Pathogenesis of Attic Cholesteatoma

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Abstract

In this paper, the clinical and basic studies were designed to understand the pathogenesis of attic cholesteatoma. Among 101 ears of 51 patients who had been treated at our OME outpatient clinic, the incidence of retraction pocket (RP) was 42.6% in OME group, which was statistically significantly higher than that of control (2.2%). All but one ear showed type A or C1 tympanogram, indicating normalized mesotympanum and CT scan revealed the clear attico-antrum. These findings indicated that RP persisting or developing after the resolution of OME was not caused by the disorder of surrounding structure but rather by the pars flaccida itself.

The study of the epithelial cell kinetics in the pars flaccida of the tympanic membrane revealed that epithelial cells in the pars flaccida was smallest in shape and strongest in its cell proliferation activity in the entire tympanic membrane. This observation indicated that the pars flaccida bears very active generation of epithelial cells, which is likely to produce RP under some pathologic circumstances. As a matter of fact, immunohistochemical studies demonstrated that ICAM-1, EGF receptor, IL-6 receptors, and the increased density of Ki-67 antigen were present in the epidermis of inflamed cholesteatoma. Furthermore, high concentration of IL-1 and IL-6 were measured in cholesteatoma debris by ELISA. These findings indicated that cytokines were likely to play a role in the proliferation of epidermis of the pars flaccida to form cholesteatoma.

Key words: Cholesteatoma, Cytokine, Otitis media with effusion, Ear drum, Retraction pocket

Introduction

Attic retraction pockets (RP) were frequently observed among patients with otitis media with effusion (OME) and occasionally persisted even after a complete resolution of OME. As suggested by Tos et al., such ears bear a potential risk for development of attic cholesteatoma and we experienced five cases of cholesteatoma during the follow-up periods of more than 1000 OME patients in our outpatient clinic. In this paper, clinical and basic studies were designed to understand pathogenesis of RP and cholesteatoma. Special attentions were paid to how much the negative middle ear pressure is responsible for RP, how frequently RP developed in the ears with preceded episode of OME, how frequently RP transited to cholesteatoma, and how much the blockade of tympanic isthmus is responsible for the formation of cholesteatoma. Based on the obtained clinical data, the characteristic feature of epithelia in pars flaccida was studied in Mongolian gerbil and the detailed inflammatory process was examined in human cholesteatoma specimens at the molecular level to understand the pathogenesis of RP and of cholesteatoma.

Materials and Methods

1. Clinical study of RP and cholesteatoma

First, an effect of a tympanostomy tube insertion was examined on the resolution of retraction of pars tensa and pars flaccida to know how much the negative middle ear pressure is responsible for the formation of RP. Twenty-five ears, which could be followed more than 10 years, were served for this study. The retraction of pars tensa and of pars flaccida was evaluated according to the classification of Sade and Tos, respectively, and they were followed periodically before and during
the intubation and after the extubation.

Second, 101 ears of 51 patients older than 15 years and with an episode of OME which was treated at our OME outpatient clinic were examined by otoscopy to understand the incidence of RP after the resolution of OME. In cases that RP was found, their middle ear condition was characterized by a eustachian tube function test and by tympanometry. The average age was 16.2 years ranging from 15 to 20 years. Forty-six ears of 23 age-matched patients without any history of OME were served as control.

Third, patency of tympanic isthmus and aeration of attico-antrum were evaluated by computerized tomography (CT) in a series of 53 ears with RP of Tos grade 2 or deeper, including 28 RP and 25 cholesteatomas.

2. Basic study of pars flaccida epithelium of Mongolian gerbil and of human cholesteatoma.

The epithelial cell kinetics in pars flaccida of ear drums were examined in six Mongolian gerbils. Mongolian gerbils were chosen for this experiment because their pars flaccida was quite similar to that of human tympanic membrane and had potential to develop spontaneous cholesteatoma. The shape and surface area of epithelial cells of ear drum were traced using the camera lucida equipment after silver staining. Cell proliferation in epithelial layer of ear drum was monitored using 5-bromo-2'-deoxyuridine (BrdU) incorporated into cellular DNA to detect S-phase cells.

Cholesteatoma epithelium and keratin debris were obtained from 12 patients with cholesteatoma either in an inflamed condition or in a static condition. Normal external ear canal skin and impacted cerumen were also obtained for a control study. Cholesteatoma epithelium with underlying granulation tissue were processed for the immunohistochemical analysis of IL-6 receptor, EGF receptor, ICAM-1, T cell marker (CD43), and Ki-67 nuclear antigen, which is present in proliferating cells. The keratin debris were isolated from epithelium and granulation tissue, homogenized in phosphate buffered saline, and centrifuged. The contents of IL-1α, IL-1β, IL-6, TGF-α were measured from the obtained supernatant by ELISA.

Results

While most of the retraction of pars tensa were normalized after the tympanostomy tube insertion, the effect of tympanostomy tube insertion on the retraction of pars flaccida varied with cases. RP of Tos grade 1 normalized even after the extubation in 83.3%, however, this ratio became worsened as the Tos’s grading, like 70% in RP of Tos grade 2 and 33.3% in RP of Tos grade 3. Several cases showed that their RP became deeper after the extubation, leaving pars tensa intact.

The incidence of RP was 42.6% in patients older than 15 years and with an episode of OME, which was statistically significantly higher than that of control (2.2%). All ears with RP showed type A or C tympanogram except for one ear, and nearly half of the RP cases showed positive for sonotubometry. Cholesteatoma was developed from RP in only 5 cases with a relatively long latent period ranging from 2 to 11 years and received surgery. The tympanostomy tube insertion could not prevent the transit process from RP to cholesteatoma. While the CT scan revealed 92.9% of RP cases showed clear attico-antrum, the lack of aeration of the attic was demonstrated in 60% of the cholesteatoma. Good patency of the aditus and a pneumatized antrum were, however, observed in early stages of most cases of cholesteatoma.

Pars flaccida of Mongolian gerbil was covered with the many-layered epithelium in series with the epithelium of external ear canal. Silver staining revealed that the surface area of epithelial cells was the smallest in pars flaccida and that they gradually increased their size in postero-superior quadrant and in antero-superior quadrant. The distribution of BrdU-positive cells were the densest in pars flaccida and sparse in postero-superior quadrant, along the handle of malleus, and at umbo.

IL-1α was found in keratin debris at the relatively high concentration of 7.28pg/mg in average, which is statistically significantly higher than that of cerumen, horny tissue from normal and psoriasis skins. While the amount of IL-1β was very low in cholesteatoma debris, compared to that of horny tissue from skin (Fig. 1). The contents of IL-6 and TGF-α in cholesteatoma debris differed greatly with cases, so that there was no
statistical difference between any group, however, keratin debris obtained from the inflamed cholesteatoma contained high level of IL-6 and TGF-α.

![Graph showing IL-1α and IL-1β levels](image)

**Fig. 1** The contents of IL-1α and IL-1β in cholesteatoma debris, cerumen, and horny tissue from normal and psoriasis skin.

Immunohistochemical studies demonstrated that ICAM-1 was expressed 100% on the epidermis of inflamed cholesteatoma, and trafficking of T cells was also observed in 75% of ICAM-1 expressing epidermis. While only half of static cholesteatoma showed ICAM-1, any T cells were observed in such epithelium. Immunohistochemical study also demonstrated IL-6 receptors in the inflamed cholesteatoma epithelium, however, they were negligible in the static cholesteatoma and in the normal external ear canal skin. EGF receptors were observed on the basal layer of normal skin and were distributed throughout the epidermis of the inflamed cholesteatoma. The mitotic figures were hardly seen in cholesteatoma epithelium, however, increased density of Ki-67 antigen was found in the inflamed cholesteatoma matrix compared to that of normal ear canal skin and of static cholesteatoma. Ki-67 score, quotient of Ki-67 positive cells and total cells, was 4.15% in normal meatal skin and 14.6% in cholesteatoma epithelium.

**Discussion**

Present clinical observations revealed that the retraction of both pars tensa and pars flaccida initially resulted from the negative middle ear pressure, however, other factors may be involved when the RP became deeper, since this process took place independent from the condition of pars tensa in ears with normal tympanogram and normal eustachian tube function. Our CT findings of the cases of RP and cholesteatoma revealed the patent tympanic isthmus in most of the cases, as shown before and did not support the hypothesis that a blockade of tympanic isthmus causes isolation of the attic from the adjacent middle ear spaces resulted in negative pressure in these spaces, leading to a formation of RP and eventually cholesteatoma. These findings indicated that RP was not caused by the disorder of surrounding structure but rather by the pars flaccida itself.

The pathogenesis of attic cholesteatoma is considered to be a migration of tympanic membrane of pars flaccida into attic-antrum. The cell kinetics of pars flaccida has not been well documented, however, present study clarified that they are very active. The epithelial cells in pars flaccida were the smallest in shape and the largest in its cell proliferation activity in the entire tympanic membrane. This observation indicated that pars flaccida bears very active generation of epithelial cells, which is likely to produce RP or cholesteatoma under some pathologic circumstances.

The distinct pathological difference between the retraction pocket and attic cholesteatoma is absence or presence of accumulated keratin debris. However, the information about the biological activity of the keratin debris are very limited. Previous study reported that it contains endotoxin, EGF, macrophage chemotactic activity, and stimulatory effect on macrophages. Present study revealed that cholesteatoma debris are also rich in IL-1α, IL-6, and TGF-α. Each of them are known to induce hyperproliferation of keratinocytes.

Fig. 2 summarized the cytokine network related to keratinocytes in normal and in inflamed skin. Circled ones were already proved in the cholesteatoma epidermis. It is well known fact that the epidermis contains biologically active IL-1α at rest. As long as the epidermis is intact, IL-1α is eliminated by normal desquamation. Once an inflammation take place, however, IL-1α is not only released from keratinocytes, but also soaked from keratin to exudates, since IL-1α is water soluble. Released IL-1α together with...
endotoxine if present, manifested their complicated biological activities on keratinocytes and subepithelial granulation tissues.

The very initial change of keratinocytes was known to be the expression of ICAM-1. The present study demonstrated that the inflamed cholesteatoma epidermis expressed ICAM-1 and contacted with T cells in the epithelial layer. Such an interaction between T cells and keratinocytes has also been observed in other skin diseases and is thought to be crucial in altering keratinocytes to produce cytokines and in up-regulating the expression of receptors.

The cholesteatoma matrix is known to produce IL-1 and other cytokines, as we pointed out their importance in bone absorption by cholesteatoma. Moreover, EGF receptor and IL-6 receptor, which were normally negligible in ear drum and in ear canal skin, showed distinct localization in the inflamed cholesteatoma matrix. Several cytokines, such as IL-1, IL-6, and TGF-α, were recently reported to play an important role in an epithelial proliferation. Hyperproduction of these cytokines from inflammatory cells and keratinocyte itself and hyperexpression of receptors for these cytokines on keratinocytes have been observed in the lesion of skin disease characterized by hyperproliferation such as lichen planus, psoriasis, and so on. Since similar findings were observed in cholesteatoma, one can speculate that these cytokines were also important in the proliferation of epidermis of pars flaccida to form cholesteatoma. As a matter of fact, immunohistochemical study demonstrated high Ki-67 scores in cholesteatoma epithelium. Mitotic figures were hardly seen in cholesteatoma epithelium, however, high Ki-67 score indicated active cell proliferation took place in inflamed cholesteatoma epithelium.

Judging from preceding results, our concept on the pathogenesis of cholesteatoma is schematically summarized in the Fig. 3. Retraction pocket is initially formed by the negative middle ear pressure. The pathologic change in pars tensa normalized as the resolution of otitis media with effusion, but retraction pocket persisted probably due to the mild chronic inflammation in Prussak’s space. As far as the retraction pocket is free from keratin debris or as far as a clinician can remove the debris from retraction pocket, pars flaccida retracted but cause any trouble. However, once the keratin debris accumulates in retraction pocket and gets infected, IL-1α probably with endotoxine induce the alteration of cytological feature of retraction pocket to produce several cytokines and to express receptors, which causes epithelial proliferation and eventually bone destruction, two characteristic pathological feature of cholesteatoma.
Atelectasis and RP due to negative middle ear pressure

chronic inflammation in Prussak's space causes persistent RP

cytokines (IL-1β, IL-6, TGFβ) induced epithelial hyperproliferation

cytokines (IL-1α, TNFα) induced middle ear destruction

Fig. 3 Our concept of pathogenesis of transition from retraction pocket to attic cholesteatoma.

References


