Expression of cytokeratin polypeptides during development of the rat inner ear


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Summary. The expression of cytokeratin polypeptides in the different epithelia of the developing inner ear of the rat from 12 days post conception to 20 days after birth was analysed immunohistochemically, using a panel of monoclonal antibodies. Throughout the development of the complex epithelial lining of the inner ear originating from the otocyst epithelium, only cytokeratins which are typical of simple epithelia were expressed. Cytokeratins 8, 18, and 19 were detectable shortly after the formation of the otocyst from the ectoderm (12 dpc), whereas cytokeratin 7 expression was delayed and first appeared in the vestibular portion and subsequently in the developing cochlear duct. During the development of the different types of specialized cells, differentiation-dependent modulation of the cytokeratin expression patterns was observed. In the mature inner ear, the specialized cell types displayed a function-related cytokeratin expression profile, both in the cochlear and vestibular portion. Cytokeratin expression in the flat epithelium of the vestibular portion suggests a more complex composition of this epithelium than has been established from routine morphology. Remarkably, the cochlear sensory cells were apparently devoid of cytokeratins, but no final conclusion could be drawn on the presence of cytokeratins in the sensory cells of the vestibular portion, because of the difficulty to delineate the cell borders between sensory cells and supporting cells.

Introduction

The membranous inner ear consists of the endolymphatic duct system which is lined with epithelium and situated in the temporal bone. It is filled with endolymph, a peculiar extracellular fluid with an intracellular cation composition (Johnstone et al. 1963; Bosher and Warren 1971).

The membranous inner ear arises from the auditory placode, a thickening of the embryonal ectoderm, which, by invagination, forms the otic vesicle. Via a complicated process of cell proliferation, this vesicle is transformed into a series of interconnected compartments which can be divided into a cochlear part, the cochlear duct, concerned with the perception of sound and a vestibular part, consisting of the otolithic organs (saccule and utricle) and the ampullar organs concerned with function of equilibrium.

The epithelial lining, derived from the epithelium of the otic vesicle, shows a large variety of structurally and functionally different cell types. All the compartments are provided with an area of neurosensory epithelium: the organ of Corti in the cochlea and the saccular and utricular maculae and the cristae ampullares in the vestibular part (Fig. 1).

In addition to the sensory cells, the sensory areas also contain supporting cells. In the vestibular part, both types of cells are closely packed (Fig. 1 b), whereas the organ of Corti shows a rather complicated construction with inner and outer hair cells and various types of supporting elements, which are separated by fluid-filled spaces (Fig. 1 a).

The remaining part of the epithelial lining of the endolymphatic duct system consists of different types of epithelium, part of which has been shown to be actively involved in the maintenance of the peculiar cation composition of endolymph (Kuijpers 1969; Kuijpers and Bonting 1969).

The major part of the endolymphatic duct system is surrounded by a fluid-filled perilymphatic space. It originates from resorption of the mesenchymal tissue during development and is lined by, so-called, "mesothelium".

A new and valuable tool in the classification of different cell types is the typing of their intermediate filament proteins. Cytokeratins are the main intermediate filament constituents of epithelia and specific subsets of different cytokeratin polypeptides are expressed in different epithelia depending on their origin or the type of differentiation (Franke et al. 1981; Moll et al. 1982; Tseng et al. 1982; Quinlan et al. 1985; Sun et al. 1985). Studies on embryonic development have shown that the
expression of cytokeratins is developmentally regulated (Banks-Schlegel 1982; Moll et al. 1982; Andrews et al. 1985; Lane et al. 1985; Clausen et al. 1986; Viebahn et al. 1987).

As the highly specialized epithelia in the various parts of the inner ear are derived from the simple epithelium of the otic vesicle, monitoring of cytokeratin expression during development can contribute to a better understanding of the process of differentiation during development of this organ.

Studies on the distribution of cytokeratin polypeptides both in the adult inner ear and during development are rather scarce. Except for the study conducted by Raphael et al. (1987) dealing with the expression of cytokeratin 18 and 19 during the development of the guinea pig cochlea, other studies on the expression of intermediate filament protein in the developing inner ear are limited to one distinct developmental stage in the mouse and human inner ear. Moreover, the data are conflicting in many respects (Anniko et al. 1986, 1987, 1989a, 1990).

This report presents a detailed immunohistochemical study on the expression of cytokeratin polypeptides throughout the development of the inner ear of the rat. To overcome the shortcomings in previous studies we have used an extended panel of polyclonal and monoclonal antibodies and applied them to the most important stages of inner ear development from 12 days before birth up to 20 days after birth.

Materials and methods

Animals

This study was performed on Wistar rats. The development of the inner ear was followed from 12 days post conception (dpc) to 20 days after birth (dab). The gestational period of the rat is 21 days. The day of the vaginal plug was considered as day 1.

Immunohistochemistry

Immunohistochemical staining procedures were performed on 7 µm thick frozen sections. For this purpose, embryos were collected from pregnant female rats under Nembutal anesthesia and freed from their membranes. Small embryos were frozen as a whole in liquid nitrogen, whereas only the head was frozen of larger embryos. In the case of newborn and young rats, a tissue block containing the middle and inner ear was dissected from the skull after decapitation.

As the cartilaginous capsule surrounding the membranous inner ear shows progressive osseification after birth, all postnatal specimens were stored in a decalcification solution immediately after dissection, containing 10% EDTA (disodium salt) and 7.5% polyvinylpyrrolidone in 0.1 M Tris-HCl buffer (pH 7.2) (Johnson et al. 1986) at 4°C for a period ranging from 1 h to 2 days, depending on the age of the specimen. After rinsing in the same solution but without EDTA, the specimens were frozen in liquid nitrogen and sectioned on a cryostat. Although the epithelial lining of the inner ear is a very delicate structure, the morphological preservation was generally satisfactory, except for the cochlear duct epithelium during final maturation. During that period the epithelium was frequently found to be disrupted, notably in the area of the organ of Corti. Fixation procedures applied to improve morphology, often resulted in severe loss of immunoreactivity. Only antibody RCK102 was found to resist short fixation in a fixative (Tonnier et al. 1990) modified after McLean and Nakane (1974). For that purpose, fresh specimens were fixed for two hours at 4°C in 0.04 M phosphate buffer (pH 6.2) containing 0.1 M lysine-HCl, 0.01 M metaperiodate and 2% paraformaldehyde, prior to storage in the decalcification solution. Comparison of immunoreactivity on sections obtained from freshly frozen or dissected specimens revealed that EDTA treatment up to two days for the 20 day specimens had no detrimental effect on the immunostaining reactions with the applied antibodies. It even revealed an enhancing effect on
cytokeratin detectability, which has been suggested to be due to an unmasking effect of the epitopes (Tonnaer et al. 1990). A comparable effect was observed in the prental specimens after EDTA exposure. The cryostat sections were placed on poly-L-lysine coated slides, dried for 30 min in a cold air stream and subsequently stored at -70°C until required. For immunostaining, sections were fixed in acetone (5 min at 4°C) washed in PBS and thereafter exposed to the polyclonal or monocolonal antibodies for 45 min in a moist chamber, either as undiluted culture supernatants or diluted in PBS, as indicated in Table 1. The sections were then washed in PBS and incubated for 30 min with horse-radish peroxidase conjugated rabbit anti-mouse for monocolonal antibodies or swine anti-rabbit immunoglobulin (Dakopatts, Glostrup, Denmark) for polyclonal antibodies diluted (1:40) in PBS and containing 5% rat serum for monocolonal antibodies or 10% goat serum for polyclonal antibodies. After washing in PBS, followed by washing in Na-acetate buffer (0.05 M; pH 4.9), the peroxidase activity was detected using 0.02% 3'-amino-9-ethylcarbazole, 5% dimethylformamide and 0.01% hydrogenperoxide in Na-acetate buffer (pH 4.9). Control sections were incubated in nonimmune serum.

Counterstaining was performed with Mayer's hemalum. The specificity and references to the antibodies used are given in Table 1. Mouse monoclonal antibodies E2 was a kind gift from Dr. S. Troyanovsky (Moscow) and reacts specifically with the rat cytokeratin 8 equivalent. It should be kept in mind that most of the antibodies used were characterized for their specificity to human cytokeratins, which have been numbered 1–20 (Moll et al. 1982, 1990). When indicating a certain cytokeratin polypeptide by a number, we refer to the rat analogue of its human counterpart.

For orientation, adjacent sections were stained with haematoxylin-eosin.

Results

At twelve dpc, shortly after otocyst formation, the cytokeratin (CK) expression pattern was already different from that seen in the primitive epidermis. With the antibodies pKer, RCK102 (CK5+8), E2(CK8) LP2K (CK19) and CK18-2(CK18) the whole epithelial lining of the otocyst, consisting of pseudostratified epithelium, revealed a rather weak staining reaction except for the basal and apical parts, which stained more intense. RGE53(CK18) showed only a weak local staining, while no reaction was found with antibody RCK105(CK7) (Fig. 2).

Antibodies 1C7(CK13), 6B10(CK4) and RKS60 (CK10) were negative in all inner ear cell types at all stages of development. At other sites of the rat body these antisera showed a specific positive reaction. In general, antibody RGE53 directed against cytokeratin 18, reacted with less cells as compared to the antibody CK18-2, which recognizes the same cytokeratin protein.

Subsequently, the otocyst develops into a duct system, which can be subdivided into a vestibular and cochlear portion, lined with different-sized epithelium. The development of the cytokeratin expression patterns in these portions will be described separately.

Cochlea

At 15 dpc the transverse section of the cochlear duct is ovalshaped. The whole epithelial lining of the developing cochlear duct displayed a positive reaction to antibodies pKer, RCK102(CK5+8), E2(CK8), LP2K (CK19) and CK18-2(CK18), although the staining pattern was different in the posterior wall as compared to the anterior wall (Fig. 3a–d). The cells of the posterior wall were much less reactive. No reaction was found with antibody RCK105(CK7). With RGE53(CK18), the staining was limited to the anterior wall, showing a weak to moderate reaction (Fig. 3a–d).

At 18 dpc, the delineation of Reissner’s membrane, the stria vascularis and external sulcus was visible in the anterior wall of the cochlear duct (Fig. 3c).

Antibodies pKer, RCK102(CK5+8), E2(CK8), LP2K(CK19) and CK18-2(CK18) exhibited a distinct reaction in these structures, although the presumptive external sulcus stained only weakly with CK18-2(CK18).

The reaction to RGE53(CK18) was limited to Reissner’s membrane and the stria vascularis, whereas RCK105(CK7) only revealed weak staining of the stria vascularis and external sulcus (Fig. 3f–i).

At this age, the posterior wall had been transformed into the so-called greater and lesser epithelial ridges, harbouring the developing organ of Corti (Fig. 3e). This transformation was accompanied by a change in the expression of various cytokeratins (Fig. 3f–i). The lesser epithelial ridge displayed homogeneous positive staining with antibodies pKer, LP2K(CK19), RCK102(CK5+8) and E2(CK8), whereas CK18-2(CK18) only showed a scattered reaction. In the greater epithelial ridge, pKer, RCK102(CK5+8), E2(CK8) and LP2K(CK19) showed a scattered reaction, mainly confined to the basal part of the cells. Staining with CK18-2(CK18) was very weak in this area, whereas no reaction was found with the antibodies RGE53(CK18) and RCK105(CK7). The area of the developing organ of Corti displayed an inconsistent weak reaction to antibodies pKer, LP2K(CK19), RCK102(CK5+8), E2(CK8), CK18-2(CK18) and RGE53(CK18) in the basal part, the site of the developing supporting cells.

At 1 day after birth (dab), the cochlear duct showed its typical triangular form in transverse section (Fig. 4). The whole epithelial lining showed a positive reaction to antibodies pKer, RCK102(CK5+8), E2(CK8), LP2K(CK19) and CK18-2(CK18). With RGE53(CK18), staining of the non-sensory epithelium was confined to Reissner’s membrane and the stria vascularis, whereas RCK105(CK7) only stained the stria vascularis and the external sulcus cells (Fig. 4b–f). With regard to the organ of Corti, all the antibodies, except for RCK105(CK7), showed a weak positive reaction in the area of the developing supporting cells.

At 5 dab the cytokeratin profile of the cochlear duct epithelium did not differ significantly from that observed on day 1. At this age, the basolateral part of the cell membrane of the marginal cells of the stria vascularis had developed numerous corrugations, whereas the external sulcus cells displayed basal extensions which penetrated deeply into the spiral lamina (Fig. 5a–e). In the organ of Corti, the developing supporting elements revealed staining with antibodies pKer, RCK102(CK5+8), E2(CK8), LP2K(CK19), RGE53(CK18) and CK18-2(CK18) but not with RCK105(CK7). No cytokeratin expression could be established in the sensory cells (Fig. 6a, c).
During further maturation, pronounced changes took place in the cytokeratin expression pattern. Staining with antibody RCK105(CK7) gradually disappeared from the stria vascularis and external sulcus cells, whereas staining with RGE53(CK18) disappeared from Reissner's membrane and the organ of Corti. In the mature cochlear duct all non-sensory cell types revealed staining with the antibodies pKer, RCK102(CKs5+8) (Fig. 6b), E2(CK8), LP2K(CK19) and CK18-2(CK18), although staining intensity of the various cell types varied with the different antibodies. In contrast, staining with RCK105(CK7) was limited to a few transitional cells at the margins of the stria vascularis, while with RGE53(CK18) only the stria vascularis displayed a positive reaction (Fig. 5f-j). In the organ of Corti, staining with cytokeratin antibodies remained confined to the supporting cells (Deiters cells, pillar cells, inner border cells, Hensen cells). With none of the antibodies applied staining could be established in the sensory cells (Fig. 6b, d).

**Vestibular compartment**

At 15 dpc the duct system of the vestibular portion was lined with epithelium of different size. At this stage the whole epithelial lining stained with antibodies pKer, RCK102(CKs5+8), E2(CK8), LP2K(CK19) and CK18-2(CK18) with regional differences in intensity. Only part of the epithelium displayed a weak reaction with the antibodies RGE53(CK18) and RCK105(CK7) (Fig. 7a-d). At 18 dpc the vestibular duct system showed a clear subdivision into sacculus, utricle and ampullar organs, while the areas of developing sensory epithelium (macu-
Fig. 4a–f. Immunohistochemical staining of cochlear duct at 1 dab with the antibodies LP2K(CK19) (b); RCK102(CKs3+4) (c); CK18-2(CK18) (d); RGE53(CK18) (e); RCK105(CK7) (f); (a) Haematoxylin-eosin stained section; e: Cells of Claudius; ex: external sulcus; t: Primitive inner sulcus; m: "Mesothelium" of Perilymphatic space (p); r: Reissner's membrane; s: Stria vascularis; sl: Spiral lamina; o: Sensory cells; s: Supporting cells. Bar indicates 40 μm

Fig. 5a–j. Immunohistochemical staining of the lateral wall of the cochlear duct at 5 dab (a–e) and at 15 dab (f–j) with the antibodies LP2K(CK19) (a, f); RCK102(CKs5+8) (b, g); CK18-2(CK18) (c, h); RGE53(CK18) (d, i); RCK105(CK7) (e, j). ex: External sulcus; p: Spiral prominence; s: Stria vascularis. Upper bar indicates 25 μm; lower bar 30 μm
lae and crista) could clearly be distinguished (Fig. 8a, g). Immunostaining exhibited a strong positive reaction in the whole epithelial lining to pKer, RCK102(CKs5+8), E2(CK8), CK18-2(CK18) and LP2K(CK19). With the antibodies RGE53(CK18) and RCK105(CK7) the non-sensory epithelium stained heterogeneously, but the reaction with RGE53(CK18) was more pronounced. The developing maculae and crista revealed only a weak reaction with both antibodies (Fig. 8b-f, h-l).

At 1 dag, immunostaining with the various antibodies did not differ significantly from that observed at 18 dpc. Within 5 days after birth the epithelial lining of the vestibular portion reaches morphological maturation. During this period of time an area of so-called dark cells and an area of cuboidal cells adjacent to maculae and crista became visible (Fig. 9c, i). Immunostaining of the mature vestibular portion with the antibodies pKer, RCK102(CKs5+8), E2(CK8) and CK18-2(CK18) displayed homogeneous staining of the whole epithelial lining (Fig. 9a, d, g, j). A comparable staining pattern was found with LP2K(CK19), but this antibody failed to react with the cuboidal cells (Fig. 9b, h). With the antibody RGE53(CK18) both dark and cuboidal cells intensely stained while the remaining part of the non-sensory epithelium stained heterogeneously (Fig. 9e, k). A weak heterogeneous staining of the non-sensory epithelium was observed with antibody RCK105(7), but no reaction was found in the cuboidal and dark cells (Fig. 9f, l).

In the sensory areas, the staining pattern obtained with the antibodies pKer, RCK102(CKs5+8), LP2K(CK19), E2(CK8) and CK18-2(CK18) mirrors the position of the supporting cells and staining reactions were most pronounced at the base and at the apex of these cells (Fig. 9a, b, d, g, h, j, m). With RGE53(CK18) only the apical surface was positive (Fig. 9e, k), whereas with RCK105(CK7) only a distinct small population of cells stained (Fig. 9f, l, arrowheads). No final conclusion could be made on basis of these staining patterns, with respect to the intermediate filament content of the sensory cells, because of the close proximity of supporting and sensory cells. Throughout development no cytokeratin expression was found in the so-called "mesothelial lining" of the perilymphatic space of the cochlea and vestibular compartments (Figs. 3, 4, 6, 8, 9).

Discussion
The present study provides a comprehensive description of the distribution of cytokeratin polypeptides during development of the complex epithelial lining of the rat inner ear. Although most of the antibodies used in this study were raised against human cytokeratin proteins, the antibodies which normally react to cytokeratins 7, 8, 18 and 19 in human simple epithelia were found to be positive in the simple epithelial lining of the rat inner ear. This specificity of these antibodies, however, has not been described for epithelia of other organs of the rat (Hereman et al. 1985; Verhagen et al. 1988; Ramaekers et al. 1989; Vennix et al. 1990). Although antibodies CK18-2 and RGE53 are both specific to CK18, RGE53 only stained part of the otocyst epithelium and this difference was maintained throughout development. An explanation for this finding could be that the configuration of the epitopes recognized by
Fig. 7a–d. Immunohistochemical staining of the developing vestibular compartment at 15 dpc with the antibodies LP2K(CK19) (a); CK18-2(CK18) (b); RGE53(CK18) (c); RCK105(CK7) (d). Bar indicates 55 μm

Fig. 8a–l. Immunohistochemical staining of vestibular portion (utricle b–f; ampulla b–l) at 18 dpc with the antibodies RCK102(CK5+8) (b, h); LP2K(CK19) (c, i); CK18-2(CK18) (d, j); RGE53(CK18) (e, k); RCK105(CK7) (f, l); (a, g) Haematoxylin-eosin stained sections; m: Macula; c: Crista. Bar indicates 50 μm
these antibodies is structurally different in the various cell types. This phenomenon has been reported before by Franke et al. (1987) and by Schäfers et al. (1989).

Developmentally regulated cytokeratin expression patterns have so far been shown in other epithelia such as epidermis and the urogenital system (Banks-Schlegel 1982; Moll et al. 1982; Andrews et al. 1985; Lane et al. 1985; Clausen et al. 1986; Viebahn et al. 1987). This study demonstrates an alteration in the expression of cytokeratin polypeptides, in relation to structural and functional maturation of some cell types in both the cochlear and vestibular partition.

**Cochlea**

Throughout the development of the cochlear duct, the uniform distribution pattern of cytokeratins 8, 18, and 19 was maintained in the main part of the epithelial lining. However at 18 dpc, distinct changes occur in the cytokeratin expression patterns in the posterior wall of the cochlear duct accompanying cell differentiation. This is particularly obvious in the area of the developing organ of Corti. At this stage and during further maturation of this structure, it became apparent that the sensory cells were virtually devoid of cytokeratins. This apparent
absence of cytokeratin expression is quite remarkable in epithelial cells and has also been reported in the adult organ of Corti of the guinea pig (Raphael et al. 1987) and recently in man (Arnold and Anniko 1990). On the contrary, Anniko et al. (1987) showed the presence of cytokeratin polypeptides in the sensory cells of the human inner ear at 4 months gestation, but failed to show cytokeratins in the newborn mouse at a comparable stage of development (Anniko et al. 1986, 1989a). The absence of cytokeratins in distinct epithelial cells has also been observed in the sensory cells of the olfactory epithelium (Voirath et al. 1985) which develops in a similar way from embryonal ectoderm, but differs from the sensory cells of the organ of Corti in so much as they differentiate into bipolar neuronal cells. We can only speculate about the functional significance of the lack of cytokeratin polypeptides in the sensory cells of the cochlea. Possibly, the active contractions these cells exhibit during depolarisation (Zwicker 1979; Zener et al. 1988), involve the presence of a less rigid cytoskeleton structure, including intermediate filament cytoskeleton.

In the anterior wall the expression of cytokeratins 8, 18 and 19 remained unchanged throughout differentiation of this epithelium into stria vascularis, Reissner's membrane and external sulcus. However, with the cytokeratin 18 antibody, RGE53, staining disappeared from Reissner's membrane and the organ of Corti, leaving only the stria vascularis stained in the mature cochlea. Regarding the stria vascularis, this structure is composed of three cell layers in the mature inner ear: 1. an outer layer of marginal cells bordering the endolymphatic space, 2. an intermediate cell layer and 3. a layer of basal cells. The expression of cytokeratins in the marginal cells confirms their epithelial nature and corresponds with their origin from the otocyst epithelium. The intermediate cells are assumed to originate from melanocyte precursor cells migrating from the neural crest (Schart and Spoendlin 1987; Schrott et al. 1988), whereas basal cells are derived from the mesenchym. The lack of cytokeratin expression in these two latter cell types is in agreement with these assumptions.

The marginal cells of the stria vascularis and external sulcus cells showed a remarkable behaviour with respect to the expression of cytokeratin 7. As compared to cytokeratins 8, 18 and 19, the expression of cytokeratin 7 was delayed during inner ear development. It first appeared at 15 dpc in part of the epithelium of the developing vestibular portion and at 18 dpc it was only expressed in the part of the anterior wall of the cochlear duct, representing the developing stria vascularis and external sulcus. This limited expression was maintained up to five days after birth. During final morphological maturation cytokeratin 7 disappeared from these structures, except for a few transitional cells between stria and adjacent epithelium. During the same period staining with the antibody RGE53(CK18) disappeared from Reissner's membrane and organ of Corti, leaving only the stria vascularis stained. From the biochemical and physiological studies by Bosher and Warren (1971), Kuipers (1974) and Romand (1983) it appears that these changes in cytokeratin expression coincide with the functional maturation of the cochlea, i.e. the typical intracellular cationic composition of the endolymph, the Na-K-ATPase activity of the stria vascularis and the electric activity of the cochlea reach adult conditions. It is therefore tempting to speculate about a possible relation between the functional condition of the stria vascularis, which is shown to be responsible for the maintenance of the cationic gradients between endolymph and serum, necessary for cochlear function, and the intermediate filament composition of these cells.

**Vestibular portion**

As in the cochlear portion, homogeneous expression of cytokeratins 8, 18 and 19 was found throughout development in the major part of the epithelial lining of the vestibular compartment. During the final stages of maturation of the different cell types expression of CK's 8 and 18 did not change, but cytokeratin 19 disappeared from the developing cuboidal cells. During the same period cytokeratin 7 disappeared from both cuboidal and dark cells, while staining with RGE53(CK18) became more pronounced in these areas. In contrast to the organ of Corti a limited expression of cytokeratin 7 and staining with RGE53(CK18) persisted in maculae and cristae. These changes coincide with the development of neural responses evoked by appropriate stimulation of the vestibular sensory epithelium (Curthoys 1979). Owing to the fact that it was difficult to delineate the cell borders between the sensory and supporting cells no definite conclusion could be drawn regarding the cytokeratin expression in the sensory cells.

**Cochlea versus vestibular portion**

Comparison between the different cell types in the cochlear duct and the vestibular portion revealed similar cytokeratin expression patterns in the stria vascularis in the cochlear duct and the dark cells in the vestibular portion. The morphology of the dark cells resembles that of the marginal cells of the stria vascularis. Both cell types show numerous interdigitations of the basolateral cell membrane and a high Na-K-ATPase activity (Kuipers 1969; Kuipers and Bonting 1969). They are assumed to regulate the high potassium and low sodium concentration of the endolymph (Kuipers and Bonting 1969; Kimura 1969). The cuboidal cells, shown to be involved in the secretion of glycoproteins (Mann and Kuipers 1987) do not have a counterpart in the cochlear duct. Their cytokeratin expression pattern differs as compared to those of the other secretory structures, the stria vascularis and dark cells, in particular with respect to CK19.

The supporting cells in the sensory areas of both cochlea and vestibular portion have in common a pronounced expression of cytokeratins 8, 18, 19. In contrast to the organ of Corti, staining for RGE53 persists in the adult maculae and cristae, while a small population of supporting cells expresses cytokeratin 7. With respect
to the sensory cells of the vestibular compartments, no final decision could be made on the absence of cytokeratin polypeptides in these cells as established in the cochlea, because of the close proximity of heavily immunoreactive supporting cells. The flat epithelium of the vestibular compartments can be considered as the counterpart of the epithelium of Reissner's membrane, which main function is assumed to prevent passive leakage of sodium ions into and potassium ions out of the endolymphatic space. Although part of the epithelial lining of the vestibular compartments shows a similar cytokeratin expression as compared to Reissner's membrane, the cytokeratin profile in the remaining part of the vestibular epithelium suggests a more complex tissue organisation than known so far.

Apart from the developmental study on the expression of cytokeratins 18 and 19 in the cochlear duct of the guinea pig (Raphael et al. 1987), which is in line with the present observations in the rat, several data have been reported on cytokeratin expression in the developing mouse and human inner ear (Anniko et al. 1986, 1987, 1989a, b, 1990). However, these data are mostly confined to the description of one developmental stage and differ in several aspects from the observations made in the present study, even with the use of antibodies recognising the same antigens. Although these discrepancies might be ascribed to differences between species, it seems more likely that technical aspects may play a role. The use of mouse monoclonal antibodies on frozen mouse sections can lead to specific reactions because of the presence of mouse immunoglobulins which will be detected by the secondary anti-mouse IgG antibodies used in the immunochemical procedures. In addition, prolonged exposure to a paraformaldehyde containing fixative for the human tissue (Anniko et al. 1990) can seriously interfere with the detectability of cytokeratin polypeptides in the inner ear epithelium (Tonraer et al. 1990).

In summary, this study demonstrates that the developmentally-regulated cellular differentiation in the rat inner ear is accompanied by function-related changes in cytokeratin expression patterns within different types of epithelial cells.

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