Early Fetal Development of the Human Cochlea

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ABSTRACT

The cochlear scalas are differentiated from a single tube with a lining by the tall epithelium, that is, the cochlear duct. However, we have no information about the mechanism involved in the formation of the scalas. We evaluated histological sections taken from 20 fetuses: eight each at 8–9 weeks [early stage; 28–45 mm crown–rump length (CRL)] and 11–12 weeks (middle stage; 52–74 mm CRL), and four at 14–15 weeks (late stage; 90–110 mm CRL) of gestation. In four of eight early-stage and in all eight middle-stage specimens, we observed irregular perilymphatic spaces and their fusion; these spaces tended to be larger in the future scala tympani than in the future scala vestibuli. The cochlear duct epithelium was positive for cytokeratin 19 in contrast to the other parts of the cochlea. The tectorial membrane appeared in two of eight middle-stage and all four late-stage specimens. After 16 weeks, mesothelial lining of the scala may follow the development of aquaporin-positive thin blood vessels along the scala wall. Notably, gap formation of the cochlear duct epithelium at a site facing the scala tympani consistently occurred before the development of S100 protein-negative organ of Corti. This gap is likely to correspond to a site occupied finally by Hensen's cells. All these steps likely started in the basal coil and extended to the apical side of the cochlea. These findings suggest that leakage through the epithelial gap of endolymph, with a high concentration of potassium ions, causes mesenchymal cell death, leading to the coalescence of vacuoles containing low potassium perilymph. Anat Rec, 294:996–1002, 2011. © 2011 Wiley-Liss, Inc.

Key words: cochlear duct; scala tympani; organ of Corti; endolymph; perilymph; aquaporin; human fetus

Grant sponsor: The National R & D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea; Grant number: 0620220-1.

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Received 19 October 2010; Accepted 3 March 2011
DOI 10.1002/ar.21387
Published online 28 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

The cochlear scalas are differentiated from a single tube with a lining by the tall epithelium, that is, the cochlear duct. However, we have no information about the mechanism involved in formation of the scalas. Transverse sectioning of each coil has shown that the membranous labyrinth of the inner ear cochlea is composed of three regions: the scala vestibuli at the apical side, the scala tympani at the basal side, and the scala media containing the organ of Corti. The scala media contains "endolymphatic fluid" (or endolymph), whereas the scala vestibuli and the scala tympani contain "perilymphatic fluid" (or perilymph). The difference in terms might be based on the morphology in the history of research, such as the "endo"-lymph attaching to the organ of Corti in contrast to the "peri"-lymph separated from the specific nerve terminal. The endolymphatic fluid is known to resemble the intracellular fluid with high potassium ion and relatively low sodium (Williams, 1995). Hirt et al. (2010) reported the difference in osmotic pressure: 322 mOsm/kg H_2O in the endolymphatic fluid; 289 mOsm/kg H_2O in the perilymphatic fluid.

According to Streeter (1917), the scala tympani forms before the scala vestibuli, with scala formation occurring at 8–17 weeks of gestation. At these stages, the cochlea is composed of the basal and second coils, with the apical part added after 15 weeks (Arnold and Lang, 2001; Jeffery and Spoor, 2004; Yasuda et al., 2007). Although the cochlear nerve reaches the cochlear duct at 11 weeks (Yamashita et al., 1993), myelination starts much later than the stages (Ray et al., 2005). Before the differentiation of the organ of Corti in fetuses, and, in some cases, the established scala media, the fetal scala media is called the "cochlear duct" (Fig. 1), in which tall epithelial cells separate the potassium-rich endolymphatic fluid from the surrounding sodium-rich perilymphatic fluid. Thus, in the scala vestibuli and scala tympani, these spaces or subdivisions are called the "perilymphatic space," in contrast to the "endolymphatic space," which corresponds to the cochlear duct and its subdivisions, including the organ of Corti and inner tunnel. In the previous descriptions on the early development of the scalas in the cochlea (Hamilton and Mossman, 1978; Moore and Persaud, 1998), there seems to be no agreement as to whether the early scalas carry the definite mesothelial lining (Fig. 1A–C) or they are formed by the fusion or coalescence of irregularly shaped perilymphatic spaces or vacuoles without lining mesothelium (Fig. 1D–F). However, we considered this point as a clue to provide better understanding of how the cochlear scala forms.

In the inner ear immunohistochemistry, previous researchers have paid attention to connexin-26 in the organ of Corti because the molecule seems to be responsible for hearing loss (Kikuchi et al., 1994; Fish et al., 2001; Kammen-Jolly et al., 2001). However, there has been no or few immunohistochemical study on the cochlear mesenchymal cells around the scala. Therefore, we used immunohistochemistry to examine the human fetal membranous labyrinth, using frontal or sagittal sections, with special emphasis on the formation of the scalas. The immunohistochemistry would help us to demonstrate site-dependent differences in cell differentiation along the scala. The major question is what and how makes the fluid space scala without definite epithelia such as the cochlear duct.

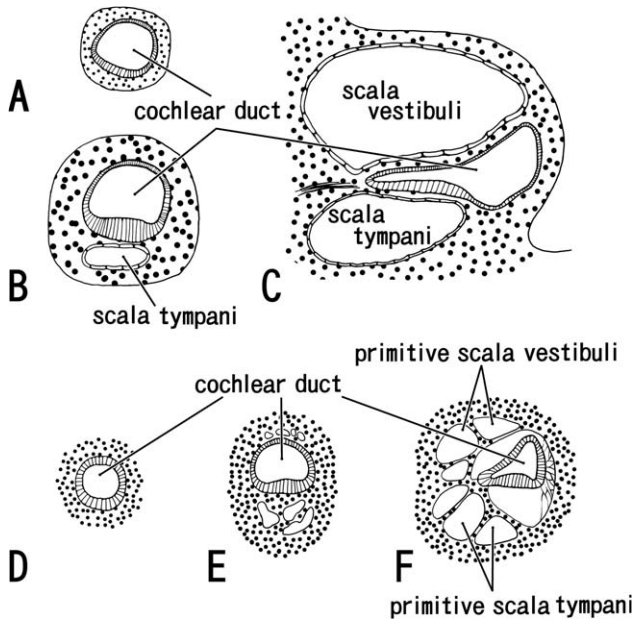

Fig. 1. Two hypotheses of the development of the scala tympani and scala vestibuli. (**A–C**) Cross-sections of a coil of the human fetal cochlea at 9 weeks (A, CRL 35 mm), 11 weeks (B; CRL 50 mm), and 17 weeks (C; CRL 130 mm), showing the development of the two scalas as definite spaces enclosed by a mesothelial lining (modification from Hamilton and Mossman, 1978). (**D**, **E**) Cross-sections of a coil of the human fetal cochlea at 8–12 weeks showing absence of mesothelium resulting from fusion of perilymphatic spaces appearing in the mesenchymal tissue (modification of Moore and Persaud, 1998).

MATERIALS AND METHODS

We used paraffin-embedded specimens of 20 fetuses, eight at 8–9 weeks of ovulational age [early stage; crown–rump length (CRL) 28–45 mm], eight at 11–12 weeks (middle stage; CRL 52–74 mm), and four at 14–15 weeks (late stage; CRL 90–110 mm). All specimens were part of the large collection kept at the Embryology Institute of the Universidad Complutense, Madrid and were the products of miscarriages and ectopic pregnancies at the Department of Obstetrics of the University. The study was approved by our university ethics committee and was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh, 2000). Because the specimens were taken from the products of miscarriages and ectopic pregnancies, they may have had abnormal pathology. However, we have attempted to describe the morphology commonly present in each group.

After routine procedures for paraffin-embedded histology, most of the specimens were cut frontally at a thickness of 5 μm and at intervals of 20 μm. However, sagittal sections were made for one specimen in each group of early, middle, and late stages. Depending on the size of each specimen, we needed to examine approximately 30–100 sections, including almost the entire cochlea, of each. Most sections were stained with hematoxylin and eosin, while some sections in all stage groups were used for immunohistochemistry (see below).

The primary antibodies used were (1) rabbit monoclonal anti-human S100 protein (dilution 1:100, Dako

Cytomation, Kyoto, Japan), (2) rabbit monoclonal anti-human aquaporin-4 (dilution 1:50, Santa Cruz Biotechnology, Santa Cruz, CA), (3) rabbit monoclonal anti-human CD68 (dilution 1:100, Dako, Glostrup, Denmark), (4) rabbit monoclonal anti-human alpha-1 smooth muscle actin (dilution 1:100, Dako); (5) mouse monoclonal anti-human CD34 (dilution, 1:100; Dako, Glostrup, Denmark); (6) mouse monoclonal anti-human podoplanin or D2-40 (dilution, 1:100; Nichirei, Tokyo), and (7) mouse monoclonal anti-human cytokeratin 19 (dilution, 1:100; Dako, Glostrup, Denmark). In most cases, paraffin sections were not pretreated, including microwave treatment. However, for D2-40, we used a ligand activator (Histofine SAB-PO Kit, Nichirei, Tokyo, Japan) with autoclave treatment (105°C, 10 min). Using Dako Envision ChemMate, the second antibody was labeled with horseradish peroxidase (HRP), and antigen–antibody reactions were detected using the HRP-catalyzed reaction with diaminobenzidine (with hematoxylin counterstaining). In addition, to identify cell death with DNA fragmentation, we conducted the deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) method using Millipore ApopTag Plus Peroxidase in situ Apoptosis Kit (Merck, Darmstadt, Germany).

We assayed fetal specimens for expression of S100 protein, a marker of glial cells (Ludwin et al., 1976) and of developing cartilage and ossification (Chano et al., 1996; Duarte et al., 2003). Foster et al. (1994) and Yamashita et al. (1995) reported that S100 protein is a maturation marker of the fetal inner ear membranes. We also assayed the specimens for expression of the water channel protein aquaporin-4, expressed by specific cells in the organ of Corti (Takumi et al., 1998), CD-68, a macrophage marker, and smooth muscle actin, which is expressed by the endothelium of arteries and veins, smooth muscle cells, and cochlear pericytes, but not by lymphatic endothelium (Hayashi et al., 2008; Shi et al., 2008). CD34 is a famous marker for the vascular and mesenchymal progenitor cells (Xu et al., 2003; Zambidis et al., 2007). D2-40 is a limited but effective marker for the mesothelium and lymphatic epithelium (Ishikawa et al., 2006; Jin et al., 2010). Finally, cytokeratin 19 is generally expressed in epithelial or epithelium-like cells (Moll et al., 1982; Makin et al., 1984). To our knowledge, there was no previous data on immunohistochemistry of the membranous labyrinth using CD34, D2-40, or cytokeratin 19.

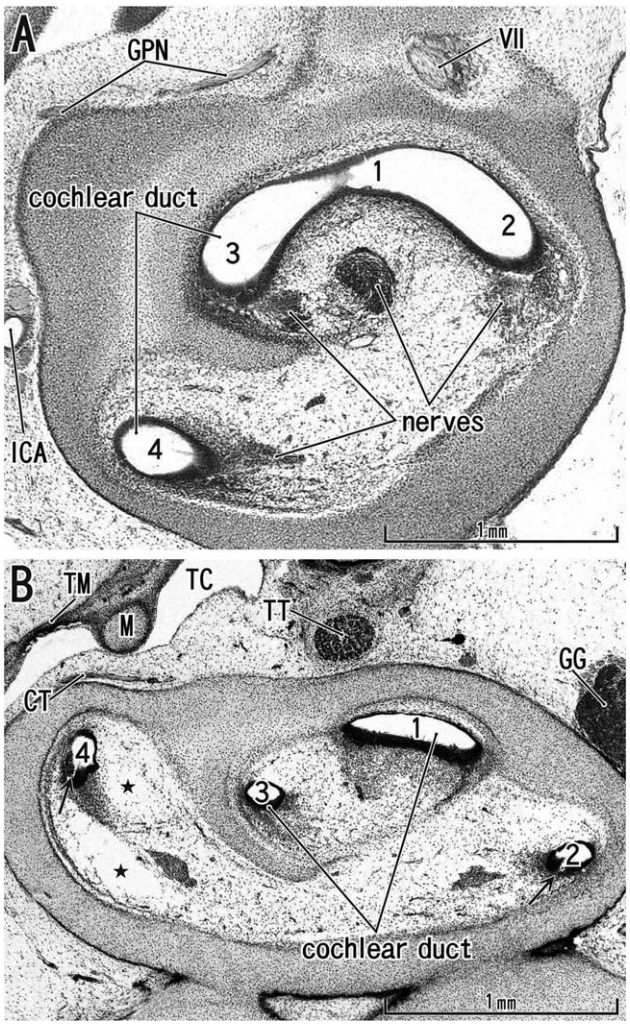

Fig. 2. Frontal sections of the developing cochlea in the early-stage group. (**A**) Cochlear mesenchymal tissue without space formation at CRL 38 mm. (**B**) Cochlear mesenchymal tissue at CRL 45 mm, containing two spaces (stars), corresponding to the scala vestibuli or scala tympani, respectively. The arrows at sites 2 and 4 show epithelial gaps in the cochlear duct. The four sites along the cochlear duct are indicated as 1–4, for correspondence among Figs. 2–5. CT, chorda tympani nerve; GG, geniculate ganglion; GPN, greater petrosal nerve; ICA, internal carotid artery; M, malleus; VII, facial nerve; TC, tympanic cavity (middle ear); TM, tympanic membrane; TT, tensor tympani muscle.

RESULTS

Early-Stage Group (CRL 28–45 mm)

Examination of the cochleae showed that the second loop had already formed, even in the smallest specimen (CRL 28 mm). The developing nerve elements were identified as mesenchymal cell condensations, reaching the cochlear duct before the formation of scalas (Fig. 2A). However, ganglion cells were not yet differentiated. In four of eight specimens in this group, we observed irregular perilymphatic spaces or vacuoles and their fusion or coalescence in the basal coil. All four of these specimens had a large space after fusion in the future scala tympani, and two of four had another smaller space in the future scala vestibuli (Fig. 2B). Notably, in two specimens of this group (CRL 29 mm and CRL 45 mm or Fig. 2B), we found a small gap in the cochlear duct epithelium at a site facing the future scala tympani (see also the description of the "gap" at later stages). This epithelial gap was attached to the mesenchymal condensation or nerve element. The tectorial membrane was not seen in this group.

Middle-Stage Group (CRL 52–74 mm)

In all the eight specimens, we found irregular perilymphatic spaces or vacuoles and their fusion or coalescence at the basal coil around at least one cut surface of the cochlear duct. In five of the eight specimens, the large space present after fusion was restricted in the basal coil and on one side of the second coil (Fig. 3A).

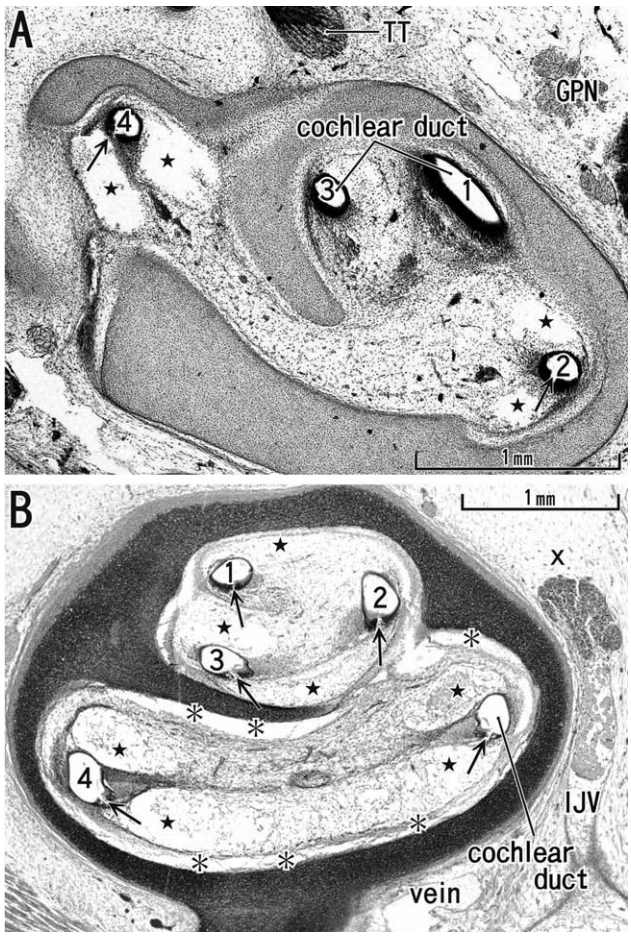

Fig. 3. Frontal sections of the developing cochlea in the middle-stage group. (**A**, **B**) Cochleae at CRL 52 mm (A) and 74 mm (B), each showing a pair of spaces (stars) for each of the transversely cut cochlear ducts. The four sites along the cochlear duct are indicated by 1–4 (see legend to Fig. 2). The arrows represent epithelial gaps in the cochlear duct, and the asterisks represent artificial spaces made by the histological procedure. IJV, internal jugular vein; X, vagus nerve superior ganglion. Other abbreviations are the same as in the legend to Fig. 2.

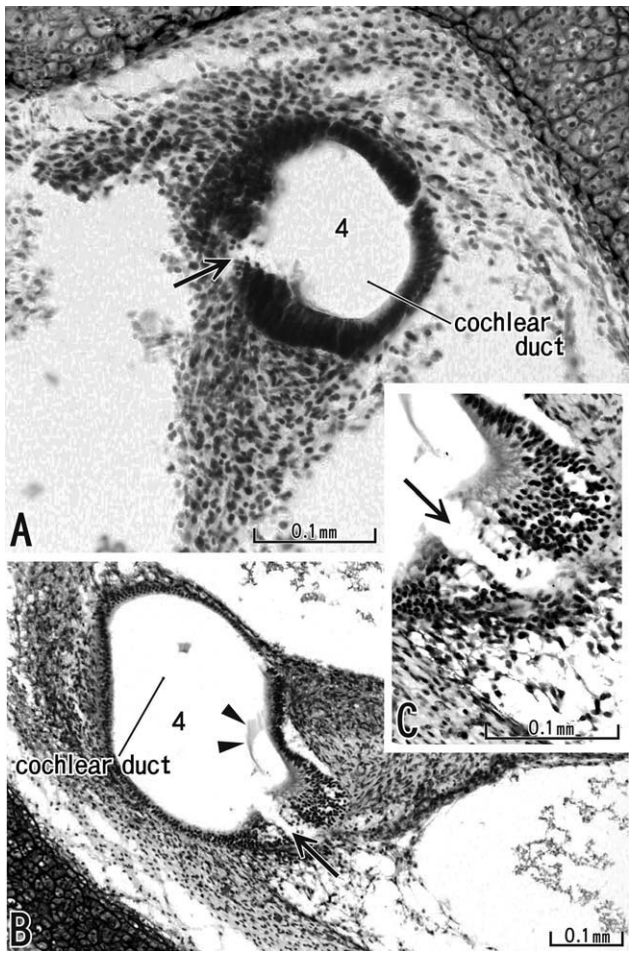

Fig. 4. Higher magnification views of site 4 in the cochlear ducts shown in Fig. 3. (**A**, **B**) Cochleae at CRL 52 mm (A) and 74 mm (B), containing epithelial gaps (arrow). Dispersing epithelial cells with small round nuclei near the gaps suggest cell death. The arrowheads represent tectorial membranes. (**C**) Higher magnification of the central part of panel B. Along and near the epithelial gap (arrow), the cell nuclei tended to be small and round, in contrast to the large oval nuclei in supporting cells of the organ of Corti.

However, in the remaining three specimens, there was a large space in all sections of the coils, from the basal site to the apical site (Fig. 3B). All these spaces were present in pairs, corresponding to the future scala tympani and scala vestibuli. The future scala tympani was not always larger than the future scala vestibuli (Fig. 3A). A gap in the cochlear duct epithelium was almost always (7/8) present at a site facing the scala tympani (Figs. 3 and 4). The gap was located at and opened to the margin of the mesenchymal condensation or nerve element. The cochlear duct epithelial cells appeared to disperse at the edges facing the gap. In contrast to cells in the epithelium, the dispersed cells appeared round and contained little cytoplasm (Fig. 4A). The tectorial membrane appeared at the basal coil in only two of these eight specimens possibly due to its formation following the complete differentiation of the organ of Corti (Fig. 4B), but it did not extend laterally (i.e., to the bony side) to reach the level of the epithelial gap. Along the scala wall, thin vessels containing red blood cells were present.

Late-Stage Group (CRL 90–110 mm)

The tectorial membrane was observed in all four of these specimens (Fig. 5A). We were able to identify the organ of Corti in all four: it was composed of (1) the tectorial membrane and (2) extremely tall supporting cells with the nuclei at the basal side, but (3) no inner tunnels or pillars (Fig. 5B). The supporting cell layer differed in cell height from that in other parts of the cochlear duct epithelium. Notably, the gap formation of the cochlear duct epitelium was consistently followed by the aforementioned differentiation of the organ of Corti. We found that at this stage, Reissner's and basilar membranes were differentiated from the cochlear duct epithelium: the former was thin but still contained two to three layers. However, mesothelial lining of the scalas

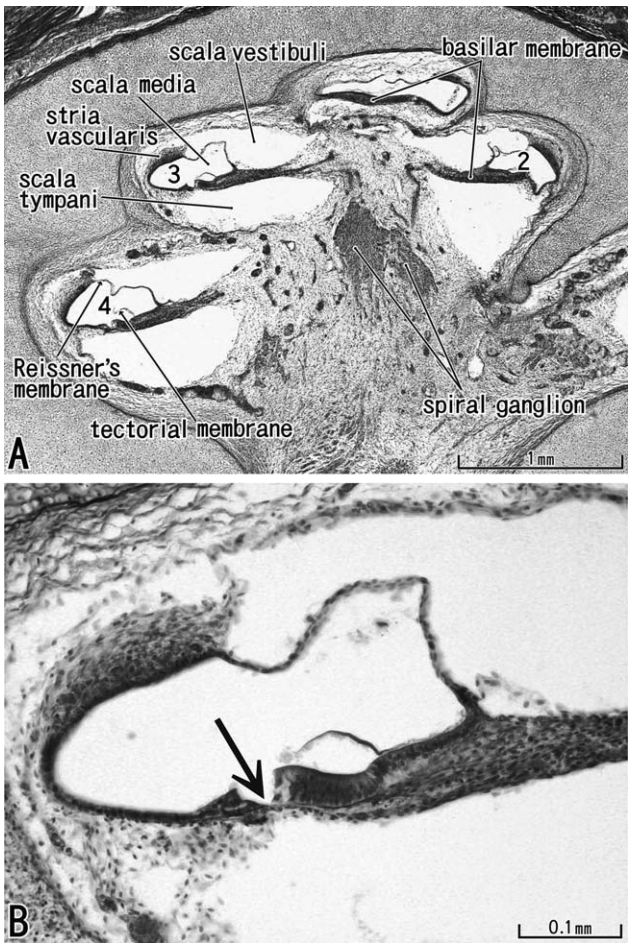

Fig. 5. Frontal sections of the developing cochlea in the late-stage group. (**A**) Cochlea showing all basic structures of the membranous labyrinth, including the organ of Corti (CRL 100 mm). The epithelium of the cochlear duct has already differentiated into Reissner's membrane, basal membrane, and stria vascularis, but the mesothelial lining of the scala has not yet formed. The four sites along the cochlear duct are indicated as 1–4 (see legend to Fig. 2). (**B**) Higher magnification of site 3 in panel A. The epithelial gap (arrow) in the organ of Corti is similar to those found in the middle stage (see Fig. 4).

tympani and vestibuli was not yet complete, with the surrounding mesenchymal cells appearing to face the scala at many sites. All these steps appeared to start in the basal coil, afterward extending to the apical side of the cochlea.

Immunohistochemistry

Among the three staged groups, the late-stage group specimens showed the best staining, with good preservation of tissues, although the histological procedure might usually make the tectorial membrane detached (Fig. 6). Cytokeratin 19 started expression in the early-stage group, and in the middle- and late-stage groups, it was positive in (1) the apical margin of the tall epithelial cells of the cochlear duct (Fig. 6A), (2) thin epithelia of the semicircular ducts, and (3) the epithelium of the middle ear and the skin. S100 protein was expressed in the basilar membrane, including the cochlear nerves, scala walls, and mesenchymal cells along and near the inner ear cartilage but not expressed in the organ of Corti, including in the scala wall facing the cartilage and containing thin vessels (Fig. 6B). In contrast, most of the supporting cells of the organ of Corti and in the future stria vascularis were positive for aquaporin-4 (Fig. 6C). Aquaporin was also expressed in the vascular endothelium along the scala wall near the cartilage. These thin vessels were negative for smooth muscle actin in contrast to vessels in and along the spiral ganglion. All these vessels were positive for CD34 (not shown), but loose mesenchymal cells were negative around the developing scala (data will be shown in the separated paper). Cells along the epithelial gap were negative for both S100 protein and aquaporin-4. Abundant CD68-positive macrophages were present in loose mesenchymal tissues around the scala (Fig. 6D) and along the cochlear nerve. D2-40 did not express in the membranous labyrinth including the loose mesenchyma in and around the scala, but it was positive in the cartilaginous cochlea (figure, not shown). We found no positive cells in the cochlea using TUNEL method although we tried twice.

DISCUSSION

Our findings indicate that, in the human fetal cochlea, scala formation occurs via fusion of small perilymphatic spaces. In contrast to the fetal cochlear duct, in which epithelial cells are tightly attached to each other, the primitive scala carries no definite mesothelial lining, even during later stages. A gap junction protein, connexin 26, which has been associated with many types of hearing loss, acts by maintaining high concentrations of potassium ions in the endolymph (Kikuchi et al., 1994). In human fetuses of 11–31 weeks gestation, connexin 26 is expressed in the mesothelial layer of the scala vestibuli, stria vascularis, and supporting cells of the organ of Corti (Kammen-Jolly et al., 2001). The mesothelial lining for scala perilymph seems to form much later than the epithelial lining system for endolymph although the lining was negative for a mesothelial marker D2-40. Mesothelial development likely follows the development of aquaporin-positive thin blood vessels along the scala walls starting at 11–12 weeks of gestation. In contrast to the scala wall, the epithelium of the cochlear duct expressed cytokeratin 19. This cytokerain seems to be available for a marker to identify a difference between the cochlear duct epithelium and the other scala walls. Conversely, until the middle stage, the scalas tympani and vestibuli are unlikely to carry the definite lining cells. Actually, Reissner's and basilar membranes were differentiated during the late stage.

One of our most striking findings was the observation of a gap in the cochlear duct epithelium, which was present in more than half of the cut surfaces (in number) of the cochlear ducts at and around CRL 50–55 mm. The earliest such finding was in the basal coil of a fetus with CRL 29 mm, suggesting that the same shape and site are maintained throughout the stages examined. Composite cells appeared to disperse from both edges of the cochlear duct epithelium facing the gap, possibly due to the cell death process (see below). In fetuses at 14–15 weeks of gestation, the epithelial gap delimited a lateral or bony side of S100 protein-negative organ of Corti.

Thus, due to the topographic relationship between the gap and organ, the epithelial gap most likely corresponds to a site containing Hensen's cells, a supporting cell population (Fawcett, 1994). Although Hensen's cells have been reported to strongly express the water channel protein aquaporin-4 (Takumi et al., 1998), we found that cells along the gap were "selectively" negative for aquaporin, suggesting that Hensen's cells, which act as a plug in the epithelial gap, develop during the latest stages of cochlear development. Although, we originally regarded the epithelial gap as the primitive inner tunnel of the organ of Corti, we found that the tectorial membrane never extended laterally to reach the gap. Therefore, we hypothesize that this epithelial gap is the first sign of the formation of the organ of Corti, suggesting that this organ starts to develop at the same fetal stage as the start of scala formation.

To our knowledge, Streeter (1917) was the first to describe the appearance of small perilymphatic spaces or vacuoles around the fetal semicircular duct, as well as their fusion or coalescence in the loose mesenchymal tissue. In the developed inner ear, the duct is filled with endolymphatic fluid, while the mesenchymal cells are embedded in the perilymphatic fluid. Thus, due to the specific function of the developing duct epithelium, even at 8–15 weeks of gestation, there seems to be a difference in osmotic pressure and ion contents between endolymph and perilymph (Hirt et al., 2010). Due to the presence of an epithelial gap, starting during early steps of scala formation, it is likely that the endolymph leaks through this gap, causing mesenchymal cell death and resulting in the coalescence of vacuoles containing perilymph. Moreover, this gap is likely to be closed by cells strongly positive for aquaporin-4 (Hensen's cells). Because the cochlear nerve may induce the organ of Corti (Takebayashi et al., 2007) and the organ physiologically faces the endolymphatic space (i.e., the cochlear duct and inner tunnel), the developing nerve element passing through the mesenchymal tissue should be resistant to exposure to endolymph. There is therefore a question regarding the type of cell death occurring in the mesenchymal cells surrounding the cochlear duct.

In the inner ear, apoptosis has been shown to occur in epithelial cells and in epithelium-derived cells in the surrounding mesenchyme (Represa et al., 1990; Nishokori et al., 1999). The present examination of mesenchymal tissues in the cochlea showed no TUNEL-positive mesenchymal cells, although Yasuda et al. (2007) reported apoptotic body-like fragments in the cochlear duct epithelial cells as well as in the surrounding early-stage mesenchymal tissue. TUNEL-positive mesenchymal cells have been reported in the middle ear but not in the inner ear (Palva et al., 2003). Although acid phosphatase-positive cells were observed along the bony or cartilaginous wall of the cochlea (Anderson et al., 1969), they were later shown to be osteoclast-like cells used for remodeling of the hard tissue (Jin et al., 2010). However,

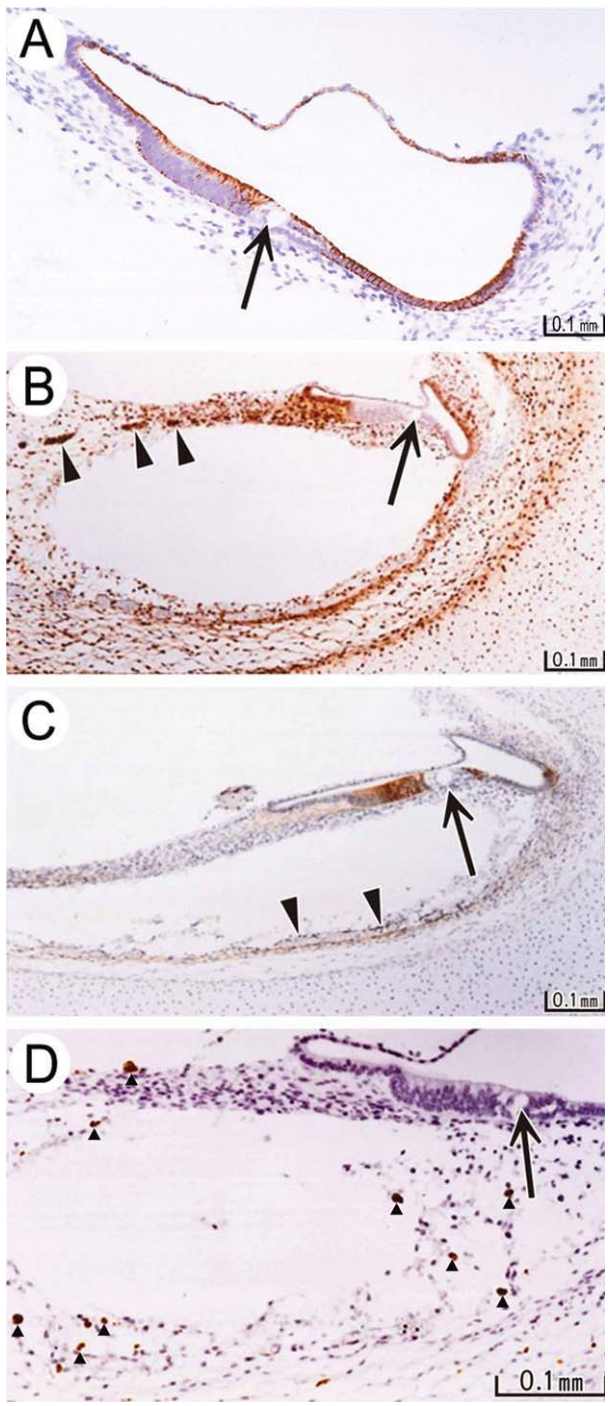

Fig. 6. Immunohistochemistry of frontal sections of the developing cochlea in a late-stage fetus. Expression of (**A**) cytokeratin 19, (**B**) S100 protein, (**C**) aquaporin-4, and (**D**) CD68 in the organ of Corti and scala tympani of a basal coil (CRL 90 mm), corresponding to site 2 in the other figures. Panel A displays a specimen different from that in panels B–D. The scala media may have been shrunk by the histological procedure. The epithelial gap is indicated by an arrow. (A) Cytokeratin 19 was selectively positive in the cochlear duct epithelium especially at the lumen side. (B) S-100 was expressed by the basilar membrane, including the nerves, the scala walls including the thin vessels, and the mesenchymal cells along and near the inner ear cartilage. (C) Aquaporin-4 was expressed by supporting cells of the organ of Corti and by vascular endothelium (arrowheads) along the scala tympani wall. (D) CD68 was expressed by macrophages (triangles) in loose mesenchymal tissues around the scala tympani. Panels A–C are different in magnification from panel D.

we observed abundant macrophages in cochlear mesenchymal tissues along and around the scala. Overall, mesenchymal cell death during scala formation may occur according to a TUNEL-negative mechanism, for example, without DNA fragmentation (e.g., Castro-Obregón et al., 2002), due to exposure to the endolymph. We believe that, after the initial endolymph leaks through the epithelial gap into the scala tympani, a potassium flow can quickly reach the apex of the coil to extend into the scala vestibuli. All nerve elements near the scala walls should be safe becasue of no toxicity of high potassium against nerve cells.

LITERATURE CITED

Anderson H, Matthiessen ME, Jørgensen MB. 1969. The growth of the otic cavities in the human foetus. Acta Otolaryngol 68:243–249.

Arnold WH, Lang T. 2001. Development of the membranous labyrinth of human embryos and fetuses using computer aided 3D-reconstructuin. Ann Anat 183:61–66.

Castro-Obregón S, del Rio G, Chen SF, Swanson RA, Frankowski H, Rao RV, Stoka V, Vesce S, Nicholis DG, Bredesen DE. 2002. A ligand–receptor pair that triggers a non-apoptotic form of programmed cell death. Cell Death Differ 9:807–817.

Chano T, Matsumoto K, Ishizawa M, Morimoto S, Hukuda S, Okabe H, Kato H, Fujino S. 1996. Analysis of the presence of osteocalcin, S-100 protein, and proliferating cell nuclear antigen in cells of various types of osteosarcomas. Eur J Histochem 40: 189–198.

Duarte WR, Shibata T, Takenaga K, Takahashi E, Kubota K, Ohya K, Ishikawa I, Yamauchi M, Kasugai S. 2003. S100A4: a novel negative regulator of mineralization and osteoblast differentiation. J Bone Miner Res 18:493–501.

Fawcett DW. 1994. Bloom and Fawcett. A textbook of histology. 12th ed. New York: Chapman & Hall; Chapter 35.

Fish JH, III, Scholtz AW, Hussl B, Kammen-Jolly K, Ichiki H, Kreczy A, Schrott-Fischer A. 2001. Immunohistochemical and morphological studies on the human fetal cochlea: a comparative view on methods. Tissue Cell 33:189–199.

Foster JD, Drescher MJ, Harfield JS, Drescher DG. 1994. Immunohistochemical localization of S-100 protein in auditory and vestibular end organs of the mouse and hamster. Hear Res 74:67–76.

Hamilton WJ, Mossman HW. 1978. Human embryology. 4th ed. London: Williams & Wilkins.

Hayashi S, Murakami G, Ohtsuka A, Itoh M, Nakano T, Fukuzawa Y. 2008. Connective tissue configuration in the human liver hilar region with special reference to the liver capsule and vascular sheath. J Hepatobiliary Pancreat Surg 15:640–647.

Hirt B, Penkova ZH, Eckhard A, Liu W, Rask-Andersen H, Műller M, Löwenheim H. 2010. The subcellular distribution of aquaporin 5 in the cochlea reveals a water shunt at the perilymph–endolymph barrier. Neuroscience 168:957–970.

Ishikawa Y, Akasaka Y, Kiguchi H, Akishima-Fukasawa Y, Hasegawa T, Ito K, Kimura-Matsumoto M, Ishiguro S, Morita H, Sato S, Soh S, Ishii T. 2006. The human renal lymphatics under normal and pathological conditions. Histopathology 49:265–273.

Jeffery N, Spoor F. 2004. Prenatal growth and development of the modern human labyrinth. J Anat 204:71–92.

Jin ZW, Nakamura T, Yu HC, Kimura W, Murakami G, Cho BH. 2010. Fetal anatomy of peripheral lymphatic vessels: a D2–40 immunohistochemical study using an 18-week human fetus (CRL 155 mm). J Anat 216:671–682.

Kammen-Jolly K, Ichiki H, Scholtz AW, Gsenger M, Kreczy A, Schrott-Fischer A. 2001. Connexin 26 in human fetal development of the inner ear. Hearing Res 160:15–21.

Kikuchi T, Adams JC, Paul DL, Kimura RS. 1994. Gap junction systems in the rat vestibular labyrinth: immunohistochemical and ultrastructural analysis. Acta Otolaryngol 114:520–528.

Ludwin SK, Kosek JC, Eng LF. 1976. The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase labelled antibodies. J Comp Neurol 165:197–208.

Makin CA, Boblow LG, Bodmer WF. 1984. Monoclonal antibody to cytokeratin for use in routine histopathology. J Clin Pathol 37:975–983.

Moll R, Franke WW, Schiller DL. 1982. The catalog of human cytokeratins: pattern of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24.

Moore KL, Persaud TVN. 1998. The developing human: clinically oriented embryology. 6th ed. Philadelphia: Saunders; Chapter 19.

Nishokori T, Hatta T, Kawauchi H, Otani H. 1999. Apoptosis during inner ear development in human and mouse embryos: an analysis by computer-assisted three-dimensional reconstruction. Anat Embryol 200:19–26.

Palva T, Pääkkö P, Ramsay H, Chrobok V, Šimáková E. 2003. Apoptosis and regression of embryonic mesenchyme in the development of the middle ear spaces. Acta Otolaryngol 123:209–214.

Ray B, Roy TS, Wadhwa S, Roy KK. 2005. Development of the human fetal cochlear nerve: a morphometric study. Hearing Res 202:74–86.

Represa JJ, Moro JA, Gato A, Pastor F, Barbosa E. 1990. Patterns of epithelial cell death during early development of the human inner ear. Ann Otol Rhinol Laryngol 99:482–488.

Shi X, Han W, Yamamoto H, Tang W, Lin X, Xiu R, Trune DR, Nuttal AL. 2008. The cochlear pericytes. Microcirculation 15:515–529.

Streeter GL. 1917. The factors involved in the excavation of the cavities in the cartilagenous capsule of the ear in the human embryo. Am J Anat 22:1–25.

Takebayashi S, Yamamoto N, Yabe D, Fukuda H, Kojima K, Ito J, Honjo T. 2007. Multiple roles of Notch signalling in cochlear development. Dev Biol 307:165–178.

Takumi Y, Negelhus EA, Eidet D, Matsubara A, Usami S, Shinkawa H, Nielsen S, Ottersen OP. 1998. Select types of supporting cell in the inner ear express aquaporin-4 water channel protein. Eur J Neurosci 10:3584–3395.

Williams PL. 1995. Gray's anatomy. 38th ed. Edinburgh: Churchill Livingstone. p 1384.

Xu H, Edwards J, Banerji S, Prevo R, Jackson DG, Athanasou NA. 2003. Distribution of lymphatic vessels in normal and arthritic human synovial tissues. Ann Rheum Dis 62:1227–1229.

Yamashita H, Sekitani T, Moriya K, Bagger-Sjöbäck D. 1993. Gliar fibrillary acidic protein-like immunoreactivity in the human fetal inner ear. Acta Otolaryngol Suppl 506:18–23.

Yamashita H, Takahashi M, Bagger-Sjöbäck D. 1995. Expression of S-100 protein in the human fetal inner ear. Eur Arch Otorhinolaryngol 252:312–315.

Yasuda M, Yamada S, Uwabe C, Shiota K, Yasuda Y. 2007. Three-dimesnional analysis of inner ear development in human embryos. Anat Sci Int 82:156–163.

Zambidis ET, Sinka L, Tavian M, Jokubaitis V, Park TS, Simmons P, Péault B. 2007. Emergence of human angiohematopoietic cells in normal development and from cultured embryonic stem cells. Ann NY Acad Sci 1106:223–232.