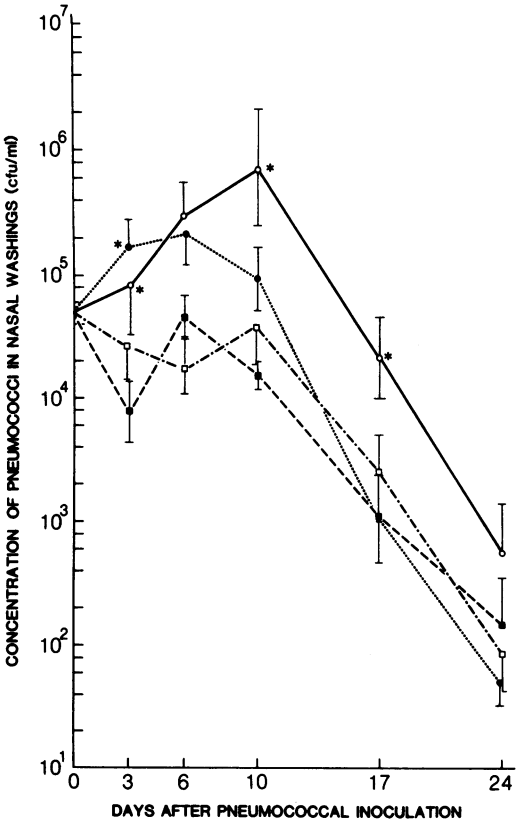

FIG. 4. Mean (± standard error) concentration of pneumococci in nasal washings of influenza A virus-infected chinchillas and non-influenza-infected saline control chinchillas at intervals after pneumococcal inoculation. Statistical comparisons of mean concentrations in virus-infected animals versus those in control groups were made with Student's t test (*, $P < 0.05$). O——O, H3N2; ●·····●, CR-29; □-·-·-□, H1N1; ■----■, saline control.

cociliary apparatus (20); increased adherence of pathogenic respiratory bacteria to upper respiratory epithelial cells (8); and depressed oxidative, chemotactic, phagocytic, and bactericidal activities of phagocytic cells (1, 7, 9, 10, 14, 21, 22, 30, 31, 33). Influenza A virus may adversely influence other host defense factors, such as local and systemic immunoglobulin and complement production, which have not been investigated to date.

Because the phagocytic cell represents an important defense against bacterial infection and PMNs are abundant in the ME effusion of animals and humans with purulent otitis media during the first weeks of infection, we have speculated that influenza A virus may enhance host susceptibility to purulent otitis media by depressing phagocytic cell function. In fact, the frequency of experimental pneumococcal otitis media in the chinchilla model progressively increases as the time of pneumococcus inoculation approaches the time of virus-induced PMN dysfunction (2).

In our previous experiments, in which an influenza A/NWS/33 (H1N1) strain was used, eustachian tube dysfunction with negative ME pressure developed before, during, and after peak PMN dysfunction (12). Since compromised eustachian tube function is known to lead to negative ME pressure and ME effusion (4, 18, 28), a second, equally probable etiological factor in the development of pneumococcal otitis media was present in influenza virus-infected animals.

Tympanostomy tube ventilation of the ME fossa during influenza A/NWS/33 infection in chinchillas did not prevent the occurrence of pneumococcal otitis media, suggesting that negative ME pressure may not have been required in this animal model for the development of pneumococcal otitis media (2). More recently, however, histopathological analysis of the temporal bones from these animals showed reduced inflammation of the ME mucoperiosteum from ventilated ears as compared with nonventilated ears (24). Thus, both eustachian tube and phagocytic cell dysfunction appear to contribute to the enhanced susceptibility of influenza A virus-infected chinchillas to pneumococcal otitis media.

To further evaluate the relative contributions of influenza-induced eustachian tube and phagocytic cell dysfunction, we speculated that current influenza A virus strains might show different virulence in chinchillas, as they appear to do in humans and infant rats (3). Our previous experiments were performed with a multiply passaged laboratory H1N1 strain, and the susceptibility of chinchillas to wild-type strains of known pathogenicity in humans had not been examined. Three strains, representing a wild-type H3N2 strain which caused otitis media in young children, a wild-type H1N1 strain which caused influenzal illness in adults but without an association with otitis, and a cold-adapted, avirulent H3N2 vaccine strain (CR29), were chosen. The doses of virus selected ($10^{5.8}$ to $10^{6.3}$ PFU) were in the range of doses used to study influenza infectivity in other vertebrates and have been shown to be 100% infective in rats, mice, ferrets, and humans. The dose of H1N1 virus, which is fourfold lower than that of H3N2 virus, and the presence of preexisting H1N1 antibodies in 24% of the chinchillas may have influenced the responses of the chinchillas to this agent. However, the dose used was $10^{1.5}$ PFU higher than that which caused illness in adult volunteers (27).

Chinchillas infected with these viruses showed responses which closely paralleled those seen in humans. Infectivity in chinchillas was measured by the titer of virus in nasal washings and by the serological response. The mean peak virus titer among chinchillas infected with the H3N2, H1N1, and CR29 strains was $10^{2.9}$, $10^{1.4}$, and $10^{1.2}$ PFU/ml, respectively. Virus shedding persisted on day 7 in 2 of 10, 3 of 11, and 3 of 9 animals infected with the H3N2, H1N1, and CR29 strains, respectively. Likewise, in infant and adult mice infected with another H3N2 strain (A/PC/1/73), peak nasal wash virus titers were $10^{3.5}$ 50% egg infective doses and virus shedding ceased before day 11 (29). Although virus titers from nasal turbunate homogenates were higher in infant rats infected with the H3N2, H1N1, and CR29 strains (3), Reuman et al. found that virus titers were significantly lower in nasal homogenates than in nasal washes performed on the same animals (29).

The incidence of TM inflammation, negative ME pressure, PMN oxidative dysfunction, and susceptibility to pneumococcal otitis media was significantly greater in H3N2-infected chinchillas than in H1N1- or CR29-infected chinchillas. Since the H1N1- and CR29-infected animals that were challenged with pneumococcus were all seronegative before inoculation and had virus shedding or an antibody response after inoculation, the lower incidence of pneumococcal disease in H1N1- and CR29-infected chinchillas was not due to lack of virus infection. Similar observations were made with these influenza strains in an infant rat model of *Haemophilus influenzae* type b meningitis (3). The incidence of *H. influenzae* type b bacteremia was greatest (100%) in influenza A/Alaska/6/77-infected rats, intermediate (33%) in influenza A/Hong Kong/123/77-infected rats, and lowest (8 to 15%) in CR29-infected rats. Likewise, the incidence of *H. influenzae* type b meningitis was 64, 8, and 0 to 8%, respectively.

As in previous chinchilla studies with the influenza A/NWS/33 strain, the occurrence of negative ME pressure during week 1 of virus infection showed a strong correlation with the subsequent development of otitis media. The significant reduction in occurrence of otitis media in the CR29-infected animals compared with wild-type H3N2-infected animals is encouraging for the future development of attenuated influenza vaccines. A recent report indicates that intranasal vaccination of susceptible children with live, attenuated, cold-adapted influenza A viruses efficiently stimulates both systemic and local antibody responses (26).

Coincident with the increased incidence of pneumococcal otitis media during H3N2 infection was an increased incidence of labyrinthitis and higher mortality. Labyrinthitis may have been due to inner ear infection or, more likely, to diffusion of a bacterial, viral, or host cell toxin into the scala tympani via the round window membrane (17, 32). Influenza virus infection may have enhanced the permeability of the round window membrane.

The increased titer of pneumococcus in nasal washings of H3N2-infected animals compared with that in other groups suggests that this virus may have opened epithelial receptor sites for pneumococci, thereby leading to higher concentrations of pneumococci in nasal washings from these animals. It is possible that the increased occurrence of pneumococcal otitis media in this group was simply due to higher concentrations of pneumococci in the nasopharynx.

The results of these experiments suggest that purulent pneumococcal otitis media develops in influenza virus-infected chinchillas without a prior ME effusion. Only 3 of 30 animals infected with virus alone developed effusion or showed otoscopic and tympanometric evidence of effusion during the study period. These results suggest that influenza virus disrupts other local defense mechanisms, possibly ciliary function, allowing recently inoculated pneumococci to gain access to the ME fossa. Virus-induced PMN and eustachian tube dysfunction might then enhance the capacity of the pneumococcus to establish an infectious focus in the ME.

These experiments support the concept that normal PMN function and eustachian tube ventilation of the ME fossa are important in maintaining the health of this organ. Respiratory virus infections which do not alter these two defense mechanisms are not likely to enhance susceptibility to otitis media.

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