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# Influenza A Virus-Induced Polymorphonuclear Leukocyte Dysfunction in the Pathogenesis of Experimental Pneumococcal Otitis Media

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The role of influenza A virus-induced polymorphonuclear leukocyte and eustachian tube dysfunction in the pathogenesis of acute purulent otitis media was studied in chinchillas. Polymorphonuclear leukocyte function, middle ear pressure, and the incidence of pneumococcal otitis media were observed after intranasal inoculation with influenza A virus, *Streptococcus pneumoniae*, or both. Results showed that depressed negative middle ear pressure and polymorphonuclear leukocyte chemiluminescence and chemotactic activity occurred after influenza inoculation, but not after inoculation with pneumococcus alone. The greatest incidence of pneumococcal otitis media occurred when the pneumococcus was inoculated just before the time of influenza-induced polymorphonuclear leukocyte dysfunction and negative middle ear pressure. Animals that had unilateral tympanostomy tubes placed before inoculation of influenza with pneumococcus showed no difference in the occurrence of pneumococcal otitis media in ventilated and nonventilated ears, suggesting that polymorphonuclear leukocyte dysfunction contributes more to the pathogenesis of pneumococcal otitis media than does negative middle ear pressure in this animal model.

The pathogenesis of otitis media is linked in a complex way to the presence of pathogenic microbes in the upper respiratory tract, compromised middle ear ventilation secondary to eustachian tube dysfunction, and immune defense mechanisms. Viral upper respiratory tract infections have been associated with an increased risk of otitis media, suggesting a role for certain respiratory viruses in the pathogenesis of otitis media (3, 4, 10, 18, 31, 37). Viruses in the upper respiratory tract and antibody seroconversion to these viruses have been found in approximately 25% of patients with acute otitis media (24).

Respiratory viruses could be involved in the development of otitis media by several mechanisms. Upper respiratory tract infections can induce markedly negative middle ear pressure via eustachian tube dysfunction, which could lead to the development of otitis media (6, 34). Other studies have shown that respiratory viruses can induce phagocytic cell dysfunction which might increase susceptibility to secondary infections (1, 7, 8, 23, 25, 26, 33, 35, 36, 38).

To further define the role of leukocyte dysfunction and negative middle ear pressure in the pathogenesis of acute purulent otitis media, the chinchilla model of experimental otitis was used (10, 11, 13). Chinchillas were inoculated intranasally with influenza A virus alone, with *Streptococcus pneumoniae* alone, or with both microbes to study the effect of each on polymorphonuclear leukocyte (PMN) function. The development of negative middle ear pressure was studied after intranasal inoculation of chinchillas with either influenza virus or pneumococcus. The time interval between intranasal inoculation of pneumococcus with influenza was varied to study the effect on the frequency of pneumococcal otitis media. Unilateral tympanostomy tubes were placed in the ears of chinchillas receiving pneumococcus with influenza to determine whether otitis media would still occur in the absence of negative middle ear pressure. Results showed that animals developed leukocyte dysfunction and negative middle ear pressure 4 to 6 days after influenza inoculation, but not after inoculation with pneumococcus alone. The greatest incidence of pneumococcal otitis media occurred in animals receiving pneumococcus with influenza in whom pneumococcal inoculation occurred just before the time of maximal PMN dysfunction and the onset of negative middle ear pressure. The incidence of pneumococcal otitis media in ventilated and nonventilated ears was not different, suggesting that PMN dysfunction plays an important role in the pathogenesis of pneumococcal otitis media.

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## MATERIALS AND METHODS

Using an animal model of otitis media described by Giebink et al. (10, 11, 13), we randomly assigned healthy 1- to 2-year-old chinchillas to receive intranasal inoculation with 0.5 ml of influenza A virus ($H_1N_1$, A/NWS/33), *S. pneumoniae* type 7F (type 51 by American nomenclature), or *S. pneumoniae*, followed 2 days later by influenza A virus. Preparation of the influenza isolate has been described (10), and the viral suspension used in these experiments had a hemagglutination titer of 1:128 and a tissue culture infectivity dose of $10^{2.5}$. Preparation of the *S. pneumoniae* has been described (14), and an infecting dose of $2 \times 10^5$ bacteria per ml was reached by making a 100-fold dilution of previously frozen pneumococcus with phosphate-buffered saline. Previous work has shown that animals infected intranasally with saline do not have an increased incidence of disease or alterations in leukocyte function (1, 10). Two to three times before microbial inoculation and approximately every other day thereafter for 18 days, heparinized blood (25 U of heparin in 2.5 ml of blood) was obtained by cardiac puncture from ketamine hydrochloride-anesthetized chinchillas. Leukocyte counts were done on a Coulter Counter (model ZBI; Coulter Electronics, Inc., Hialeah, Fla). Phagocytic cell chemiluminescence was performed on duplicate samples, using luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St. Louis, Mo.) amplification, $2.5 \times 10^4$ PMN/ml, and preopsinized zymosan or phorbol myristate acetate (PMA) as the stimulus (1). PMN chemotactic activity was studied under agarose with zymosan-activated serum as the chemoattractant (1). Triplicate determinations were done, and chemotactic activity was calculated by subtracting random migration from chemoattractant migration.

The time interval between inoculation with influenza virus and type 7 pneumococcus was varied to determine what effect this would have on incidence of pneumococcal disease. Chinchillas were randomly assigned to receive pneumococcus 2 days before, concurrent with, or 2, 4, 6, and 12 days after influenza inoculation. Before microbial inoculation and at 2- to 4-day intervals thereafter for 3 weeks, the tympanic membranes of each chinchilla were examined with an operating microscope, and tympanometry was performed with a Grason-Stadler model 1722 middle ear analyzer (Grason-Stadler, Inc., Littleton, Mass.). Bone wax (Lukens Laboratory, St. Louis, Mo.) was liberally applied to the tympanometer tip to ensure an airtight seal. The tympanometric tracing began between 0.25 and 1.25 mohm. Duplicate readings were taken of each ear. Tympanic membrane color and opacity were described during each otoscopic exam, and presence of an air-fluid level, bubbles, or perforation was noted. A highly sensitive and specific algorithm for detecting middle ear effusion in this animal model has been formulated that uses otoscopy and tympanometry. Ears with a yellow tympanic membrane and flat tympanogram yield effusion in 96% of cases with 89% specificity for detecting effusion (G. S. Giebink, K. A. Heller, and E. R. Harford, Ann. Otol. Rhinol. Laryngol., in press). Tympanocentesis and bacterial cultures of middle ear effusions were performed when otoscopy exam or tympanometry suggested effusion (11). Blood cultures were performed on selected animals, using 5% sheep blood agar, twice each week after pneumococcus inoculation. Chest roentgenograms were obtained on some animals 2 to 3 weeks after microbial inoculation. Selected animals were sacrificed at the termination of the study, and bacterial cultures of blood, middle ear fossa, lung tissue, and cerebrospinal fluid were performed (13).

Middle ear pressure was measured by tympanometry in a group of chinchillas inoculated with influenza or pneumococcus to measure the effect of these microbes on the development of negative middle ear pressure. Tympanometry was performed before microbial inoculation and at 1 to 4-day intervals thereafter.

The role of negative middle ear pressure in the pathogenesis of pneumococcal otitis media was studied in chinchillas that had received unilateral tympanostomy tubes 2 weeks before intranasal inoculation of pneumococcus followed 2 days later by influenza A virus. Otoscopy was performed 7, 10, 14, 21, 28, 42, and 56 days after pneumococcal inoculation, and tympanocentesis and bacterial cultures were done when middle ear disease was suspected.

In all studies, nasal washings for culture of pneumococcus and influenza A virus were obtained 4 days after inoculation of the microbe (10). Complement fixation antibodies to influenza virus were measured in serum obtained before and 3 weeks after inoculation by a modified Kolmer test, using 2 U of influenza antigen (27). A fourfold increase in antibody titer was considered evidence of infection.

The means of the preinoculation results for each PMN assay on individual animals or a group of animals were calculated by arithmetic analysis. Each postinoculation value was compared with the mean preinoculation value for individual animals or a group of animals, using the Student *t*-test for independent samples. The Student *t*-test was also used to compare PMN function between the various groups. For statistical evaluation of the tympanostomy tube experiments, the chi-square test was used. To detect differences in the incidence of pneumococcal disease occurring in animals inoculated with *S. pneumoniae* alone compared with animals inoculated with influenza virus and pneumococcus, the chi-square test was used to evaluate the frequency of occurrence of pneumococcal otitis media, and Fisher's exact test was used to evaluate pneumococcal bacteremia, meningitis, and pneumonia.

## RESULTS

A total of 238 healthy adult chinchillas were studied. One hundred eighty animals were used in the experiment which looked at the effect of varying the time interval between intranasal inoculation in animals receiving both influenza and pneumococcus. Twenty-four of these animals were also studied for the effect of microbial inoculation on PMN function, and 64 were used in the experiments measuring the effect of in-

tranasal microbial inoculation on middle ear pressure. A separate stock of 38 animals was used in the experiment in which the effect of tympanostomy tubes in preventing pneumococcal otitis media was studied. All animals inoculated with influenza virus had a significant rise in their serum complement-fixing antibody against influenza, and most animals had a positive nasal washing for the virus. All animals receiving *S. pneumoniae* had a positive nasal washing for the bacteria.

Twenty-four chinchillas were selected for PMN function studies and were randomly assigned to receive intranasal inoculation with pneumococcus, influenza, or both. Two of the animals receiving both microbes and one of the influenza-inoculated animals died during the 2nd week after inoculation, and these animals were excluded from the data analysis. Autopsies did not reveal significant pathology, and these animals were presumed to have died from hypovolemic shock. The other 21 animals survived through the study period. During the postinoculation period, the mean peripheral leukocyte count and differential did not significantly change from the preinoculation level for any of the three groups.

Minimal fluctuation in peak PMN chemiluminescent activity in response to opsonized zymosan occurred during the preinoculation period in each of the three groups of chinchillas (Fig. 1). In the pneumococcus-inoculated group, peak PMN chemiluminescent activity was increased on days 14 and 18 after inoculation compared with the preinoculation activity ($P \leq 0.02$); significantly depressed peak chemiluminescence did not occur in this group. In both the influenza-inoculated group and the group inoculated with both microbes, peak PMN chemiluminescent activity was depressed on days 4 and 6 after inoculation compared to the preinoculation activity ($P \leq 0.024$). When the three groups of chinchillas were compared with each other on identical days after inoculation, significantly depressed zymosan-induced peak PMN chemiluminescent activity occurred on day 6 in the group inoculated with both microbes compared with the pneumococcus-inoculated group.

Peak PMN chemiluminescent activity in response to PMA varied minimally during the preinoculation period in each of the three groups (Fig. 2). No significant change in peak PMN chemiluminescence occurred in the pneumococcal group when the postinoculation values were compared with the mean peak chemiluminescent activity. Depressed peak PMN chemiluminescent activity occurred on days 4 and 6 in the group inoculated with both microbes ($P \leq 0.02$) and on day 6 in the influenza group ($P = 0.038$) compared with their respective preinoculation

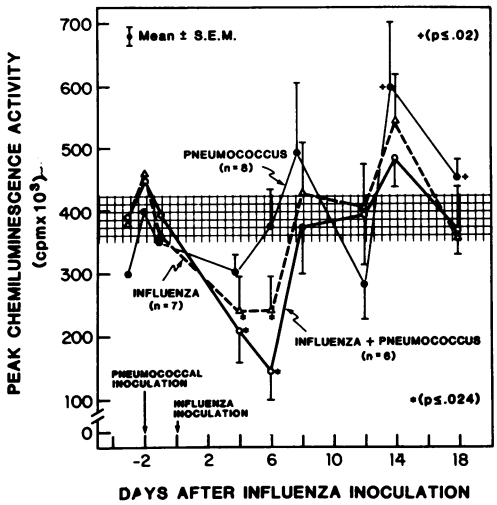


FIG. 1. Peak PMN chemiluminescence in response to zymosan stimulation in chinchilas inoculated intranasally with influenza A virus alone (---), type 7F *S. pneumoniae* alone (●), or both microbes (○). The cross-hatched area represents the 95% confidence interval for the preinoculation values. *, Significant depression ($P \leq 0.024$) on the given postinoculation day compared with the mean preinoculation chemiluminescent activity of the respective group. +, Significant increase ($P \leq 0.02$) on the postinoculation day compared with the mean of the preinoculation days for this group.

mean PMN chemiluminescent values. When the three chinchilla groups were compared with one another on identical days after inoculation, significantly depressed PMA-induced peak chemiluminescent activity occurred on day 6 in the

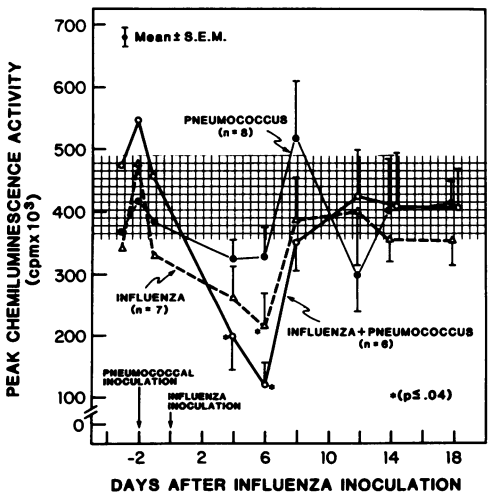


FIG. 2. Peak PMN chemiluminescence in response to PMA in chinchillas inoculated with influenza, pneumococcus, or both microbes. Symbols are as in Fig. 1.

group inoculated with both microbes compared with the pneumococcus-inoculated group.

PMN chemotactic activity did not vary significantly after inoculation of the pneumococcus group compared with the mean preinoculation value of this group (Fig. 3). Depressed chemotactic activity occurred on days 4 through 8 in the group inoculated with both microbes ($P \leq 0.001$) and on day 6 in the influenza-inoculated group ($P \leq 0.01$) compared with their respective preinoculation mean values. When the chemotactic activities of the three groups were compared with one another on identical days, the chemotactic activity of the group inoculated with both microbes was significantly depressed compared with that of the influenza group on day 4 and the pneumococcal group on day 6 ($P \leq 0.02$).

Five of the six chinchillas inoculated with pneumococcus plus influenza and five of the seven chinchillas inoculated with influenza alone had depressed chemiluminescent or chemotactic activity, or both, on days 4 and 6 after inoculation. Fewer than two animals in each group had depressed chemiluminescent or chemotactic activity on days 8, 12, 14, and 18. At least six of the eight pneumococcus-inoculated animals had normal chemiluminescent or chemotactic activity on each of the sampling days after inoculation.

Middle ear pressure was measured by tympanometry in 18 animals (36 ears) inoculated with pneumococcus and in 46 chinchillas (92 ears) inoculated with influenza virus (Fig. 4). Ears that developed middle ear effusions were excluded from this analysis, since middle ear pressures were characteristically negative or unobtainable due to tympanic membrane dampening by the middle ear fluid. Middle ear pressure gradually declined during the first 3 days after influenza inoculation and then rapidly decreased to reach a nadir on the 7th day after inoculation. Fourteen days after influenza inoculation the middle ear pressures had gradually returned to normal. In contrast, intranasal inoculation with pneumococcus was not associated with an appreciable change in middle ear pressure.

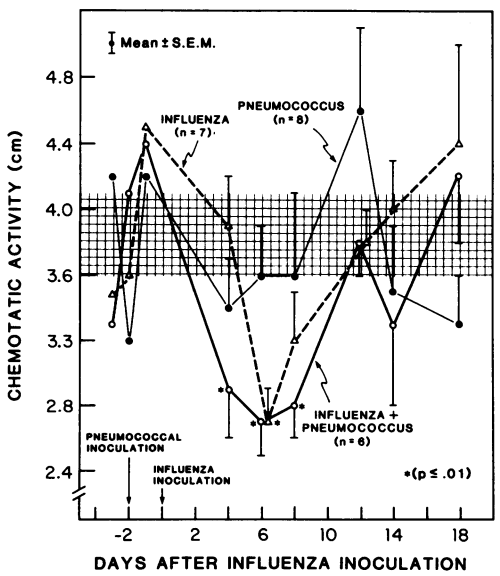


FIG. 3. PMN chemotactic activity (attractant minus random migration) in chinchillas inoculated intranasally with influenza, pneumococcus, or both microbes. Symbols are as in Fig. 1.

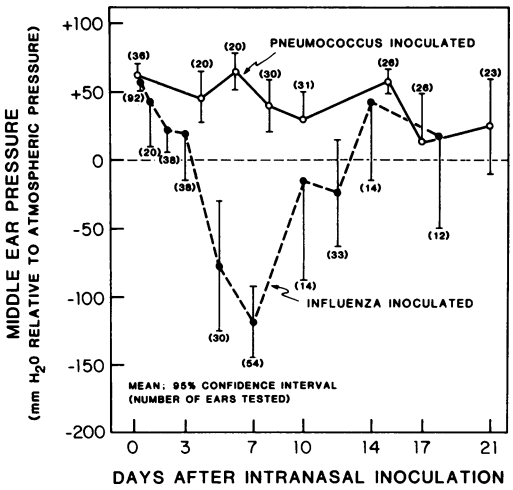


FIG. 4. Middle ear pressure in chinchillas, measured by tympanometry, at various times after intranasal inoculation of influenza (---) or pneumococcus (○). The number of animals tested on a given day is noted in parentheses. The mean and 95% confidence interval is noted by the bracketed lines.

One hundred eighty chinchillas were inoculated with influenza virus, pneumococcus, or both microbes to determine the effect of varying the time interval between inoculation of pneumococcus and influenza virus on the incidence of pneumococcal disease (Table 1). Pneumococcal disease occurred in a total of 52 animals with onset of disease occurring 5 to 17 days after intranasal inoculation. Approximately 80% of all animals with pneumococcal disease were first noted to be culture positive between days 7 and 14. None of the animals inoculated with only influenza virus developed pneumococcal infection. In those animals inoculated with only *S. pneumoniae*, 7 of 37 (18.9%) developed pneumococcal otitis media, with the infection occurring bilaterally in one of these animals. Evidence for the occurrence of pneumococcal bacteremia,

TABLE 1. Effect of varying the time interval between intranasal inoculation with influenza virus and *S. pneumonia* on the incidence of pneumococcal disease

| Type of infection[a] | | Site of positive culture for *S. pneumoniae* | | | | Positive Chest roentgenogram[b] |
|---|---|---|---|---|---|---|
| Influenza virus | *S. pneumoniae* | Ear | Blood | Cerebrospinal fluid | Lung | |
| + | | 0/40[c] | 0/9 | 0/9 | 0/9 | ND[d] |
| | + | 7/37 (1)[e] | 0/18 | 0/24 | 0/15 | ND |
| + | + (−2) | 17/56 (7) | 1/15 | 1/37 | 2/6 | 3/13 |
| + | + (0) | 3/8 (2) | 1/7 | 0/7 | ND | 1/7 |
| + | + (+2) | 7/10 (5)[f] | 4/10[f] | 1/3 | 1/3 | 2/4 |
| + | + (+4) | 9/10 (3)[f] | 2/9 | 0/2 | 0/2 | ND |
| + | + (+6) | 6/10 (3)[f] | 0/8 | 0/5 | 0/5 | ND |
| + | + (+12) | 2/9 (1) | 0/9 | 0/4 | ND | 0/8 |

[a] The infecting microbe is noted by the +, and the number in parentheses notes which day the *S. pneumoniae* was inoculated in relation to when the influenza virus was inoculated.
[b] Roentgenograms showed either lobar or segmented infiltrates.
[c] Number of animals with a positive culture/number of animals studied.
[d] ND, Not done.
[e] The number in parentheses indicates the number of animals with bilateral pneumococcal otitis media.
[f] $P \leq 0.01$ compared with the group inoculated with pneumococcus alone.

meningitis, or pneumonia was not found in this group. In the groups of animals intranasally inoculated with both influenza virus and pneumococcus at various time intervals, the greatest incidence of pneumococcal otitis media and bacteremia infection occurred in animals inoculated with pneumococcus 2, 4, and 6 days after influenza virus. Thus, the groups of animals with the greatest incidence of pneumococcal otitis media were inoculated with the pneumococcus at the time at which influenza virus caused depressed PMN function and negative middle ear pressure. The greatest incidence of pneumococcal otitis media (90%) and bacteremia (40%) occurred in those groups inoculated with pneumococcus 4 and 2 days, respectively, after influenza virus. The number of chest roentgenograms with infiltrates and the number of pneumococcus-positive cerebrospinal fluid and lung cultures were too small to draw any conclusions regarding the inoculation time interval at which these findings most often occur. Thus, the greatest incidence of pneumococcal otitis media and bacteremia occurred when *S. pneumoniae* was inoculated just before influenza-induced PMN dysfunction.

Unilateral tympanostomy tubes were inserted in 38 chinchillas 2 weeks before inoculation of both pneumococcus and influenza virus to determine the relative importance of influenza-induced negative middle ear pressure in the pathogenesis of pneumococcal otitis media. Pneumococcal otitis media occurred in eight ears with tubes and in five ears without tubes (chi square = 1.2929; $P = 0.5$). One animal had bilateral otitis media. Sterile OME occurred in one ear with tubes and in none of the ears without tympanostomy tubes.

## DISCUSSION

Viral upper respiratory tract infection and otitis media are the most common childhood infectious diseases (21, 30). Otitis media occurs in approximately two-thirds of children at least once during the first 2 years of life (22, 29). Pathogenic bacteria have been cultured from middle ear effusion in approximately 75% of the cases of acute otitis media; among these microbes *S. pneumoniae* is the most frequently isolated, and approximately 80% of pneumococcal otitis media episodes are caused by serotypes 1, 3, 4, 6, 7, 9, 14, 15, 18, 19, and 23 (2).

Several studies support the hypothesis that respiratory viruses predispose the host to an increased risk of otitis media. During an epidemic of respiratory syncytial virus in a nursery, 13 infants had this virus isolated from middle ear effusions, and 3 had concurrent isolations of pneumococcus from the effusions (16). Klein and Teele found respiratory viruses in the ears of only 4.4% of 663 patients, but 23.7% of these patients had respiratory viruses in their nasopharynx, and 28.8% had significant antibody seroconversion, suggesting that viral infection contributed to the development of otitis media in some patients (24). Experiments in chinchillas showed that intranasal inoculation of influenza A virus with pneumococcus produced a higher incidence of OME and pneumococcal otitis media than inoculation with pneumococcus alone (10). Additionally, chinchillas developed PMN dysfunction 4 through 8 days after influenza inoculation (1). The present study was designed to investigate the role of influenza-induced middle ear changes and PMN dysfunction in the

development of pneumococcal otitis media in chinchillas.

Chinchillas inoculated intranasally with influenza alone or pneumococcus with influenza demonstrated significantly depressed chemotactic and chemiluminescent activity between the 4th and 8th days after inoculation compared with their respective preinoculation mean chemotactic and chemiluminescent activities. This depression was most pronounced on day 6, when 71.4% (five of seven) of influenza-inoculated chinchillas and 83.3% (five of six) of animals inoculated with both microbes had depressed PMN function. None of the eight pneumococcus-inoculated animals had depressed PMN function on day 6 after inoculation. We have previously shown that chinchillas inoculated with influenza alone have decreased PMN chemiluminescent, chemotactic, and bactericidal activity, but normal phagocytic activity (1). In this study, PMN chemiluminescent activity was depressed to both opsonized zymosan and the soluble stimulus PMA. Since PMA stimulates the phagocytic cell by a mechanism independent of phagocytosis, these data further support the concept that influenza virus-induced PMN dysfunction is due to intrinsic cell damage and not to decreased phagocytosis.

Influenza A and B viruses have been shown to depress phagocytic cell metabolic, bactericidal, and chemotactic activity (7, 8, 25, 26, 35, 36). Studies have yielded inconsistent results regarding the effect of influenza A virus on PMN phagocytic activity (8, 25, 26, 32, 38). Other respiratory viruses, including respiratory syncytial virus and Sendai virus, also cause phagocytic cell dysfunction (7, 23). Thus, influenza and other respiratory viruses can depress phagocytic cell function and may thereby increase host susceptibility to secondary bacterial infection. Although the majority of circulating phagocytic cells in chinchillas are PMN, peripheral blood monocytes may have contributed to the leukocyte dysfunction observed in these experiments. The mechanism by which influenza causes phagocytic cell dysfunction is not currently known.

The phagocytic cell plays an important role in protecting the host from bacterial infection. PMN are found in the middle ear effusion and tissues of animals and humans with purulent otitis media and are particularly abundant during the first weeks of infection (13, 28). The migration of PMN into the middle ear when purulent otitis media occurs suggests that PMN play an important role in the immune response to bacterial infection of the middle ear. Hill et al. described 14 children with recurrent otitis media and chronic diarrhea in association with markedly abnormal PMN chemotactic activity (19). Giebink et al. reported depressed PMN chemotactic, bactericidal, and chemiluminescent activity in 18, 23, and 15%, respectively, of children with persistent otitis media with effusions (12). These studies suggest that normally functioning PMN play a role in protecting the host from otitis media and that PMN dysfunction, such as that induced by influenza virus, can result in middle ear infection.

Respiratory virus infection is also associated with eustachian tube dysfunction and negative middle ear pressure. Normal tubal function allows for proper ventilation and equalization of pressure between the middle ear and the atmosphere. Compromised eustachian tube function, whether by mechanical or functional obstruction, initiates a complex inflammatory response which can result in the development of negative middle ear pressure and middle ear effusion (5, 16, 31). Negative middle ear pressure has been reported in a majority of children with upper respiratory tract infections (6, 34). In the present study, chinchillas developed markedly negative middle ear pressure after influenza inoculation, whereas middle ear pressure remained normal in pneumococcus-inoculated animals. High negative middle ear pressure could be associated with aspiration of bacteria into the middle ear, thereby inducing acute otitis media (5). It is difficult to ascertain whether middle ear infection can occur without previous eustachian tube dysfunction, since infection would promptly cause effusion and eustachian tube dysfunction (31).

The interval between inoculation of pneumococcus with influenza virus was varied to determine the importance of virus-induced PMN and eustachian tube dysfunction in the development of pneumococcal otitis media. The frequency of pneumococcal otitis media in chinchillas progressively increased as the time of pneumococcus inoculation moved closer to the time of virus-induced PMN dysfunction and the onset of eustachian tube dysfunction. Studies in other animal species have shown that the maximal incidence of bacterial disease occurs when influenza virus infection precedes pneumococcal infection by approximately 1 week (9, 17).

The value of tympanostomy tubes in preventing the occurrence of acute otitis media is controversial (31). In this study, unilateral tympanostomy tubes were inserted in chinchillas before inoculation of pneumococcus with influenza virus to determine the relative importance of normal middle ear ventilation in the pathogenesis of pneumococcal otitis media. No difference was found in the frequency of occurrence of pneumococcal otitis media in ears with and without tubes, suggesting that negative middle ear pressure is not required in this animal model

for the development of pneumococcal otitis media.

The sequence of events leading to the development of purulent otitis media during a respiratory virus infection could be as follows. (i) Influenza virus infects the upper respiratory tract, and this coincides temporarily with the acquisition in the upper respiratory tract of a pathogenic strain of *S. pneumoniae*. Gray et al. have shown that the time during which an infant is at greatest risk of developing pneumococcal disease is during the 1st month after the bacteria colonize the infant (15). After that time, the infant will often harbor the bacteria but the risk of clinical disease is minimal. (ii) Aspiration of pneumococci into the middle ear occurs in association with high negative middle ear pressure. (iii) The influenza virus induces peripheral PMN dysfunction, which could decrease migration of PMN into the middle ear and the bactericidal capacity of the PMN which reach the middle ear. It is also possible that the virus directly invades the middle ear, causing abnormal leukocyte function in PMN which subsequently enter the middle ear. However, a previous study from our laboratory suggested that direct invasion of influenza virus into the middle ear is infrequent (10). Thus, influenza virus-induced PMN dysfunction could enhance the capacity of the pneumococcus to establish a site of infection in the middle ear. The effect of influenza viruses on other factors such as local and systemic immunoglobulin production, complement, rate of bacterial growth in the nasopharynx, and adherence of bacteria to respiratory epithelium also require investigation to determine whether other mechanisms might also contribute to the pathogenesis of middle ear disease in this model.

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