

Middle Ear Effusion and Fungi

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Objectives: Bacteria and viruses are rarely isolated from the middle ear fluid in cases of otitis media with effusion (OME). However, since endotoxins are often detected in such effusions, it is suspected that patients with OME have a previous history of gram-negative infection. Recently, fungi have drawn attention as microorganisms that cause chronic sinusitis. We investigated the involvement of fungi in the formation of middle ear effusions of patients with OME and eosinophilic otitis media, in which patients have viscous middle ear effusions and a history of adult bronchial asthma indicating definite involvement of eosinophils.

Methods: Middle ear effusions and nasal secretions were collected from patients with eosinophilic otitis media (7 patients) or OME (12 patients), and smears were prepared for methenamine silver staining. The remaining specimens were embedded in Epon and stained with toluidine blue for observation under a light microscope, and ultrathin sections were prepared for examination under an electron microscope.

Results: Fungal hyphae were detected in the middle ear fluid in all of the patients with eosinophilic otitis media or OME. Charcot-Leyden crystals (CLCs) were observed in 6 of the 7 patients with eosinophilic otitis media. In regard to the findings in the nasal secretions, fungal hyphae were also detected in the nasal secretions of all patients, whereas CLCs were detected in only 1 patient with eosinophilic otitis media.

Conclusions: It was clarified by use of the methenamine silver staining method that fungi were present in the middle ear fluid in 100% of the studied cases of eosinophilic otitis media or OME. Whether fungi are also present in the middle ear cavity of normal persons is unknown, but the possibility that they may contribute as a cause of both diseases cannot be excluded. Particularly in eosinophilic otitis media, the observation of numerous CLCs in the middle ear fluid suggests that many eosinophils have degenerated. The eosinophil granule proteins released from the degenerated eosinophils can cause epithelial injury of the middle ear. The possibility that fungi induce the eosinophils in the middle ear also cannot be excluded.

Key Words: Charcot-Leyden crystal, eosinophilic otitis media, fungal hypha, middle ear effusion, otitis media with effusion.

INTRODUCTION

The middle ear cavity and paranasal sinuses are quite similar, in that they both open directly into the upper respiratory tract. A similar spectrum of pathogenic bacteria causing infection in these cavities has also been reported.^{1,2} The bacteria include *Streptococcus pneumoniae* and *Haemophilus influenzae*, and *Moraxella catarrhalis* has also recently begun to garner attention in this respect. These pathogenic bacteria are believed to first colonize the nasopharynx and infect the middle ear cavity via the eustachian tube and thereby cause acute otitis media; then they infect the paranasal sinuses via the ostia of the sinuses, causing acute sinusitis. The nasopharynx is thus considered a source of infection of the upper respiratory tract.³ In 1999, Ponikau et al⁴ reported that fungi were isolated in the nasal secretions of nearly all healthy subjects, as well as patients with chronic sinusitis. They suggested that the cause of chronic sinusitis in all cases is induction by these fungi of local migration of eosinophils, resulting in

eosinophilic inflammation. Since, as noted above, the same pathogenic bacteria cause infection of the middle ear cavity and the paranasal sinuses, both of which have openings into the upper respiratory tract and similar histology, the fungi that are isolated in the nasal secretions in 100% of cases are also highly likely to be present in the middle ear cavity. The presence of fungal DNA in middle ear effusions has been demonstrated by polymerase chain reaction (PCR).⁵ If fungi are present in middle ear effusions, they may play important roles as pathogenic microorganisms in the middle ear. We investigated the frequency of isolation of fungi in middle ear effusions collected from patients with otitis media with effusion (OME) and patients with eosinophilic otitis media exhibiting viscous middle ear effusions and a history of adult bronchial asthma, which indicated definite involvement of eosinophils.

SUBJECTS AND METHODS

Middle ear effusions, which were observed on

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both sides in every patient, and nasal secretions were collected from patients with eosinophilic otitis media and patients with OME. The diagnosis of eosinophilic otitis media was made by medical history,⁶ and the presence of many eosinophils in the middle ear effusion was determined by microscopic examination of specimens stained with Eosino Stain TORII. Seven patients (1 man and 6 women; mean age, 60.4 years) had eosinophilic otitis media; all patients had a past history of asthma for which systemic high doses of steroids had been given, although none had received any steroid during the 6 months prior to the sample collection. Four of the 7 patients had aspirin-induced asthma. There was only 1 patient who was positive for antibodies to *Aspergillus* by radioallergosorbent test (RAST) among the 7 patients. Among the 12 patients with OME, there were 7 (4 male and 3 female; mean age, 51 years) with serous effusions and 5 (4 male and 1 female; mean age, 15 years) with mucous effusions. The middle ear effusions were bilateral in all of the OME patients. None of the patients with eosinophilic otitis media or OME had tympanic membrane perforation at the time of collection of the effusion fluid samples. The external ear canal was washed 3 times with

distilled water before collection of the effusion fluid samples. The effusion fluid was obtained by suction after myringotomy. Samples of nasal secretions were also obtained by suction. Portions of the collected middle ear effusion and nasal secretion specimens were directly smeared on glass slides, and the remaining portions were fixed in 2% glutaraldehyde (0.1 mol/L cacodylate buffer) for 1 hour. The latter samples were washed 3 times with 0.1 mol/L cacodylate buffer, fixed in osmic acid for 40 minutes, and then embedded in Epon 812. Sections with an area of 1 mm² and a thickness of 1 μ m were prepared and placed on glass slides for toluidine blue staining. After staining, 5 random sections were selected, and Charcot-Leyden crystals (CLCs) within an area of 1 mm² were counted. The Epon-embedded blocks were also sliced into ultrathin sections and stuck on a mesh grid with 117- μ m-square holes and examined by electron microscopy. The eosinophil numbers in 5 randomly selected holes were counted, and the ultrafine structures of the CLCs were observed.

The specimens directly smeared on glass slides were stained with methenamine silver for 1 hour and further stained with safranin O for 5 minutes;

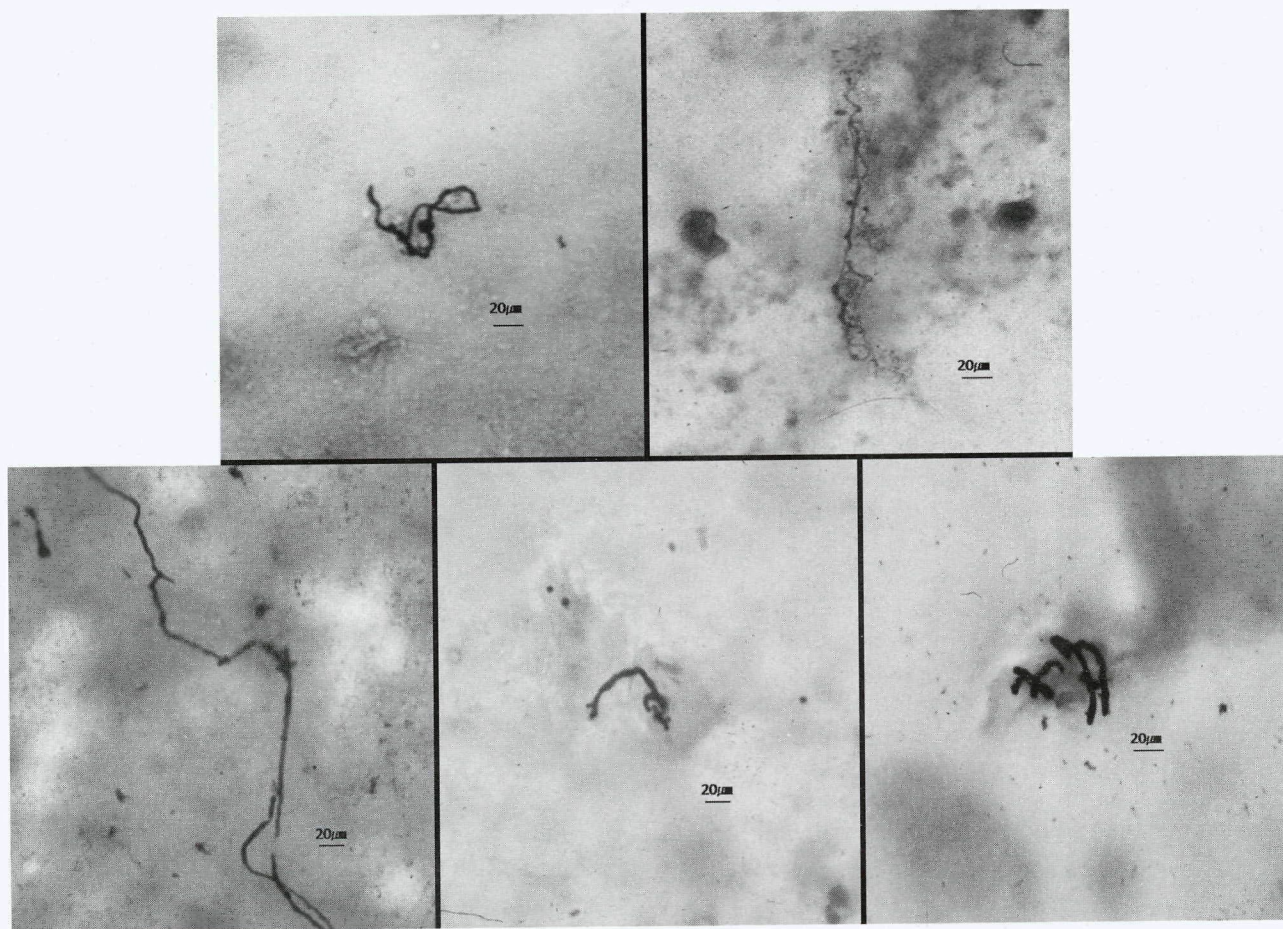


Fig 1. Smears of middle ear effusions. Fungi turn black when stained with methenamine silver.

PATIENTS WITH EOSINOPHILIC OTITIS MEDIA

Group	Patient	Age (y)	Sex	Asthma	RAST	History of Steroid Use	Eosinophils Per 1 Mesh Hole	Middle Ear Effusions		Nasal Secretions	
								Hyphae	CLCs	Hyphae	CLCs
A	1	70	M	+	—	+	25.7	+	—	+	+
	2	52	F	+	—	+	18.7	+	0.24/mm ²	+	—
	3	67	F	+*	—	+	13.7	+	0.25/mm ²	+	—
	4	57	F	+	+†	+	3.3	++	0.86/mm ²	+	—
	5	73	F	+*	—	+	12.3	+	1.65/mm ²	++	—
B	6	52	F	+*	—	+	4.5	++	3.71/mm ²	++	—
	7	52	F	+*	—	+	29.7	+	5.43/mm ²	+	—

In group A, symptoms were not controlled by transtympanic ventilation tube. In group B, symptoms were controlled by transtympanic ventilation tube.

RAST — radioallergosorbent test; CLCs — Charcot-Leyden crystals.

*Aspirin-induced.

†For *Aspergillus*.

each specimen was observed under 400× magnification in 5 fields of view, and specimens with fungi in at least 1 of the 5 fields of view were graded as +, whereas those with fungi in 2 or more fields of view were graded as ++. No examinations by fungal culture were performed. Although the rate of detection by fungal culture appears to vary greatly among test facilities, the rate by the methods routinely used in Japan is generally very low. Ponikau et al⁴ stated that the rate of detection of fungi is low unless special culture methods are used. At our facility, the rate of detection of fungi is low because routine methods are used for detection. Therefore, in this study, we adopted a method in which smears were actually stained and microscopically observed to detect fungi.

RESULTS

Eosinophilic Otitis Media. Several kinds of fungal hyphae were observed in the smear preparations (Fig 1) of the middle ear effusion fluids in all 7 patients (see Table); 2 patients received grades of ++, and 5 patients grades of +. In Epon-embedded sec-

tions sliced to 1 µm, we found no eosinophil migration to the circumference of the fungus or eosinophil binding to the fungus during the observation period (Fig 2).

Charcot-Leyden crystals were observed in 6 of the 7 patients (see Table and Fig 3). Electron microscopy revealed that the CLCs present in the cytoplasm of the eosinophils were released from the cells together with specific granules through sites of cell membrane rupture caused by eosinophil necrosis (Fig 4). None of the 7 patients with eosinophilic otitis media were found to have tympanic membrane perforation at their initial presentation. Myringotomy was performed in all patients, and the colloidal effusion retained in the middle ear was collected during the procedure. A transtympanic ventilation tube was inserted after the collection. In 2 patients (see Table, group B), the transtympanic ventilation tube was not occluded and enabled relatively good ventilation of the middle ear cavity with cleaning once every few weeks. In the remaining 5 patients (see Table, group A), however, the transtympanic

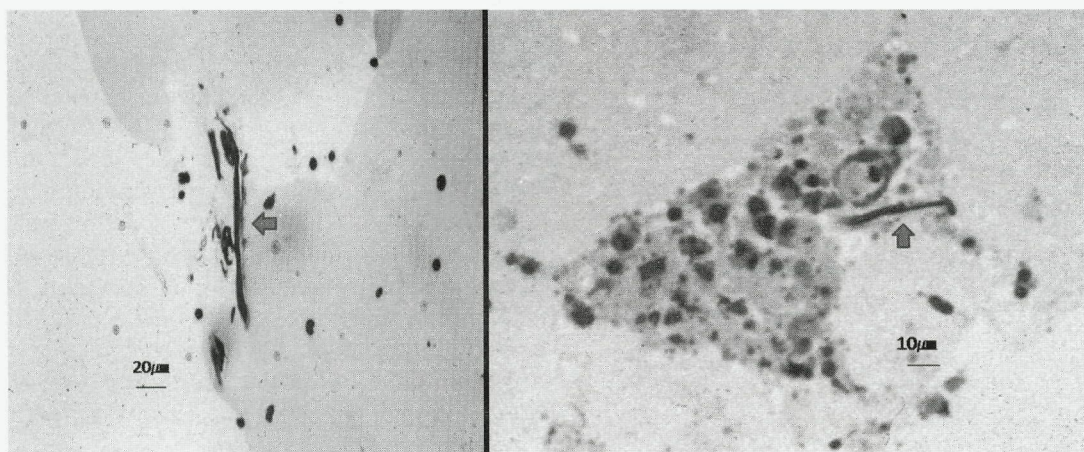


Fig 2. In Epon-embedded sections sliced to 1 µm, eosinophils do not migrate to circumference of fungus or bind to fungus. Arrows — fungal hyphae.

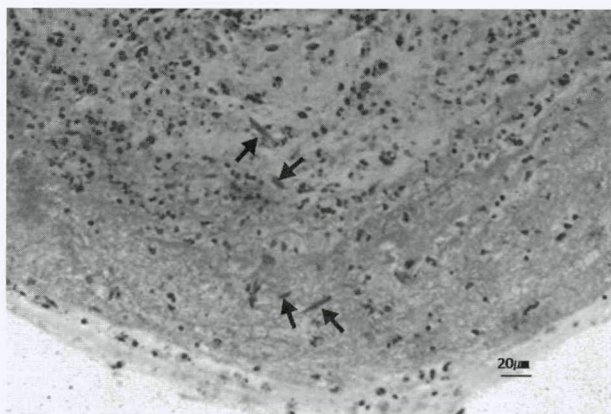


Fig 3. Charcot-Leyden crystals (arrows) in middle ear effusions.

ventilation tube became occluded immediately, and the symptoms could not be controlled by the trans-tympanic ventilation tube. The number of CLCs per unit area was 0.6/mm² in the cases that were poorly controlled despite insertion of a ventilation tube, and 4.57/mm² in those that were adequately controlled after insertion of a ventilation tube. A statistically significant difference was found between the two groups (Student's *t*-test, *p* < 0.01) although the statistical processing may have been improper, because the number of patients was low. No significant correlation could be recognized between the number of eosinophils and the number of CLCs, or between the number of eosinophils and the number of hyphae in the middle ear effusion (Pearson's correlation coefficient). There was no deterioration of hearing during the clinical course in any of the patients. The rate of detection of fungal hyphae was 100% in the nasal smears; however, CLCs were observed in the nasal secretions of only 1 of the 7 patients (see Table).

OME. The 7 patients with serous effusions were 18 to 75 years of age, and included no children. One of the 5 patients with mucous effusions was 51 years of age, whereas the other 4 were children 6 years of age or younger. The rate of detection of fungal hyphae in methenamine silver-stained smear preparations was 100% for both serous and mucous effusions. The types of detected fungal hyphae were almost the same as those in patients with eosinophilic otitis media. However, no CLCs were detected in any of the patients. Observation of methenamine silver-stained nasal smear preparations yielded a rate of detection of fungal hyphae of 100%. Neither eosinophils nor CLCs were observed in the nasal secretions of patients with OME.

DISCUSSION

Culture of the middle ear effusions of patients with

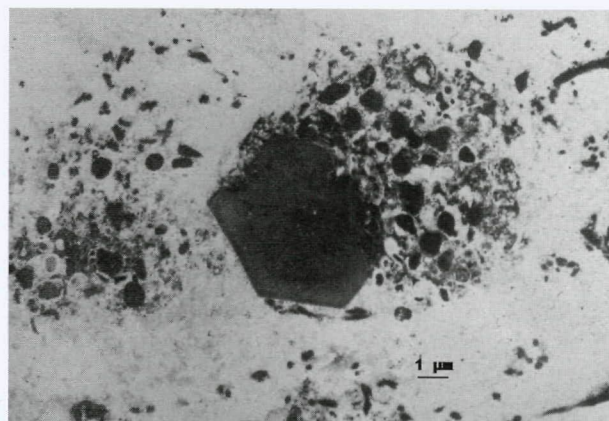


Fig 4. Charcot-Leyden crystals within disrupted eosinophil. Most eosinophils in effusions of patients with eosinophilic otitis media have become necrotic. Hexagonal columnar crystal is on verge of being released from disrupted eosinophil.

OME rarely leads to isolation of bacteria. However, as endotoxins are often detected in the effusions, previous infection with gram-negative *H influenzae* is suspected. On the other hand, the possibility of infection with pathogenic microorganisms other than bacteria cannot be ruled out. Fungi have drawn attention as microorganisms that can cause chronic sinusitis. Kim et al⁵ reported that the presence of fungi in middle ear effusions could not be demonstrated by culture, whereas fungal DNA was detected in 34% of the effusions by PCR. The isolation of fungi by PCR in the nasal secretions of patients with chronic sinusitis and normal subjects has also been reported.⁷ In that study, fungal DNA was detected in the nasal secretions of an estimated 42% of patients with sinusitis and 40% of normal subjects. Because fungi were isolated in the nasal secretions of nearly all patients with chronic sinusitis and normal subjects, according to Ponikau et al,⁴ the finding of a 34% rate of detection of fungal DNA in the middle ear effusions by PCR suggests that fungi might have been present in almost 100% of middle ear effusions, as well, if the method described by Ponikau et al⁴ was used. This corresponds to our isolation of fungi at a rate of 100% by histologic methods. The middle ear cavities in which the fungi were isolated in this study were pathologic cavities with effusions. It is unknown whether fungi can be isolated in normal middle ears without middle ear lesions. In normal middle ears, sampling of materials for examination is difficult because of the lack of fluid accumulation. If the precipitates of the solution used to wash the middle ear were examined, even though the myringotomy and irrigation of the middle ear cavity in healthy persons may not be permitted on ethical grounds, fungi residing in normal middle ears might be isolated. However, since fun-

gal hyphae were detected in 100% of the effusions of typical patients with OME, the possibility of the fungi being the cause of OME cannot be excluded. Passage of fungi into the middle ear may occur through the eustachian tube, in view of the fact that all collected middle ear effusions were from patients with no tympanic membrane perforation. The possibility of contamination at the time of collection of the samples is slight, because the myringotomy was performed carefully, with aseptic precautions taken after washing the external ear canal. The fungi might have been carried via air or mucus flow from the nasopharynx into the middle ear cavity, owing to the relationship between the middle ear pressure and atmospheric pressure. The finding of fungi in the nasal cavity in all cases suggests that they are probably also frequently present in the nasopharynx.

Eosinophilic otitis media was first proposed as a new disease entity in 1995 by Matsutani et al.⁸ Shambaugh and Glasscock⁹ described middle ear inflammation associated with allergic disease as "allergic otitis media" in their textbook, documenting the presence of an extremely viscous mucoid effusion in the middle ear containing eosinophils. However, Matsutani et al.⁸ called this condition "eosinophilic otitis media," and not "allergic otitis media," because almost all of their patients had nonatopic asthma. Nagamine et al.⁶ advocated as diagnostic criteria for eosinophilic otitis media 1) the presence of yellow and extremely viscous middle ear effusion, predominantly containing eosinophils; and 2) precedent and associated adult bronchial asthma. In our study, 6 of the 7 cases of eosinophilic otitis media were RAST-negative. Adult asthma patients in crisis are usually RAST-negative. Therefore, it is suggested that the eosinophilic otitis media in our study was a nonallergic disease, as pointed out by Matsutani et al.⁸ It is considered that steroid hormones did not affect the results of the RAST in this study, as they had not been administered during at least 6 months prior to the collection of the middle ear effusion fluids.

Ponikau et al.⁴ wrote that fungi in nasal secretions induce the migration of eosinophils in the secretions, resulting in eosinophilic inflammation of the paranasal sinuses, and they proposed to call this disease entity "eosinophilic fungal rhinosinusitis." If this were correct, fungi passing into the middle ear cavity would cause eosinophilic inflammation in the middle ear. However, eosinophils do not migrate into middle ear effusions in typical patients with OME, despite the presence of fungi. The suggestion by Ponikau et al.⁴ that fungi induce migration of eosinophils is attractive, but it cannot explain why the fungi in OME do not induce migration of eosinophils.

As eosinophilic otitis media is a very rare disease as compared to OME, the induction of eosinophils by fungi must be rare. Additional evidence is required to support the hypothesis that fungi may play roles as microorganisms in the development of OME or eosinophilic otitis media. In this study, we could not observe, in either OME or eosinophilic otitis media, eosinophils or neutrophils migrating to the circumference of the fungi and binding to them, as has been reported in nasal secretions.¹⁰ Whether fungi have pathogenicity or cause immunologic reactivity in the middle ear has not yet been definitively determined. Just as it is known that resident bacteria may suddenly begin to exhibit pathogenicity, it is possible that resident fungi may also suddenly begin to exhibit pathogenicity or cause immune reactivity.

The characteristics of eosinophilic otitis media include viscous middle ear effusion that predominantly contains degenerated eosinophils. The key factor in imparting the viscosity may be the degenerated eosinophils, which are not usually observed in OME. It has been reported that the viscoelasticity of the purulent secretions in cystic fibrosis is primarily due to the high concentrations of DNA¹¹ and actin released from the large number of necrotic neutrophils accumulated in the airways. Therefore, it is possible that viscous ear effusion is caused by the released DNA from degenerated eosinophils. However, the nasal mucus does not exhibit high viscosity in patients with nasal house dust allergy, in spite of the large number of disrupted eosinophils that are observed.¹² It may be difficult to definitively conclude that the viscous ear effusion may be caused only by DNA from degenerated eosinophils. Further research is needed to clarify the role of degenerating eosinophils in the viscoelastic characteristics of mucus in cases of eosinophilic otitis media.

Charcot-Leyden crystals are hexagonal columnar crystals with pointed edges that are frequently observed at sites of eosinophilic inflammation. There have been reports of detection of these crystals in cases of allergic fungal sinusitis,^{13,14} Kimura's disease,¹⁵ and asthma.¹⁶ Charcot-Leyden crystals are produced from proteins with a molecular weight of 17.4 kd that have lysophospholipase activity.¹⁷ Charcot-Leyden crystal proteins are present in large amounts in nuclear euchromatin and cytoplasm. Charcot-Leyden crystals are believed to crystallize gradually in the cytoplasm of eosinophils.¹⁸ In this study, CLCs being released directly from disrupted eosinophils could be observed by electron microscopy. It can therefore be supported that the CLCs observed in middle ear effusions were released directly from disrupted eosinophils. The pathologic

role of CLCs is still unknown, although it has been reported that CLC proteins may be involved in pulmonary inflammation¹⁹ or exhibit protective effects against parasites¹⁷ in mice. Investigation of whether CLC proteins may also have a protective effect against fungal infection would be a matter of interest, because these crystals were also found in cases

of fungal infection. We found CLCs in significantly greater abundance in cases in which good control of symptoms had been obtained by the use of a ventilation tube than in poorly controlled cases. Therefore, if CLCs are abundant, the placement of a trans-tympanic ventilation tube will be the therapy of first choice for eosinophilic otitis media.

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