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RESPIRATORY VIRUSES AND *MYCOPLASMA PNEUMONIAE* INFECTIONS AT THE TIME OF THE ACUTE EXACERBATION OF CHRONIC OTITIS MEDIA

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The present study was undertaken to ascertain whether or not patients with chronic otitis media are infected with viruses or Mycoplasma at the time of sudden increase in otorrhea.

From 26 patients with acute exacerbation of chronic otitis media, sera were collected at the time of sudden increase in otorrhea and three to four weeks later. These paired sera were examined for antibody titer to respiratory viruses (21 species) and *Mycoplasma pneumoniae*. Of them, influenza B virus and RSV infections were demonstrated in four and two cases, respectively. Examinations showed no infection in 10 control cases without acute exacerbation.

In 36 cases of acute exacerbation of chronic otitis media, attempts were made to isolate viruses and *Mycoplasma pneumoniae* from the pharynx and otorrhea. Consequently, influenza B virus was detected in pharyngeal mucous scrapings in two cases and RSV in one.

The probability of respiratory virus infection leading to acute exacerbation of chronic otitis media appears to be lower than that provoking acute otitis media in children and infants. However, the present data suggest that the development of respiratory virus infection in patients with chronic otitis media may cause an increase in the otorrhea, eventually resulting in an acute exacerbation of inflammation.

It is widely recognized by clinicians that non-specific upper respiratory infection is often associated with otitis media. Furthermore, it is known that even chronic otitis media is often acutely exacerbated with increase in the amount of otorrhea if a patient with this disease catches a cold. A common cold with such symptoms as cough, snivel, *etc.*, is caused by various factors. One of the primary

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causative factors is respiratory virus or Mycoplasma infection. The present study was undertaken to ascertain whether or not patients with chronic otitis media are infected with viruses or Mycoplasma at the time of sudden increase in otorrhea.

MATERIALS AND METHODS

Patients, specimen collection and titration for specific antibodies. Forty-six patients visiting this outpatient clinic for the acute exacerbation of chronic otitis media were studied. They experienced sudden appearance of otorrhea from the middle ear cavity having been dry or suddenly increased otorrhea. Peripheral blood samples were collected and titrated for specific antibodies to such viruses and *Mycoplasma pneumoniae* (*M. pneumoniae*) that were suspected of infecting the respiratory system. Blood was sampled again three to four weeks later from 26 cases. Blood sampling was repeated at intervals of a month whenever possible. The first and the subsequent samples were examined in the same way. From the other 20 cases, paired samples were not obtainable.

When blood samples were collected for the first titration of the specific antibodies, it was attempted to isolate the same viruses and *M. pneumoniae* and also other bacteria from otorrhea and pharyngeal mucous scraping. Ten patients with chronic otitis media with little otorrhea or with the dry tympanic cavity served as controls.

The serum samples were titrated with the following viruses and *M. pneumoniae* by the method indicated:

Complement fixation test (CF): Influenza virus A, B. Adenovirus. Respiratory syncytial virus (RSV).

Hemagglutination inhibition test (HI): Parainfluenza virus 1, 2, 3, 4.

Neutralization test (NT): Echovirus 1, 3, 4, 7, 9, 13, 23. Reovirus. Coxsackievirus 2, 3, 4, 5, 6.

Mycoplasma pneumonia: CF.

The titrations of serum samples for specific antibodies to viruses and Mycoplasma and isolation of bacteria were performed at the Central Laboratory of this Hospital.

Isolation and identification of viruses. To isolate virus and bacteria, otorrhea specimens were collected by the aspiration technique detailed elsewhere (SUGIYAMA, TANABE, CHANG, and NAKAI, 1981) and specimens from the pharynx were taken with swabs. These specimens were each inoculated into HEK and MK tubes, which were immediately sent to Special Reference Laboratory, Tokyo for virus isolation. At this laboratory, small aliquots of each medium were inoculated without delay into tubes of MA104, WI38, Vero and Hep-2. All cell cultures (HEK, MK, MA104, WI38, Vero and Hep-2) were incubated at 33°C on a roller drum and examined every day for the cytopathic effect (CPE). The virus multiplication was confirmed by CPE and haemadsorption. Haemadsorption, using

guinea pig erythrocytes incubated at 4°C for 20 min and at room temperature for an hour, was performed on all cell cultures before passaging into fresh cells. Cultures were considered to be negative when there was no evidence for CPE and for haemadsorption after three blind passages.

The isolates were identified by the neutralization, hemagglutination-inhibition, and complement fixation tests.

RESULTS

When the antibody titer at the second examination was higher than 4 times that at the first examination, the case was judged to be infected. Six cases out of the 26 having given paired sera were judged to be infected. Four of them (cases 1, 4, 7 and 16) were considered to be at onset of influenza B virus infection when their otorrhea increased (Fig. 1). Coincidentally, influenza B virus prevailed in December, 1981–March, 1982 in this country. The rest two were cases of RSV

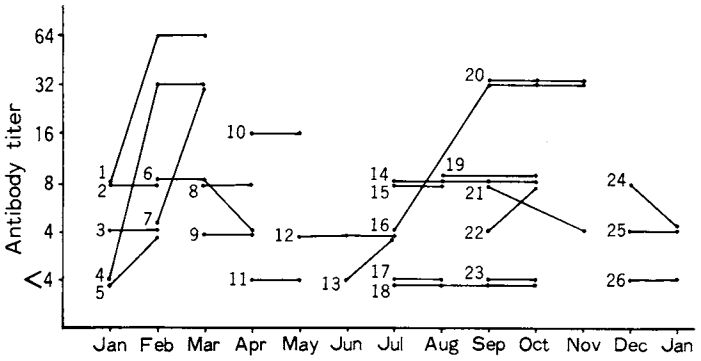

Fig. 1. Influenza B virus antibody titers in paired sera. The figure represents a case number. The titer is expressed as the reciprocal of the highest serum dilution giving positive reaction.

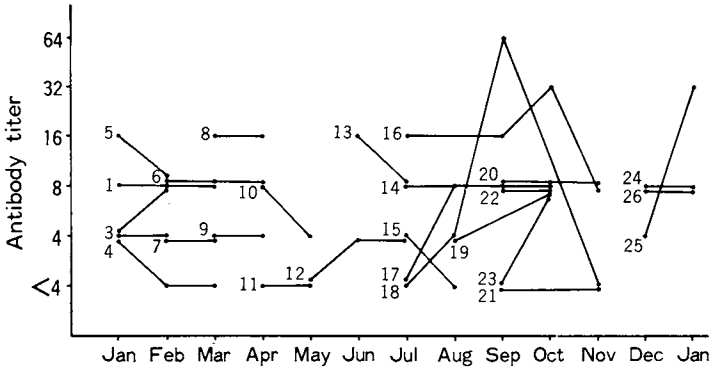

Fig. 2. Respiratory syncytial virus antibody titers in paired sera. The figure caption are the same as in Fig. 1.

Table 1. Monthly parainfluenza 3 virus antibody titers in sera from cases of acute exacerbation of chronic otitis media.

Antibody titer	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
512		1								1		
256	1	1	1	1	1		1	1	2	2	1	1
128	3	2	2	1		1	3	1	3	3	2	2
64	4	3	2	3	2	1	2	4	1	6	3	2
32	1	1	1	1	1	1	1	1	2	1		1
<32												

The antibody titers were dertermined by the with hemagglutination inhibition test. Each figure represents the number of cases.

infection. One (case 18) of them visited this clinic in July and August, complaining of sudden increase of otorrhea (Fig. 2). The titer of serum RSV antibody determined in September was as high as 16 times that determined in August. RSV infection seemed to have occurred in August in this case. The other one, case 25, showing similarly sharp increase in the antibody titer may probably have been infected in December. Cases 17 and 23 were excluded from the infected group for the following reason: they both showed titer increase by 2 tubes (from the level below 4 to 8), but the latter value itself was not so high as to give a firm basis for diagnosis of an infection.

These studies were made during the period from July, 1979 to May, 1982, but Figs. 1 and 2 refer to only months when antibody titer was determined. The titer of 83 serum samples collected from 46 patients of chronic otitis media who visited this department complaining sudden increase in otorrheal output were classified by month of examination. There was no seasonal factor affecting the titer. Among these titers, the specific antibody titers to parainfluenza 3 virus are given in Table 1.

Virus isolation was tried with 38 cases. Influenza B virus was isolated from two cases and RSV from one case; both from the pharyngeal cavity. None of the otorrheal samples yielded any virus. One of the two cases yielding influenza B virus (case 4 in Fig. 1) was diagnosed as influenza B virus infection on the basis of the rise in the titer. In the other of the two cases yielding influenza B virus and the case yielding RSV virus, titration for antibody was made only once at the initial examination. The titer of influenza B virus antibody was 16 in the former case and that of RSV antibody 32 in the latter.

All 83 serum samples gave antibody titer below 40 with respect to *M. pneumoniae*. *M. pneumoniae* was isolated from the pharyngeal cavity in one of the 38 cases, on which isolation was tried. The case in question, however, was not con-

sidered to be of *M. pneumoniae* infection since the titers of the paired sera were lower than 40.

Virus or *M. pneumoniae* infection was not demonstrated by examination of paired sera taken from any of the 10 control patients with chronic otitis media. None of them had any virus or *M. pneumoniae* in the pharyngeal cavity.

SUGITA (1977) isolated bacteria from otorrhea and pharyngeal mucous scrapings in cases of chronic otitis media, reporting that more than two species of bacteria were isolated from many of otorrheal specimens. When one of these species from otorrhea was the same as that isolated from the pharyngeal mucous scrapings, the case was called the consistent one. He reported also that the consistent ratio (ratio of consistent cases to the total cases examined) was 55.6% (40/72) in the group of cases complicated with upper respiratory inflammation, whereas only 3.6% (1/28) in the group of no complication. In the present study, we tried to isolate bacteria from otorrhea and pharyngeal mucous scrapings of 43 cases of acutely exacerbated chronic otitis media and, according to the criterion he used, the consistent ratio was 46.5% (20/43). This ratio was close to that reported by SUGITA (1977) for the complicated group. The followings were consistent cases in this study: 1) Six cases which were considered to have a virus infection in the light of the antibody titers in paired sera, and 2) two cases in which viruses isolated from the pharyngeal cavity though paired sera not obtained.

As a general symptom, we noticed fever of approximately 37–39°C in four cases of influenza B virus infection (cases 1, 4, 7 and 16) belonging to the group of virus infection and also in five cases judged to be uninfected through examinations of paired sera. Of the cases in which paired sera could not be obtained, five were also feverish (37–38°C). These cases included the one in which influenza B virus was isolated from the pharyngeal cavity. As a whole, 14 of 46 cases suffered from pyrexia.

DISCUSSION

Examinations of paired sera from 26 patients suffering from acute exacerbation of chronic otitis media revealed that six of them had some respiratory virus infections. In contrast, rise in the antibody titer was not demonstrated in any of the paired serum samples from 10 control patients with chronic otitis media (whose inflammatory condition was not exacerbated).

There has been no report describing association of respiratory virus infection with acute exacerbation of chronic otitis media, but many authors (BERGLUND, SALMIVALLI, and GRÖNROOS, 1967; GRÖNROOS, VIHMA, SALMIVALLI, and BERGLUND, 1968; DANIELEWICZ, 1975; KLEIN and TEELE, 1976; Sloyer HOWIE, PLOUSSARD, BRADAC, HABERCORN, and OGRA, 1977; ARBESMAN, 1979; BLÁHOVÁ, FEDOVÁ, and SYRÀCEK, 1980; CARLSEN and ØRSTAVIK, 1980; MEURMAN, SARKKINE, PUHAKKA, VIROLAINEN, and MEURMAN, 1980; KLEIN, 1981; HENDERSON, COLLIER, SANYAL,

WATKINS, FAIRCLOUGH, CLYDE, and DENNY, 1982) correlated respiratory virus infection with acute otitis media. It is not justifiable to discuss acute exacerbation of chronic otitis media and acute otitis media on an equal level. It is presumable, however, that respiratory virus infection has an adverse effect on the pathological condition of the middle ear in our cases of chronic otitis media as reported with acute otitis media.

KLEIN and TEELE (1976) reviewed ten reports of case of acute otitis media complicated with virus or Mycoplasma infection of the upper respiratory system, and found that out of 249 cases examined for antibodies in paired sera, 72 cases (28.9%) were suspected of respiratory virus infection and 15 of 164 cases (9.1%) had Mycoplasma. It was further found that viruses were isolated from the nasopharynx or throat in 59 of 249 cases (23.7%) and from the middle ear effusion in 29 of 663 cases (4.4%), and that Mycoplasma was isolated from the nasopharynx or throat in 5 of 116 cases (4.3%) and from the middle ear effusion in 1 of 721 cases (0.13%). BLÁHOVÁ *et al.* (1980) reported that out of 126 children with acute otitis media, 60 patients (47.8%) had a respiratory virus infection and viruses were isolated from the nasopharynx in 25 patients and from the middle ear in 5. From 50 cases with secretory otitis media, ADLINGTON and HOOPER (1980) isolated respiratory viruses from the pharynx and nasopharynx of four (8%), but none from the middle ear mucosa. CARLSEN and ØRSTAVIK (1980) stated that, of 422 children diagnosed as RSV infection, 76 had complication with otitis media. The low incidence reported hitherto may be ascribed at least in part to the failure in examining for some pathogens probably affecting the respiratory system, such as rhinovirus, coronavirus, *etc.* HENDERSON *et al.* (1982) disclosed two factors influencing the occurrence of acute otitis media with effusion (OME), namely viral respiratory infection and nasopharyngeal colonization with *Streptococcus pneumoniae* and *Haemophilus influenzae*, and concluded that respiratory infection with RSV, influenza A or B and adenovirus conferred a greater risk of OME than nasopharyngeal colonization with the bacteria mentioned above. These findings attest the association between respiratory virus infection and otitis media and suggest that some patients with acute otitis media harbor viruses in their upper respiratory tract, but rarely in the middle ear. Although the frequency of respiratory virus infection differed among investigators, many of them postulated the significance of respiratory viruses in the etiology of acute otitis media.

In the present study, respiratory virus infection was not included in cases of chronic otitis media without exacerbation, but it was demonstrated in some cases of acute exacerbation of chronic otitis media. The ratio of these cases to the total cases examined was lower than that reported in acute otitis media. It is conceivable that acute exacerbation of chronic otitis media may occur following respiratory virus infection by the same mechanism involved in acute otitis media. GIEBINK, BERZINS, MARKER, and SCHIFFMAN (1980) reported that acute otitis media was hardly induced in experimental animals by exposing them to viruses through the

nasal cavity but those exposed to both viruses and bacteria were vulnerable to inflammatory disease. This may be explained as follows: the function of Eustachian tube is disordered due to virus infection (STUART-HARRIS and SCHILD, 1976) and the bacteria present in the nasal cavity easily get into the middle ear cavity. In addition, the virus infection weakens the defensive power of the lesion (HAMILTON, OVERALL, and GLASGOW, 1976; JAKAB and GREEN, 1976; WARSHAUER, GOLDSTEIN, AKERS, LIPPERT, and KIM, 1977; GARDNER, 1980), creating an environment suitable for bacterial infection. GIEBINK *et al.* (1980) stated further that virus isolation from the middle ear was difficult in experimental otitis media, probably because the viruses, having entered the middle ear, disappeared in an early stage before middle ear effusion was produced. MEURMAN *et al.* (1980) reported that antibodies to respiratory viruses were detected in middle ear and nasopharyngeal secretions from child patients with secretory otitis media, and that the titers of middle ear secretions were generally higher than those of nasopharyngeal secretions. They alluded local production of specific antibodies in the middle ear.

From these observations in man and animals, otitis media is likely to develop through the following processes: 1) The viruses having affected the upper respiratory tract migrate to the middle ear *via* Eustachian tube and infect that organ. 2) The middle ear infected with viruses becomes liable to infection with bacteria from the nasopharynx. If a patient with chronic otitis media has respiratory virus infection, the middle ear may be affected by acute inflammation induced by the bacteria through the processes mentioned above. In fact, the same bacteria were isolated from the pharynx and otorrhea in many cases of acute exacerbation of chronic otitis media and in all cases having virus infection in this study. Chronic otitis media may be exacerbated in a similar way as in Mycoplasma infection. However, the incidence of such infection is much lower than that of virus infection. Furthermore, Mycoplasma infection usually prevails in only a limited district. Naturally, cases of Mycoplasma infection are very few. In such circumstances, data so far obtained varied widely among authors.

All the patients of chronic otitis media we studied were adults, whereas most patients of acute otitis media and of secretory otitis media referred to in this report were children or infants including newborn babies with immature immune mechanisms. The younger the patient is, the higher the susceptibility to infection with the same species of virus is. The lower the age of patients is, the wider the airway is affected. In most of adult patients, viruses stay at the upper respiratory tract and general symptoms are mild.

From the foregoing consideration, the probability of respiratory virus infection leading to acute exacerbation of chronic otitis media appears to be lower than that provoking acute otitis media in children and infants. It is possibly considered that chronic otitis media can be acutely exacerbated by a certain factor (s) other than respiratory virus infection. However, the present data suggest that the development of respiratory virus infection in patients with chronic otitis media may in-

crease otorrhea, eventually resulting in an acute exacerbation of inflammation. More specifically, respiratory virus infection may have played some etiological role in 6 of 26 cases of chronic otitis media with acute exacerbation accompanying increased otorrhea. Presumably, however, the probability of respiratory virus infection suddenly worsening the inflammatory condition of the middle ear in chronic otitis media will be higher than 6/26. In any case, it is of vital importance to take necessary measures for the protection of patients with chronic otitis media against common cold in preoperative as well as postoperative stages.

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