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Morphophysiology of the Eustachian Tube Related to Otitis Media

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Pathogenesis of otitis media is not clearly understood, and not much attention has been paid to it even though otits media is one of the most common diseases in otolaryngological field. There is abundant epidemiological and clinical evidence in support of the idea that eustachian tube problems are important predisposing factors in the pathogenesis of otitis media. In this paper, I would like to review functional morphology of the eustachian tube related to otitis media. This reveiw will provide a morphological basis for future eustachian tube and middle ear research concerning the pathogenesis of otitis media. (Ajou Med J 1998; 3(2): 96~105)

Key Words: *Eustachian tube, Otitis media, Functional morphology*

INTRODUCTION

A special interest has been focused in recent years on the mucosal biology of the eustachian tube that is related to the protection of the middle ear from infection and the formation of middle ear effusions. Because of the importance of otitis media, the morphology and function of the eustachian tube and middle ear lining have been intensively investigated. Numerous laboratory and clinical data support the notion that local defense mechanisms, in addition to systemic immunity, protect the tubotympanum from invading organisms. The eustachian tube is protected by the mucosal defense system common to all mucous membranes which are exposed to the external environment. The mucosal defense system comprises: (1) mechanical defense (e.g., mucociliary transportation system that contains mucous blanket, ciliated cells, and secretory cells); (2) biologic defense (e.g., antibacterial enzyme secretion); (3) immunodefense (e.g., humoral and cellular immune system); and (4) phagocytosis (polymorphonuclear leukocytes, macrophages). The entire length of the eustachian tube and middle ear mucosa near the tubal opening are provided with a mucociliary as well as a secretory defense system. It is important to understand that when any one of these systems is compromised, the person becomes predisposed to otitis media. The purpose of this article is to review the available histological, immunohistochemical, and ultrastructural data of eustachian tube to provide a broad morphological and cellular basis for understanding the pathogenesis of otitis media.

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FUNCTIONAL MORPHOLOGY OF THE EUSTACHIAN TUBE

In adult humans, a cartilaginous portion distinct from the bony portion can be seen, but in young infants (and in some adults) the cartilage often extends to the bony portion, making it difficult to delineate the two. In laboratory animals, particularly rodents and felines, the tubal cartilage extends directly to the tympanum. In general, these two parts are joined by a narrowing known as the tubal isthmus in humans and primates. The diameter of this isthmus is smaller in children(about 2.4 mm $\times$ 0.8 mm) than in adults (about 4.3 mm $\times$ 1.7 mm) and also varies from an individual to an individual.

The cartilaginous portion of the tube on cross-section

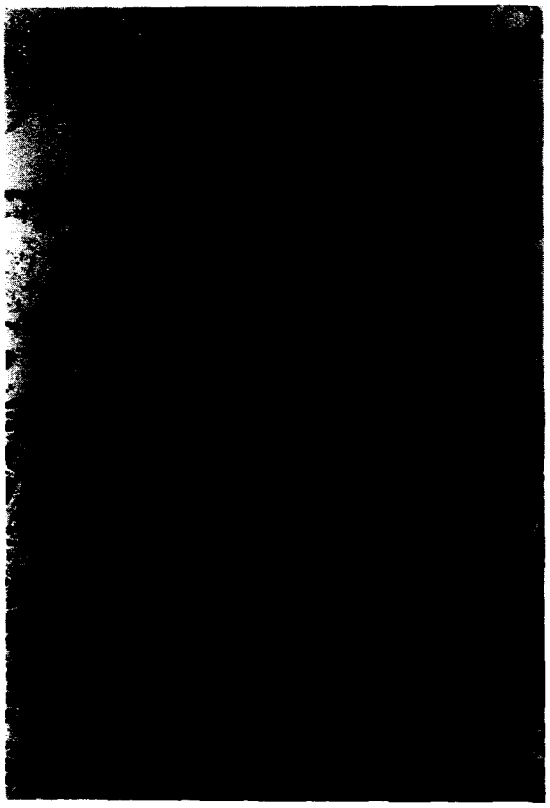

Fig. 1. Cross section of the eustachian tube in the rabbit shows slit-like lumen and shephard's crook-like carilage. The epithelial cells of the tube are composed of typical respiratory epithelium, pseudostratified columnar epithelium with interpersed goblet cells (H&E stain, original X40).

Fig. 2. Transmission electron micrograph of the mucosa lining of the eustachian tube shows epithelial cells and secretory cells which are classified into dark granulated cells (D), light granulated cells (L), and mixed cells (M) (X1,650). Bar=1.0 μm.

shows a slit-like lumen that is partially reinforced by cartilage that resembles a shephard's crook (Fig. 1). The lower portion of the main body of the cartilage becomes thicker toward the pharyngeal orifice. The epithelial cells of this portion of the tube are formed mainly of pseudostratified tall columnar ciliated epithelium with secretory, nonsecretory, and basal cells. Occasionally, a simple squamous mucosal epithelium lines the lumen, particularly near the pharyngeal orifice and the upper part of the tubal lumen. The eustachian tubal epithelium is composed of pseudostratified columnar cells; interspersed among these cells are goblet cells and the underlying basal cells (Fig. 1).

The secretory cells are classified according to their morphological basis: light granulated cells, dark granulated cells, and mixed granulated cells (Fig. 2). Futher autoradiographic studies indicate that the light granulated cells are mucous, because radioisotope-labeled glucose is taken up largely by these cells, whereas the dark granulated cells are protein-secreting cells (serous), because they incorporate isotope-labeled amino acid leucine.[1,2] The subepithelial connective tissue contains blood vessels, lymph capillaries, myelinated nerve fibers, fibrocytes, occasionally mast cells, macrophages, and plasma cells.

The mucus-propelling cilia of the tube (columnar cells) are approximately 4 to 7 μm, whereas the cilia in the middle ear (often cuboidal or squamous type) are much shorter: 3 to 4 μm. Each ciliated cell in the tympanum possesses 40 to 60 cilia, whereas in the tubal lumen each can possess up to 250. The shape of the ciliated cells varies from tall columnar to cuboidal, or even squamous. The densities of the ciliated cell populations vary considerably. For example, in the human tubal lumen they are over 80%; in the hypotympanum and near the tubal orifice of the tympanum between 51% and 80%; in the promontory and epitympanum, 11% to 60%; in the aditus, 1% to 19%; and in the mastoid, under 1%.[3] In the hypotympanum, a major track formed of groups of ciliated cells is connected with the tubal lumen. A second track is formed by ciliated-cell clusters along the epitympanic recess and peripheral area of the tympanic membrane (pars tensa) which connects to the tubal opening area.[4] It has been found that secretory cells always accompany the ciliated cells, even those that form an isolated island.[5]

The cartilaginous part of the tube contains numerous

seromucinous glands, and the serous acini are termed demilunes because of their half-moon shape caused by compression by round mucous glands. These mixed glands open to the tubal lumen through secretory ducts. Secreta pours out as viscous mucous droplets or column which subsequently forms a thin mucous blanket.

There are two main tubal muscles: tensor veli palatini (TVP) muscle and levator veli palatini (LVP) muscle. The TVP muscle is attached to the hook end of the tubal cartilage, thus, its contraction helps open the tubal lumen. The contraction of the LVP muscle is thought to elevate the lower luminar portion of the tube, thus, the lower edge of the median side of the tubal cartilage is pushed upward, helping the opening of the tube in concert with the outward pulling motion of the TVP muscle action. These muscles are thought to be poorly developed in young children. The closing of the tubal lumen is provided by the relative stiffness of the tubal cartilage. In young children, this tubal cartilage is floppy because of its poor development, causing collapse of the tube.[6] It is well established that the tubal anatomy undergoes drastic changes during infancy and early childhood and that the tubal cartilage attains its adult form by age 8 or 9, when the incidence of otitis media decreases dramatically.

In considering the tubal function, several factors are important: a) tubal muscles (TVP, LVP) which help open or close the tube; b) tubal cartilage which provides the stiffness required to prevent collapse of the tubal lumen; c) mucociliary system of the tubal mucosa which is required to prevent the entrance of bacteria and to eliminate unwanted debris or secreta from the tympanum; d) secretory products which provide bacteriostatic of bactericidal substances such as lysozyme and lactoferrin and provide a surface-active substance to make tubal opening easier; and e) mucosal immunocytes in the tube, particularly near the pharyngeal orifice, that help protect the tubotympanum. The presence of immunocytes in the tubal connective tissue is well documented, and their numbers increase following middle ear infection.

MUCOCILIARY TRANSPORTING SYSTEM

The function of a mucociliary system is directly related to the presence of ciliated and secretory cells, and the cilia of the ciliated cells must move in metachronal fashion in order to move the mucus blanket, indicating that the series of cilia move in sequence. The mucous blanket is suggested to be a nonhomogeneous mixture of different types of secretion (mucous and serous) forming a viscoelastic hydrophilic non-Newtonian gel.[7] However, it has also been suggested that the mucous blanket (viscoelastic layer) is formed largely of secreted mucus, whereas the serous fluid (low viscosity layer) bathes the periciliary space, allowing free ciliary motion.[8,9] Regulation of mucociliary transport depends on: (a) variations in the fluidic load produced by mucus on the cilia, (b) variations in the energy output of the ciliary engine, and (c) variations in the energy transfer from ciliary movement to mucus flow.[7]

Because mucociliary coupling has a significant bearing on transport efficiency, the length of the cilium that penetrates the mucus and the thickness of the periciliary layer could have a "clutching" effect on regulating the degree of mechanical coupling between the mucus and the cilia during their active stroke.[10] The tips of the cilia are known to penetrate about 0.5 μm into the mucous layer of the blanket during their effective stroke[11] so that the mucus is propelled forward. The recovery strokes, however, take place in the periciliary fluid. If the depth of the periciliary serous layer increases so that the tips of the cilia no longer hold (penetrate) the mucous layer, the mucus will not be propelled until excess fluid is removed. If the periciliary layer becomes too shallow by loss of fluid or diminished secretion, the mucus propulsion will stop because the cilia are pressed down and can no longer properly complete their beat cycle.[11] This concept emphasizes the importance of the secretion of serous as well as mucous fluid. In this regard, it is important to note that even in the epithelial secretory cells of the tubotympanum there is a morphological as well as a biochemical heterogeneity.[12,13] In order to have a functioning mucociliary system, the serous fluid must be produced locally. A functioning mucociliary transporting system is known to exist in certain parts of the lining epithelium of the middle ear as well as in the bony tube where no mixed glands are present. By the use of lectin-gold cytochemistry, it has recently been demonstrated that the epithelial dark granulated secretory cells produce serum-type glycoprotein in the bony part of the chinchilla eustachian tube and the middle ear cavity, and that light (mucous) granules of the glandular mucous cells and

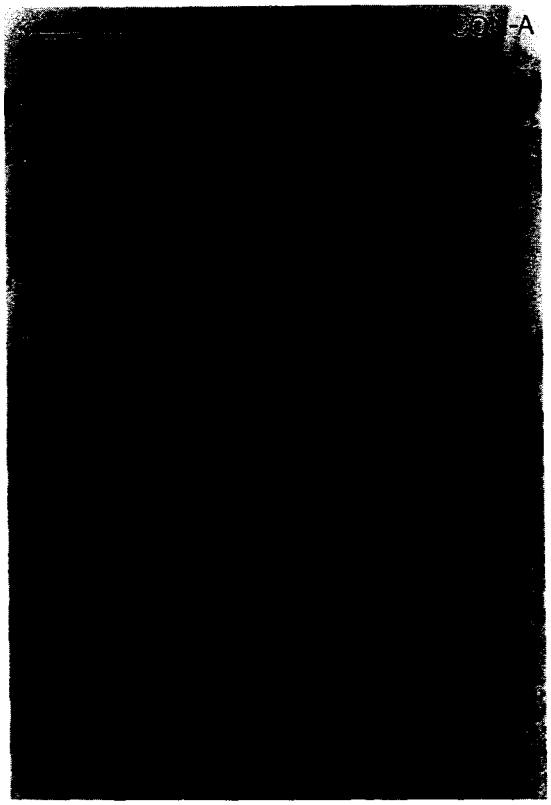

Fig. 3. Transmission electron micrograph of epithelial goblet cells of the chinchilla eustachian tube shows positive-labeled ConA specifically in dark core (D). Peripheral light portions are not labeled (arrow heads) (X16,000). Bar=1.0 μm.

goblet cells in the chinchilla tubotympanum have serum-type glycoprotein in the dark cores (Fig. 3).[14] These results suggest that the production of serum-type glycoprotein in the epithelial cells is necessary to support low-viscosity periciliary fluid in the absence of the mixed glands that produce serum as well as mucin-type glycoproteins in these regions.

There is an unequivocal evidence that nonfunctioning or disrupted cilia of the tubotympanum lead to otitis media, because patients with immotile cilia syndrome develop recurrent or chronic otitis media and maxillary sinusitis without exception. Recent evidence suggests that influenza A virus causes destruction of the ciliated epithelium of the eustachian tube in the early stage of infection, leading to a secondary bacterial invasion.[15]

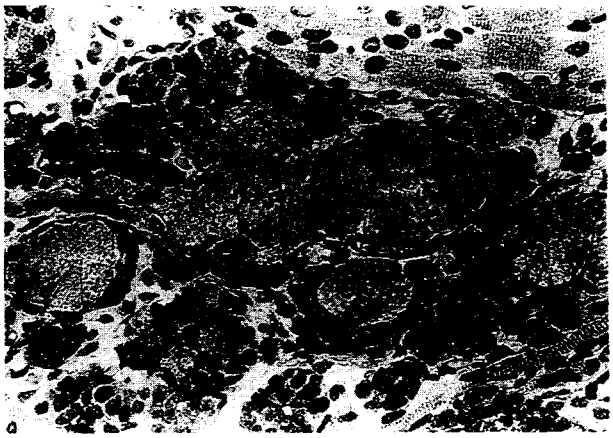

Fig. 4. Only serous part of tubal glands are positively stained with antibody to lysozyme (ABC immunostaining plus hematoxylin, original X200).

TUBAL SECRETIONS AND THEIR BIOLOGICAL FUNCTIONS

Antibacterial enzyme activity

One of the mucosal surface defense mechanics is an antibacterial secretory enzyme activity which has been demonstrated in the middle ear and eustachian tube.[16] It was demonstrated that the antibacterial secretory enzymes, lysozyme and lactoferrin, are secreted by the middle ear mucosa and eustachian tube of the normal chinchilla.[13] In the middle ear mucosa, lysozyme was produced largely by the goblet cells and localized mainly in the columnar epithelium area of the transitional zone, and lactoferrin was produced by the nonmucous secretory cells (dark granulated cells that are AB-PAS negative) and localized mainly in the cuboidal epithelium area of the transitional zone. In the eustachian tube, lysozyme was produced primarily by the epithelial goblet cells and mucous cells of the subepithelial glands, while lactoferrin was secreted primarily by the serous cells of the glands. This finding is consistent with an another observation[17] which demonstrated lactoferrin in glandular acinar cells (negative for AB-PAS staining) of the eustachian tube of the normal guinea pig. In contrast to the above observations, it was found that both lysozyme and lactoferrin were localized in the serous cells of the subepithelial glands in mice (Fig. 4).[18] In the human upper airway mucosa, lysozyme was also localized in the serous glands

and serous parts of the mixed glands.[19,20]

Whether these different results are due to species differences or something else can not be concluded. Further study is needed to clarify these important differences.

The proportion of the secretory cells producing these antibacterial agents was much greater in the eustachian tube than in the middle ear mucosa. Because the eustachian tube is the main entry port for microorganisms into the middle ear cavity, it is reasonable to suggest that lysozyme- and lactoferrin-secreting cells are strategically located in the eustachian tube to protect the middle ear from invading organisms. It is also suggested that the extent of the antibacterial enzyme secretion lies in a dynamic state, because antibacterial enzyme secretion is drastically increased following middle ear infection.

Surface-active substance of the eustachian tube

Aside from the secretion for the mucociliary blanket and enzyme activity, the tubal secretion provides also surface-active substance (SAS) to reduce the surface tension of the tubal secretion and to facilitate tubal opening. This idea could be criticized by the fact that the diameter of the eustachian tube is too large and much larger than that of the alveoli. However, the normal eustachian tube is thought to be closed for the most of time, and the diameter is very small when closed. Therefore, it is logical to assume that the tube and middle ear mucosa are covered by SAS to facilitate efficient tubal function.

Although there is good evidence to suggest that SAS is present in the tubal lumen to facilitate tubal opening (Fig. 5), their biochemical characterization remains controversial. Many investigators likened the tubal SAS to the pulmonary surfactant.[21~24] Using rat eustachian tube in vitro preparations and radioisotope-labeled choline, the synthesis of phosphatidyl choline (PC) was investigated.[25] It was found that the eustachian tube and lung synthesized PC significantly more than the control liver tissue of equal weight, suggesting that the tube may secrete a surfactant-like substance biochemically analogous to that of the lung. However, it was demonstrated that the surface activity of the tubal washings resides primarily in the aqueous phase (glycoprotein or mucoprotein) rather than the lipid phase.[26] In recent chinchilla study, it was confirmed that the biochemical content of tubal SAS is similar but not identical to that of pulmonary surfactants.[27]

Fig. 5. Autoradiogram 1 hour after injection of radioactive isotope (3H-palmitic acid) reveals black silver grain in secretory cells and lumen of the epithelium (H&E stain, original X1000).

Although an earlier cytochemical study[28] showed a secretory product and dark granules (of the serous cells) which reacted with tricomplex flocculation to suggest a possible presence of phospholipids, secretory granules resembling the lamellar secretory granules of the alveolar type II secretory cells that produce surfactant (lecithin) have not always been observed in the tubotympanum. Whether these different results are due to species differences or to methodological differences can not be answered. Further study is in need to clarify this important question.

It has been suggested that SAS plays a role for facilitating phagocytosis by macrophages of micro-organisms in the tubotympanum as well as in the lungs. An other possible action which has been suggested for the lungs is that SAS may improve mucociliary transport by improving the sliding of the gel layer over the sol layer. Still another theory is that SAS prevents effusion of serum proteins into the tubotympanum, especially under negative air pressure.

IMMUNODEFENSE SYSTEM

There is also good clinical,[29~33] immunohistochemical,[34~36] and experimental[37~39] evidence to suggest that the tubotympanum is protected by the local (humoral and cellular) as well as the systemic immune system. In the normal chinchilla tube, the glandular acini cells were positively stained with anti-IgA antibody, and a few IgA-

forming immunocytes were observed in the tubal connective tissue and in the bulla near the tube, but IgM- and IgG-forming immunocytes were rarely observed.[40] However, after an infection with S.pneumoniae (type 3 or 23), there was a dramatic increase in immunocytes in the submucosa in the bulla, the most being the IgG-forming cells followed by IgM- and IgA-forming cells. The increase in immunocytes corresponded well with an increase in immunoglobulins in the middle ear effusions.[38] These findings are consistent with results obtained in middle ear effusions. It was demonstrated that IgG was the major immunoglobulin, followed by IgA and IgM, and they increased with advancing age.[41]

The route of antigenic absorption has also been investigated by the use of a tracer. It was demonstrated that horseradish peroxidase (HRP) instilled into the guinea pig bulla was actively transported via pinocytosis on the epithelial cell surface and into the intercellular space by reverse pinocytosis.[42] The intercellular HRP was then passively transported into the submucosal connective tissue and was taken up by macrophages or by the lymphatics and venules. The tracer then found its way to the regional lymph nodes (deep cervical) within 15 minutes after the tympanal instillation.[42] These macromolecules were found in the macrophages in the lymph nodes. Thus, the regional lymph nodes seem to play an important role in the processing and presenting of the antigen to lymphocytes in the lymph nodes, thereby inducing the immune response to product antibody and evoke cellular immunity to regional antigen.

The role of cellular immunity in protecting the tubotympanum against viral and bacterial agents has not been unequivocally established. However, natural killer cells[43] have been found in large numbers in mucous tissue together with mast cells.[5] This finding suggests that local cellular immunity may be operative in the tubotympanum.

TUBAL FUNCTION AND UPPER RESPIRATORY INFECTION

Although upper respiratory viral infection has been suspected of being associated with the pathogenesis of otitis media, evidence has only recently been found to indicate a close association between respiratory viruses (such as respiratory syncytial virus, influenza A, and adenovirus) and acute otitis media.[44] Furthermore, it was demonstrated that positive secretory viral antibodies (secretory IgA) to respiratory viruses were detected in 24% of the chronic middle ear effusions studied,[45] indicating that these respiratory viruses may have an immediate as well as a long-term effect on the pathogenesis of otitis media.

Experimental evidence supporting the role of influenza A virus in the pathogenesis of acute otitis media was presented by Giebink and his collegues.[46] When the nasopharynges of chinchillas were instilled with Streptococcus pneumoniae, only 13% developed acute otitis media, whereas the incidence of acute otitis media was 67% when the nasopharynges were instilled with S. pneumoniae together with influenza A virus. The follow-up investigation documented that the influenza A virus caused immediate destruction of the tubal mucosal lining (Fig. 6), and the mucosal epithelium of the tube had not

Fig. 6. Transmission electron micrograph of the eustachian tube epithelium 3 days after transbullar viral inoculation shows detached cilium and inflammatory cells in tubal lumen (X3,300). Bar=1.0 μm.

established normal morphologic characteristics on the ultrastructural level even two weeks after viral inoculation of the middle ear.[47] I have demonstrated that histopathologic changes induced by inoculation of influenza A virus correlated well with diminished eustachian tube mucociliary activity and transport function. In this study, the analysis of ciliary beat frequency and dye transport indicated a maximal decrease approximately 7 to 14 days postinoculation, returning to the normal function by 28 days postinoculation (Fig. 7).[48] Therefore, it appears that an individual whose tubotympanum suffers from influenza A infection becomes highly vulnerable to a secondary bacterial infection from the nasopharynx. Although we are not certain whether all respiratory viruses have similar pathologic effects, it is conceivable that the pathogenic respiratory viruses may damage the integrity of the ciliated epithelium.

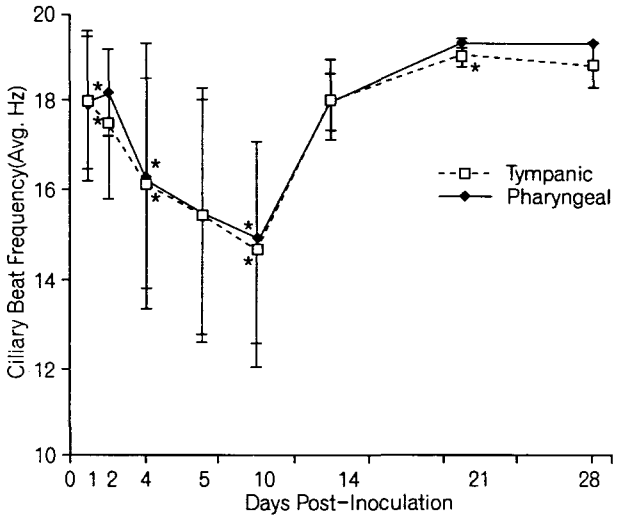

Fig. 7. Ciliary beat frequency of chinchilla eustachian tube epithelium at sites near the tympanic or pharyngeal orifice following transbullar inoculation of influenza a virus. Maximal diminution of ciliary beat frequency occurs in both the tympanic and pharyngeal portions of the eustachian tube 10 days postinoculation.

There is some evidence to show that respiratory viral infection may also enhance bacterial adherence to the host epithelial cell surfaces,[49] thereby increasing the chance of bacterial infection. This enhancement is presumably mediated by the presence of the virus-induced glycoproteins. The possible immunosuppressive effects of the respiratory viruses by various alterations of immunocompetent cell function[50] could also have an influence on the pathogenesis of acute otitis media. Although such an influence has not yet been well documented in otitis media, poor functional states of the middle ear macrophages in chronic middle ear effusions have been described.[51]

FUTURE RESEARCH OF THE EUSTACHIAN TUBE

Culture of the eustachian tube and middle ear epithelial cells

The availability of cultured eustachian tube epithelial cells provides a new avenue for research that is useful in

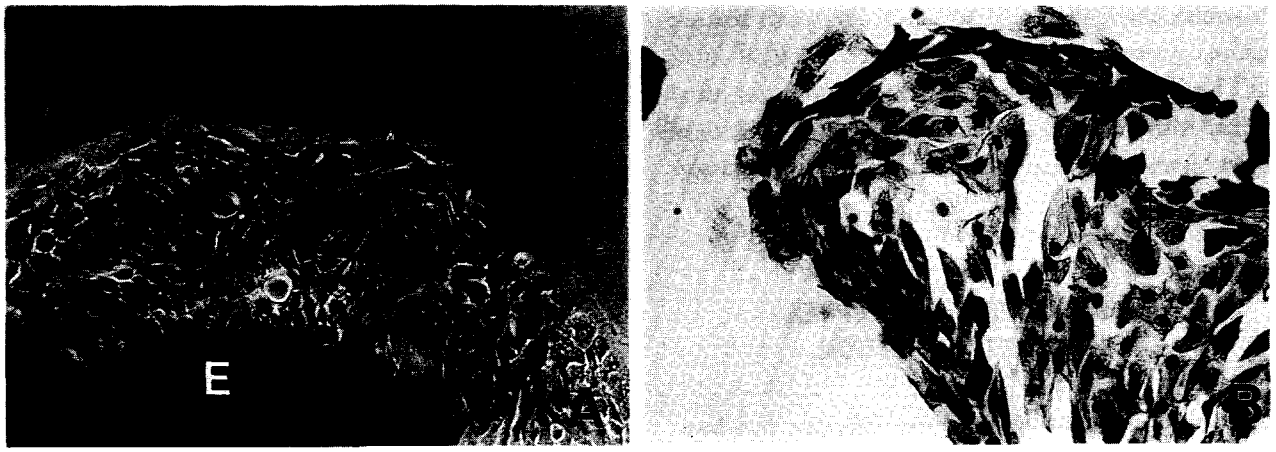

Fig. 8. A) Light micrograph of explant (E) and outgrowth (O) after 7days in culture of the human middle ear epithelium shows elongated migratory ciliated cells (original ×200). B) Cytokeratin immunostaining in cultured epithelial cells shows positive finding (original ×200). (From Moon SK et al. : Korean J Otolaryngol 1998. Reprinted by permission)

characterizing bacterial and viral receptors, hormone receptors, cell-cell interection, cell differentiation, cell transformation, and measurement of cell potential. Considerable progress has been made in recent years in cell culture techniques that allow for the successful culture of respiratory epithelial cells in many different species. A number of investigators have attempted to culture the middle ear epithelium from different species such as rat,[52] guinea pig,[53] gerbils,[54] and chinchillas.[55] Recently, the human middle ear epithelium was successfully cultured (Fig. 8).[56] This will provide a good model for the study of human eustachian tube and middle ear epithelial physiology or of secretion related to otitis media.

Mucin gene of the eustachian tube

Mucins are present in various organs such as nasal cavity and bronchotracheal tree, and it protect and lubricate the surface of mucous membranes under normal circumstances. Up to the present, nine human mucin genes have been cloned from cDNA of various human organs. The mucins in the eustachian tube, however, are unkown. Recently, Kawano et al.[57] identified mucin gene (MUC5B) in middle ear mucosa of patients with chronic otitis media. Their studies indicated that MUC5B mucin gene is expressed in the middle ear mucosa in chronic otitis media and it may play an important role in the pathogenesis of this disease. Inflammatory disorders are accompanied by excessive secretion of the mucus which contributes to pathogenesis of otitis media. Future study will clarify mucin genes in the eustachian tube which will be helpful to understand the pathogenesis and management of otitis media.

CONCLUSION

One of the important functions of the eustachian tube is to protect the middle ear from invading organisms. The host develops a number of strategies for the purpose (e.g., mucociliary protection, antibacterial secretory products, immunodefense, and phagocytosis). The bacteria also develop their strategies to evade the host protection by enhancing adherence to the mucosal surfaces, impairing mucociliary function, and evading phagocytosis.

The normal eustachian tube mucosa is protected by the mucociliary transportation system which prevents bacterial entry and by surface mucosal immunoglobulin (secretory IgA) which inhibits bacterial adhesion to the mucosal surfaces. Therefore, three conditions must be met for the occurrence of otitis media: (1) bacterial adherence to the nasopharynx, (2) bacterial entry to the tubotympanum, and (3) bacterial replication in the tympanum.

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REFERENCES

1. Lim DJ: Protein secreting cells in the normal middle ear mucosa of the guinea pig: an autoradiographic investigation. Ann Otol Rhinol Laryngol 79: 82-94, 1970
2. Lim DJ, Viall J, Birck H and St. Pierre R: The morphological basis for understanding middle ear effusions. An electron microscopic, cytochemical, and autoradiographic investigation. Laryngoscope 82: 1625-1642, 1972
3. Shimada T and Lim DJ: Distribution of ciliated cells in the human middle ear: electron and light microscopic observations. Ann Otol Rhinol Laryngol 81: 203-211, 1972
4. Lim DJ: Normal and pathological mucosa of the middle ear and eustachian tube. Clin Otolaryngol 4: 213-234, 1979
5. Lim DJ, Shimada T and Yoder M: Distribution of mucus-secreting cells in a normal middle ear mucosa. Arch Otolaryngol 98: 2-9, 1973
6. Bluestone CD, Paradise JL and Beery QC: Symposium on prophylaxis and treatment of middle ear effusions. IV. Physiology of the eustachian tube in the pathogenesis and management of middle ear effusions. Laryngoscope 82: 1654-1670, 1972
7. Verdugo P: Mucociliary function in mammalian epithelia. In:Mechanism and control of ciliary movement. BrokawCJ, Verdugo P (eds). Alan R. Liss, New York, 1982, pp 1-5
8. Lucas AM and Douglas LC: Principles underlying ciliary activity in the respiratory tract. Arch Otolaryngol 20: 518-541, 1932
9. Sade J: The mucociliary system in relation to middle ear pathology and sensorineural hearing loss. In: Sensorineural hearing loss. Wolstenholme GEW, Knight J (eds). Churchill,

London, 1970, pp 79-99
10. Verdugo P: Ca^{2+}-dependent hormonal stimulation of ciliary activity. Nature (London) 283: 764-765, 1980
11. Sleigh MA: Movement and coordination of tracheal cilia and the relation of these to mucus transport. In: Mechanism and control of ciliary movement, Brokaw CJ, Verdugo P (eds). Alan R.Liss, New York, 1982, pp 19-24
12. Lim DJ and Shimada T: Secretory activity of normal middle ear epithelium :scanning and transmission electron microscopic observations. Ann Otol Rhinol Lanyngol 80: 319-329, 1971
13. Hanamure Y and Lim DJ: Normal distribution of lysozyme and lactoferrin secreting cells in the chinchilla tubotympanum. Am J Otolaryngol 7: 410-425, 1986
14. Ueno K and Lim DJ: Heterogeneity of glycoconjugates in the secretory cells of the chinchilla middle ear and eustachian tubal epithelia: a lectin-gold cytochemical study. J Histochem Cytochem 39: 71-80, 1991
15. Giebink GS, Ripley ML and Wright PF: Eustachian tube histopathology during experimental inflnenza A virus infection in the chinchilla. Ann Otol Rhinol Laryngol 296: 199-206, 1987
16. Lim DJ, Liu YS and Birck H: Secretory lysozyme of the human middle ear mucosa: immunohistochemical localization. Ann Otol Rhinol Laryngol 85: 50-60, 1976
17. Mogi G, Maeda S and Watanabe N: The development of mucosal immunity in guinea pig middle ears. Int J Ped Otolaryngol 1: 331-349, 1980b
18. Park K and Lim DJ: Development of secretory elements in murine tubotympanum: lysozyme and lactoferrin immunohistochemistry. Ann Otol Rhinol Laryngol 102: 385-395, 1993
19. Vn den Oord JJ, De Wolf-Peeters C and Desment V: Immunohistochemical localization of lysozyme in the nasal respiratory mucosa. Arch Otorhinolaryngol 237: 1-5, 1982
20. Matsune S, Fukami K, Hanamure Y and Ohyama M: Distribution of lysozyme in the upper airway. In: Glycoconjugates in Medicine, Ohyama M, Muramatsu T(eds), 1988, pp 138-142
21. Brokler RH and Birken EA: Surface-tension lowering substance of the canine eustachian tube. Laryngoscope 81: 508-518, 1971
22. Hagen WE: The mucous blanket of the eustachian tube: a morphologic and surface property demonstration. Trans Am Acad Ophthalmol Otolaryngol 84: 242-268, 1977
23. Hagan WE: Surface-tension lowering substance in eustachian tube function. Laryngoscope 87: 1033-1045, 1977
24. Rapport PN, Lim DJ and Weiss HS: Surface-active agent in eustachian tube function. Arch Otolaryngol 101: 305-311, 1975
25. Wheeler SL, Pool GL and Lumb RH: Rat eustachian tube synthesize disaturated phosphatidylcholine. Biochem Biophys Acta 794: 348-349, 1984
26. Maves MD, Patil GS and Lim DJ: Surface-active substances of the guinea pig tubotympmum: a chemical and physical analysis. Otolaryngol Head Neck Surg 89: 307-316, 1981
27. Coticchia JM, Heiselman FA, DeMaria TF and Lim DJ: Surface active substances in the chinchilla tubotympanum: a biochemical study. Acta Otolaryngol(Stockh) 111: 1097-1104, 1991
28. Lim DJ: Functional morphology of the lining membrane of the middle ear and eustachian tube:an overview. Ann Otol Rhinol Laryngol 83(Suppl 11): 5-18, 1974
29. Bernstein JM and Ogra PL: Mucosal immune system: implications in otitis media with effusion. Ann Otol Rhinol Laryngol 89(Suppl 68): 326-332, 1980
30. Branefors-Helander P, Nylen O and Jeppson JM: Acute otitis media:assay of complement-fixing antibody against Hemophilus influenzae as a diagnostic ool in acute otitis media. Acta Pathol Microbiol B 81: 508-518, 1973
31. Juhn SK, Giebink GS, Huff JS and Mills EL: Biochemical and immunochemical characteristics of middle ear effusions in relation to bacteriological findings. Ann Otol Rhinol Laryngol 89(Suppl 68): 161-167, 1980
32. Lewis DM, Schram JL, Birck HG and Lim DJ: Antibody activity in otitis media with effusion. Ann Otol Rhinol Laryngol 88: 392-396, 1979
33. Mogi G: Secretory IgA and antibody activities in middle ear effusion. Ann Otol Rhinol Laryngol 85(Suppl 25): 97-102, 1976
34. Bernstein JM, Tomasi TB and Ogra P: The immunochemistry of middle ear effusion. Arch Otolaryngol 99: 320-326, 1974
35. Lim DJ: Functional morphology of the mucosa of the middle ear and eustachian tube. Ann Otol Rhinol Laryngol 85(Suppl): 36-43, 1976
36. Mogi G, Maeda S, Yoshida T and Watanabe N: Immunohistochemistry of otitis media with effusion. J Infect Dis 133: 125-136, 1976
37. Giebink GS and Quie PG: Comparision of otitis media due to type 3 and 23 Streptococcus pneumoniae in the chinchilla model. J Infect Dis 136: 191-195, 1977
38. Watanabe N, DeMaria TF, Lewis DM, Mogi G and Lim DJ: Experimental otitis media in chinchillas. II. Comparision of the middle ear immune responses to s.pneumoniae type 3 and 23. Ann Otol Rhinol Laryngol 91(Suppl)93: 9-16, 1982
39. Yamaguchi T, DeMaria TF and Lim DJ: Cross-reactive antibodies in type b and nontypable H. influenzae-induced experimental otitis media. Ann Otol Rhinol Laryngol 96: 112-121, 1987
40. Watanabe N, Briggs BR and Lim DJ: Experimental otitis media in chinchillas. I. Baseline immunological investigation. Ann Otol Rhinol Laryngol 91(suppel 3): 3-8, 1992
41. Lim YS, Lim DJ, Lang RW and Birck HG: Chronic middle ear effusions: immunochemical and bacteriological investigation. Arch Otolaryngol 101: 278-286, 1975
42. Lim DJ and Hussl B: Macromolecular transport by the middle ear and its lymphatic system. Acta Otolaryngol (Stockh) 80: 19-31, 1975

43. Palva T, Taskinen E and Hayry P: T lymphocytes in secretory otitis media. In: Immunology, Autoimmunity and Transplantation in Otorhinolaryngology, Veldman JE, McCabe BF, Huizing EH, Mygind N (eds). Kugler, Amsterdam, 1985, pp 45-50
44. Henderson FW, Collier AM and Sanyl MA: A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. N Engl J Med 306: 1377-1385, 1982
45. Yamaguchi T, Urasawa T and Kataura A: Secretory immunoglobulin A antibodies to respiratory viruses in middle ear effusion of chronic otitis media with effusion. Ann Otol Rhinol Laryngol 93: 73-75, 1984
46. Giebink GS, Berzins IK, Marker SC and Schiffman G: Experimental otitis media after nasal inoculation of Streptococcus pneumoniae and influenza virus A in chinchillas. Infect Immun 30: 445-450, 1980
47. Lim DJ, Ohashi Y, Chung MH and DeMaria TF: Ultrastructural pathology of the tubotympanum following experimental influenza A virus induced otitis media. In: Recent Advances in Otitis Media. Lim DJ, Bluestone CD, Klein JO, Nelson JD(eds). BC Decker, Toronto, 1988, pp 436-438
48. Park K, Bakaletz LD, Coticchia JM and Lim DJ: Effect of influenza A virus on ciliary activity and dye transport function in the chinchilla eustachian tube. Ann Otol Rhinol Laryngol 102: 551-558, 1993
49. Fainstein V, Musher DM and Gate TR: Bacterial adherence to pharyngeal cell during viral infection. J Infect Dis 141: 172-176, 1980
50. Friedman H, Spector S and Bendinelli M: Viruses and immune response. In: Bacterial and viral inhibition and modulation of host defenses. Falcone G, Campa M, Smith H, Scott GM (eds). Academic Press, London, 1984, pp 119-133
51. Yamanaka T, Bernstein JM, Cumella J and Ogra PL: Lymphocyte-macrophage interaction in otitis media with effusion. In: Recent Advances in Otitis Media with Effusion. Lim DJ, Bluestone CD, Klein JO, Nelson JD (eds). BC Decker, Toronto, 1984, pp 173-177
52. Van Blitterswijk CA, Ponec M, van Muijen GNP, Wijsman MC, Koerten HK and Grote JJ: Culture and characterization of rat middle ear epithelium. Acta Otolaryngol (Stockh) 101: 453-66, 1986
53. Takeno T, Hirakawa K and Harada Y: Tissue culture of middle ear epithelium of the guinea pig. Ear Res Jpn 19: 164-6, 1988
54. De Serres LD, Van Scott MR, Pillsbury HC and Prazma J: Culture of the middle ear epithelial cells and their ion transport. In: Lim DJ, ed. Abstracts of the Twelfth Midwinter Research Meeting, St Petersburg Beach, Fla: Association for Research in Otolaryngology, 1989, pp 80
55. Nakamura A, DeMaria T, Lim DJ and Van Blitterswijk CA: Primary culture of chinchilla middle ear epithelium. Ann Otol Rhinol Laryngol 100: 774-782, 1991
56. Moon SK, Yoon JH, Lee HK, Chung MH, and Kim HN: Culture of normal human middle ear epithelial cell using serum-free media. J Korean Otolaryngol 1998 (In Press).
57. Kawano H, Paprella MM, Ho SB, Zhang Z, Schachern PA, Morizono N, Kim Y and Lim J: Identification of mucin gene (MUC5B) in middle ear mucosa of patients with chonic otitis media. In: Lim DJ, ed. Abstracts of the Twenty first Midwinter Research Meeting, St. Petersburg Beach, Fla: Association for Research in Otolaryngology, 1998, pp 123