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Development of the Mucociliary System in the Eustachian Tube and Middle Ear: Murine Model

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In order to describe the developmental anatomy of the murine eustachian tube and its related structures, seventy six mice of ages ranging from gestational day 11 to postnatal day 21 were investigated through the light and electron microscopic observations. Development of the ciliated cells was seen concurrently in both the eustachian tube and middle ear on the 16 th gestation day, one day earlier than the epithelial secretory cells appeared in both the eustachian tube and middle ear. The number of ciliated cells and secretory cells increased rapidly after birth. Tubal glands were well identified with evidence of secretory activity around the time of birth. Thus, the findings of this study indicate that the mucociliary defense system starts to develop during the fetal stage and is well established immediately after birth.

Key Words: Developmental study, eustachian tube, middle ear, mice, mucociliary system

The mouse is an important laboratory animal for otological research, especially for developmental study. It is useful since it breeds rapidly and prolifically, and would allow rapid development of inbred strains. Furthermore mice are consistently available and not expensive to purchase, ship and maintain.

Even though the developmental anatomy of the inner ear and some parts of the murine middle ear has been described (Lim and Anniko 1985; Huangfu and Saunders 1983; Masuda *et al.* 1986), to date the description of the development of the eustachian tube and middle ear mucociliary system is sketchy. This detailed study will be useful in understanding cell differentiation and organogenesis and will provide a morphological basis for future animal studies concerning the pathogenesis of otitis media in earlier stages using the murine model.

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In this report, the developmental anatomy of the murine eustachian tube and its related structures with particular emphasis on the epithelial cells as a functional unit was investigated using light microscopy (LM) and transmission electron microscopy (TEM).

MATERIALS AND METHODS

A total of seventy six BALB/c mice ranging from 11 gestational day through 21 postnatal day were used in this investigation. Sexually mature female mice were placed with males overnight. Gestational day was determined by vaginal plug technique, establishing the first day of appearance of the vaginal plug as day one. Half of the mice of each age were prepared for light microscopy and the remainder for transmission electron microscopy. Postnatal specimens with inflammed middle ear mucosa were excluded from this investigation.

For LM observations, the specimens were decapitated without perfusion of the fixative and immersed in 10% neutral buffered formalin for 7 days at 4°C. Postnatal specimens were decalcified with 10% ethylene diamine tetraacetic acid (EDTA) in 0. 1 M Tris-buffer (pH 6.95). The end point of EDTA decalcification was checked by Seilly's chemical test

(Seilly 1982). Specimens were dehydrated with graded ethyl alcohol and were embedded in glycol methacrylate (JB-4, Polysciences, Warrington, Pennsylvania). Using glass knives, 4 micro-meter sections were made and stained with hematoxylin and eosin. Sections adjacent to the hematoxylin-stained sec-

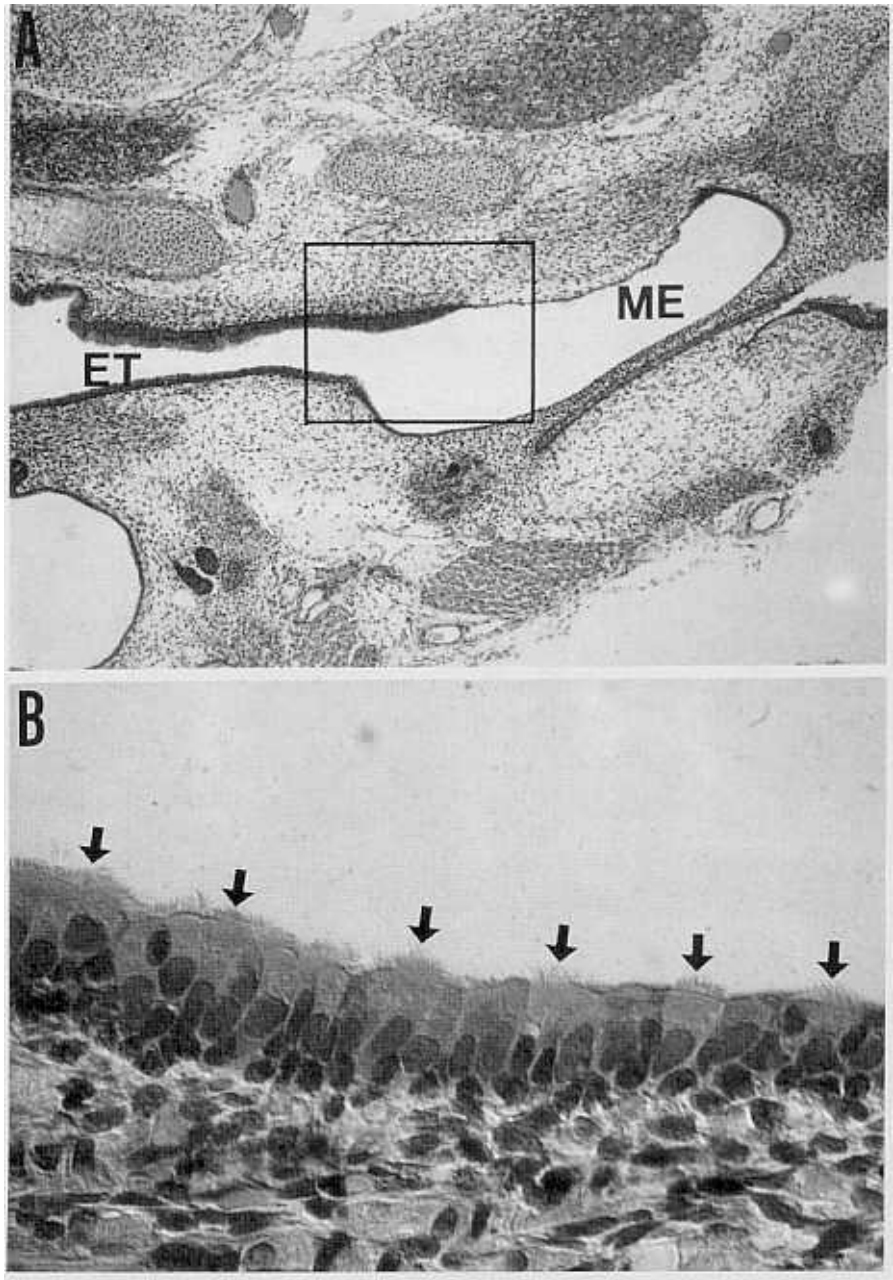

Fig. 1. *A) The tubotympanic recess divides into the eustachian tube (ET) and middle ear cavity (ME) in a 16-day gestational mouse. The tubal lumen shows the development of a ciliated pseudostratified columnar epithelium (Coronal, H & E, ×33).*

B) Close-up view of inset of A) shows the cilia (arrows) (×640).

tions were stained with alcian blue (pH 2.5)/periodic acid-Schiff (AB-PAS) to demonstrate mucosubstances of the secretory cells.

For TEM observations, the specimens were decapitated without perfusion of the fixative and immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Postnatal specimens were also decalcified with 4.5% EDTA in distilled water (pH 7.3) for 2 weeks. Specimens were dehydrated in graded ethyl alcohol, embedded in Epon 812, trimmed, and remounted for ultratome thin sectioning. Specimens were sectioned with a microtome, stained both by uranyl acetate and lead nitrate, and examined with a transmission microscope.

RESULTS

The tubal lumen, representing the persistence of the first pharyngeal pouch, is the only identificable structure in the earlier embryo. Between 12 and 13 gestational days, the first pharyngeal pouch elongated to form the tubotympanic recess, and its lumen showed a relatively smooth margin with unciliated low columnar epithelium. Between 13 and 14 gestational days, the tubotympanic recess extended to the middle ear area where the stapedial primordium and the Reichert's cartilage were continuous with each other.

Undoubtedly division of the tubotympanic recess into the eustachian tube and middle ear cavity did not occur until the 16th gestational day. By the 16th gestational day the previously smooth margin of the tubal lumen showed the development of a ciliated pseudostratified columnar epithelium (Fig. 1A, B), and ciliogenesis was noted (Fig. 2). The muscle fibers of the tensor and levator veli palatini were distinct from the surrounding tissue by the 16th gestational day although they were not tightly bound by a perimysium or epimysium. They were well identified on the 17th gestational day (Fig. 3). At this age, the epithelial secretory cells in the eustachian tube as well as in the middle ear were stained with AB-PAS (Fig. 4) and they were confirmed through TEM observations (Fig. 5), but there was still no evidence of a tubal gland. A few glands seemed to be present only around the pharyngeal orifice of the tube on the 18th gestational day, but these glandular cells were not stained with AB-PAS (Fig. 6). Tubal glands as well as tubal cartilages were well noted in the specimen from postnatal day 1 (Fig. 7), and also the tubal muscles were well developed at this age. Table I outlines the presence of the eustachian tube and its related structures ac-

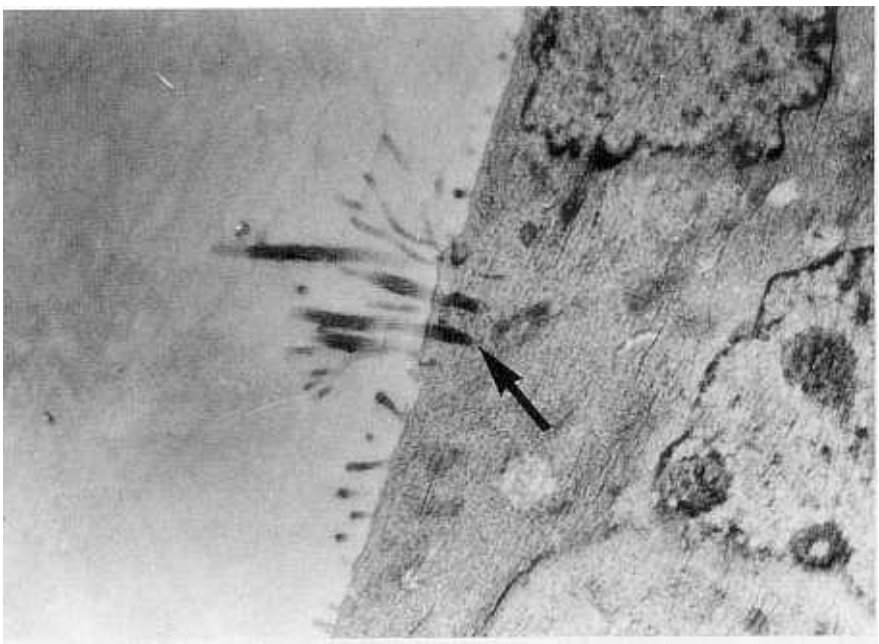

Fig. 2. TEM of the mucosa lining of the eustachian tube shows ciliogenesis (arrow) in a 16-day gestational mouse (×6,800).

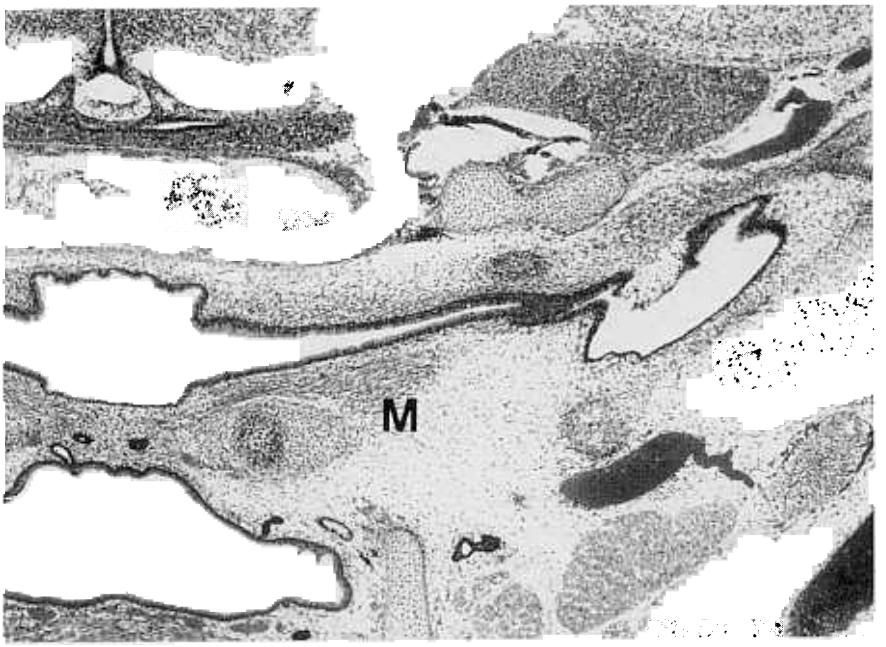

Fig. 3. The tubal muscle (M) is well identified in 17-day gestational mouse (Coronal, H & E, ×33).

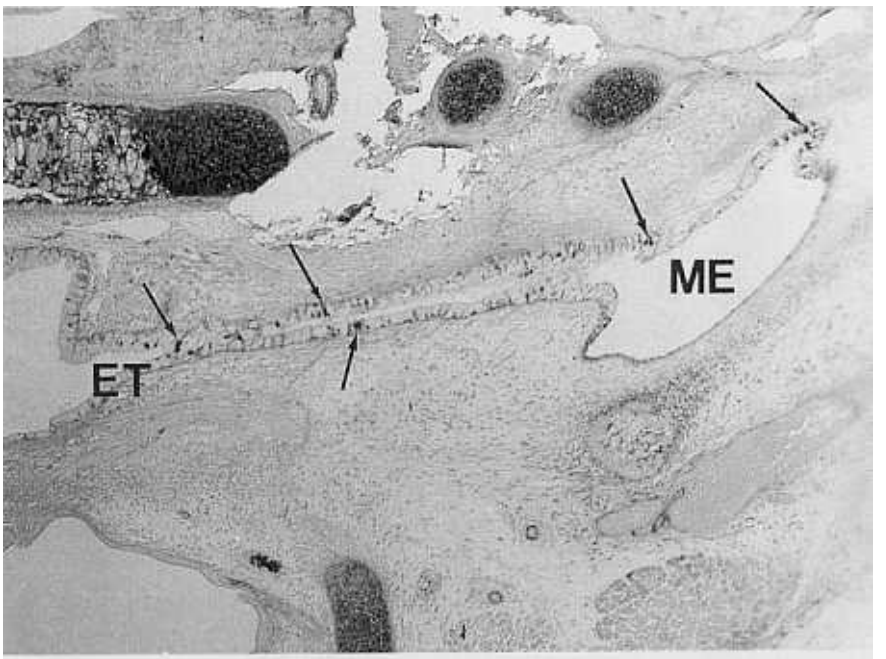

Fig. 4. A few epithelial secretory cells (arrows) are noted concurrently in the eustachian tube (ET) as well as in the middle ear (ME) in a 17-day gestational mouse (Coronal, AB-PAS, ×320).

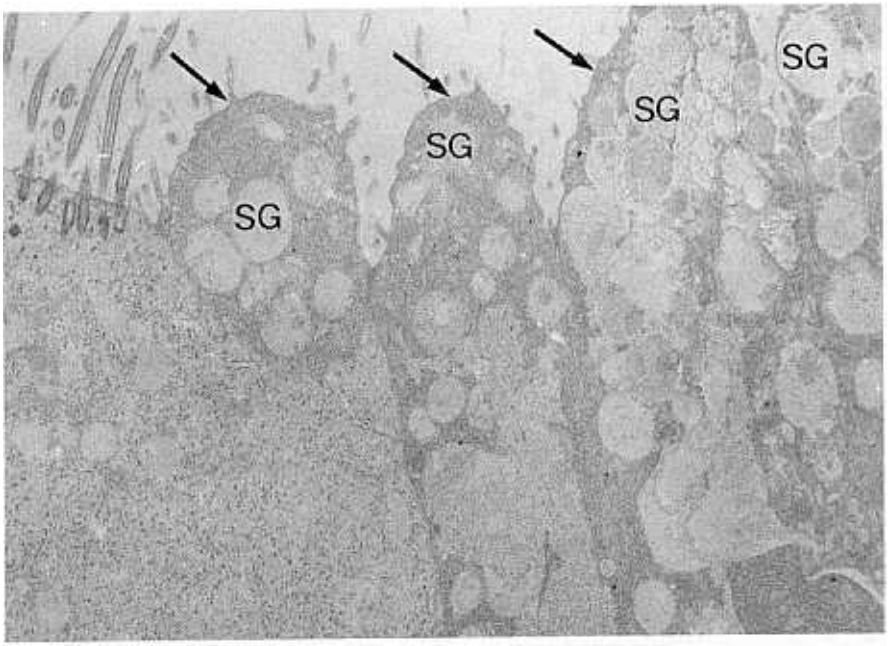

Fig. 5. *TEM of the mucosa lining of the eustachian tube shows the secretory cells (arrows) in a 17-day gestational mouse. The cell has secretory granules (SG) which are partially enclosed by a fine membrane and partially fused. These secretory granules are surrounded by abundant elongated r-ER (×3,300).*

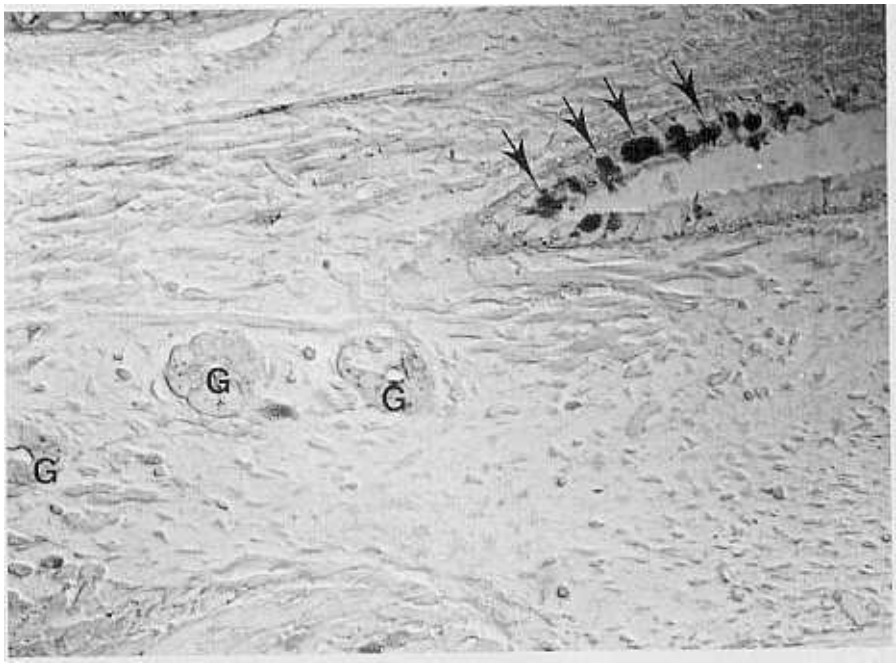

Fig. 6. *A few glands (G) with negative staining are recognized around the pharyngeal orifice of the eustachian tube in a 18-day gestational mouse. Positive epithelial secretory cells (arrows) (AB-PAS, ×320).*

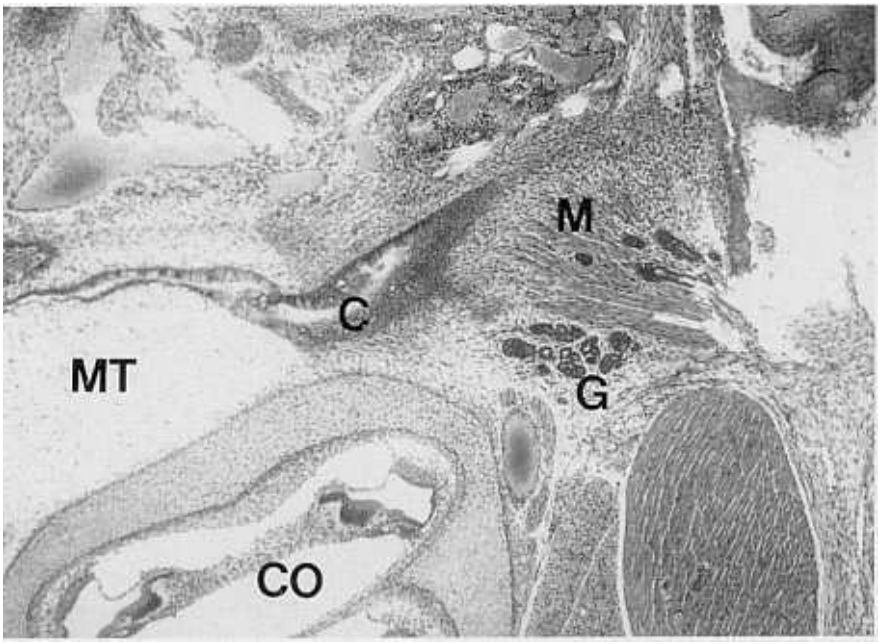

Fig. 7. Tubal glands (G), muscle (M) and cartilage (C) are well identified in a 1-day postnatal mouse. Cochlea-CO, Mesenchymal tissue in the middle ear cavity-MT (Axial, H & E, ×33).

Table 1. Presence of eustachian tube and its related structures in specimens of different gestational (G) and postnatal (P) age

Age(day)	Lumen	Epithelium		Gland	Muscle	Cartilage
		Ciliated cell	Secretory cell			
G11	−	−	−		−	
G12	+	−	−		−	
G13	+	−	−		−	
G14	+					
G15	+	−				
G16	+	+				
G17	+	+	+	−	+	
G18	+	+	+	+	+	−
P1	+	+	+	+	+	+
P3	+	+	+	+	+	+
P5	+	+	+	+	+	+
P7	+	+	+	+		
P9	+	+	+	+		
P11	+	+	+	+	+	+
P13	+	+	+	+	+	+
P15	+	+	+	+	+	+
P17	+	+	+	+	+	+
P19	+	+	+	+	+	+
P21	+	+	+	+	+	+

cording to the gestational and postnatal age.

DISCUSSION

The developmental process of the main parts of the murine eustachian tube and its related structures is very similar to that of the human described in several reports (Tos 1971; Tos 1971; Tos 1970; Swarts *et al.* 1986; Kitajiri *et al.* 1987; Bast and Anson 1949). The sequence of the development of the eustachian tube and its related structures from lumen formation, through the differentiation of epithelial cells and development of muscles, concluding with the development of glands and cartilages is consistent with that of the human except the fact that tubal muscles in the human develop earlier than any other structures. Although this study clarified the order of differentiation of the eustachian tube structures, the absolute timing of these developmental events varies. This can be attributed to difficulties in accurately ascertaining the chronological age of the specimens, to the normal variation in the timing of the differentiation of these structures, or to both.

Comparision of the developing steps of the eustachian tube and its related structures in the mouse and human are shown in Table 2. The different aspect between the human and murine eustachian tube in development is apparently that the murine eustachian tube and its related structures are completely identified at around birth in contrast to those in the human which appear by the first half of pregnancy.

The importance of the mucociliary defense system in the tubotympanum has been well established (Lim 1974; Lim 1976). However, the information on the development of the mucociliary system has been poorly documented. The present data suggest that ciliated cells appeared first at the 16th gestation day when the middle ear cleft is being formed. The ciliated cells were found concurrently in the tubal lumen as well as in the expanding tympanic cavity. This finding was unexpected because we had anticipated earlier development of the tubal lumen than that of the tympanic cavity. Another interesting observation was that epithelial secretory cells appeared 1 day later than the ciliated cells. Like in the case of ciliated cells, the secretory cells in the middle ear developed at the same time as in the eustachian tube. The epithelial secretory cells have already synthesized secretory products at this age as evidenced by AB-PAS staining characteristics. It is interesting to note that the distribution of secretory cells are parallel to that of the ciliated cells even during development. Earlier reports in human and laboratory animals demonstrated that the distribution of the secretory cells are parallel to that of the ciliated cells (Shimada and Lim 1972; Lim *et al.* 1973). This parallel distribution of ciliated and secretory cells is not surprising, considering the physiological need of both elements forming a complete mucociliary system. Based on the morphology and autoradiography (Lim *et al.* 1967; Hussl and Lim 1969; Lim and shimada 1971), several investigations suggested that there are functionally different secretory cells that are dark granulated, light granulated, and mixed. The dark granulated cells incorporated a greater amount of tritiated leucine in comparison to the light granulated cells (goblet cells), which incorporated a greater amount of tritiated glucose (Lim *et al.* 1972; Lim 1970). These findings imply that the diversity of epithelial secretory cells is needed to support the mucociliary system which requires a functioning mucous blanket and serous periciliary fluid. The present study

Table 2. Differences on mouse and human during development of structures in tubotympanal cavity

Findings of developing structures in tubotympanal cavity	Age	
	Mouse (day)	Human (week)
Formation of tubal lumen begins	G12	G 8[b]
Ciliated cell appears	G16	G15[b]
Secretory cell appears	G17	G13[a]
Tubal muscle appears	G16-17	G11[a] G10-12[b]
Tubal gland appears	G18-P1	G13[a] G15[b]
Tubal cartilage appears	P1	G12[a] G15[b]

G: Gestational
P: Postnatal

a: Tos (1971)
b: Swarts *et al* (1986)

did not include lectin labeling, and therfore, we are not able to determine wheter the diversity of secretory cells exists during development. Thus, the diversity of secretory cells in developing stage also requires further study.

In conclusion, the present study indicates that the mucociliary system in the eustachian tube and middle ear functions actively immediately after birth although it starts to develop during the fetal stage, which can be interpreted that the mucociliary defense system appears with the aeration of the lung.

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