Differentiation (1987) 35:151–162

Differentiation

Modulation of intermediate-filament expression in developing cochlear epithelium

Yehoash Raphael[1,2], Gabriel Marshak[1,3], Amihai Barash[4], and Benjamin Geiger[1]*

[1] Department of Chemical Immunology, Weizmann Institute of Science, Rehovot 76100, Israel
[2] Department of Embryology and Teratology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[3] Department of Otolaryngology and [4] Department of Obstetrics and Gynecology, Kaplan Hospital, Rehovot 76100, Israel
affiliated with the Hebrew University and Hadassah Medical School, Jerusalem, Israel

Abstract. The present study was designed to characterize the expression and distribution of intermediate filaments (IFs) in the diverse cellular elements of inner-ear epithelium in guinea pig and man. Using immunofluorescence microscopy with a battery of IF-specific monoclonal antibodies, we show that the epithelium of the otocyst expresses cytokeratin (CK) polypeptides typical of simple epithelia. Cells in the early otic ganglion were also positively labelled for cytokeratins, suggesting that they are of otocystic epithelial origin. Cytokeratin distribution was largely homogeneous in the early cochlear duct but as the epithelium differentiated, differences in the distribution of cytokeratin between the various cell types became detectable. Characteristically, cochlear hair cells became devoid of cytokeratin labelling, and remained unlabelled with antibodies specific for all other IF classes. The neural tissue of the inner ear was also devoid of cytokeratins and was typically positive for neurofilaments. Vimentin IFs were abundant in the mesenchymal tissues around the membranous labyrinth. Desmin and glial fibrillary acidic protein were not detectable in the cochlea. The apparent absence of all IFs from the cochlear hair cells in both guinea pig and man, as revealed by immunofluorescence and electron microscopy, and the possible significance of their absence for cochlear physiology, are discussed.

Introduction

The cytoskeleton of eukaryotic cells consists of complex fibrillary networks composed of microfilaments, microtubules, and intermediate-sized filaments (IF; for review see [31]). Biochemical and immunochemical studies during the past several years have shown that while IFs of all cells appear to have similar ultrastructure, their polypeptide composition may vary remarkably between cell types [2, 20, 23, 25]. IFs are commonly divided into five major classes. These include: the cytokeratin (CK) polypeptides, which are expressed in epithelial tissues; vimentin IFs, characteristic of mesenchymally derived cells; desmin filaments, specific for muscle tissues; neurofilaments, usually found in neurons, and glial fibrillary acidic protein (GFAP) in astrocytes [2, 25]. Many recent studies have shown that the cell-type-specific expression of the various IFs in normal and in transformed cells may be used to identify their embryonic origin and particular developmental stage. This feature of IF allowed the use of antibodies against them for tracing the histogenesis of normal and malignantly transformed tissues [8, 9, 24, 35]. It should be indicated that, despite their ubiquitous presence in cells, the functional involvement of IFs in specific cellular processes is still poorly understood.

In the present study we have used immunocytochemical labelling of IFs to characterize the various epithelia of the mammalian inner ear. This tissue is derived from the otocyst, an invagination product of the ectodermal otic placode [29, 30, 32]. Later in development, cells derived from this epithelium differentiate into various sensory, supporting, and secretory elements which line the cochlear scala media. External to the basement membrane, cellular as well as extracellular contributions from the surrounding mesenchyme join the epithelium to form the various functional elements of the cochlea including the stria vascularis, Reissner's membrane, and the organ of Corti (for review see [1, 17, 21]). The cochlear cytoarchitecture is striking in its high degree of organization and in the multitude of structurally and funtionally different cell types it contains, all of which are derived from the same primitive epithelium of the otocyst. This remarkable diversification renders the inner ear an attractive model with which to investigate mechanisms of epithelial segregation and differentiation. A recent report by Anniko et al. [3] suggested an unexpected profile of IF expression in the newborn mouse inner ear. According to this report, CK and desmin were not present in the inner ear, while vimentin was detected in the cochlear epithelium.

In the present study we have used a battery of monoclonal antibodies for the study of IF distribution in the developing human and guinea pig auditory organ. It was found that the expression of CK in the inner and outer hair cells gradually diminished as differentiation advanced. Since neither neurofilaments, nor any other type of IF, were detected in the mature hair cells, these cells seem to comprise a rare type of cell, completely devoid of all IFs. Moreover, immunohistochemical labelling of the otocyst pointed to the presence of CK-positive cells in the early otic ganglion. This finding suggests that epithelial cells derived from the otocyst are involved in the development of the adjacent neural elements.

* To whom offprint requests should be sent

Materials and methods

Tissues

Human and guinea pig inner ears were used in this study. Human embryos were obtained from therapeutic abortions performed at the Kaplan hospital. The developmental stage of human embryos was determined according to the reported last menstruation and confirmed by ultrasonography and staging tables [15]. The following developmental stages were chosen for the present investigation: 3-week-old embryos with the inner ear at the otocyst stage; 7- to 8-week-old embryos with an undifferentiated cochlear duct; 12- to 14-week-old embryos showing advanced differentiation of cochlear epithelium, and 18-week-old embryos displaying a nearly mature cochlea.

Pregnant guinea pigs at different gestational stages were obtained from the Experimental Animal Center of the Weizmann Institute. Embryos were from litters conceived by postpartum coitus, to enable determination of their age. In agreement with previous reports [6, 33], the otocyst stage was found in 2-week-old embryos, cochlear duct at about 3 weeks of development, early differentiation of the cochlear epithelium at 4 weeks, and advanced differentiation at 5–6 weeks of development. Sections of the mature ear were performed using neonates, and surface-preparation analysis was made on cochleas of 1- to 2-week-old guinea pigs.

Immunocytochemistry

Guinea-pig embryos were obtained under anesthesia, and whole heads (for the otocyst stage) or temporal bones (for later stages) were quickly frozen in isopentane cooled in liquid nitrogen. Frozen sections of 4–6 μ were cut at −23° C in a Frigocut 2800 cryostat (Jung Reichert, FRG). Sections were recovered on clean glass slides, air-dried, fixed in cold (−20° C) acetone, and immunolabelled with antibodies as previously described [8]. For the whole-mount labelling of the organ of Corti, guinea-pig cochleas were immersed in saline solution, the bony capsule was removed, and the surface of the organ of Corti facing the scala media was exposed by dissecting away the spiral ligament with the stria vascularis and the tectorial membrane. Half-turns of the organ of Corti were then separated from the bony modiolus, fixed in cold acetone, and immunostained using the same protocol described above for sections. Most sections and surface preparations were double-stained for both cytokeratins and F-actin, using antibodies in conjunction with fluoresceinated phalloidin (Molecular Probes, USA). Immunostained sections were dehydrated in absolute alcohol, mounted in Entellan (Merck, FRG) and examined using a Zeiss Axiophot photomicroscope equipped for epifluorescence, using ×20/0.5, ×40/1.0 oil or ×100/1.3 oil Plan-Neofluar objectives.

Immunochemical reagents

The following reagents were used: 1. A broad spectrum monoclonal antibody KG 8.13 [12] directed against human cytokeratin (CK) polypeptides nos. 1, 5, 6, 7, 8, 10, 11, and 18 according to the classification by Moll et al. [23]. In guinea pig this antibody has been shown to react with several polypeptides of molecular weights 61 Kd, 56.5 Kd, 53 Kd, 50 Kd, and 43 Kd [22].
2. Monoclonal cytokeratin antibody KM 4.62 specific to polypeptide no. 19 in man [13].
3. Monoclonal cytokeratin antibody Ks B.18 reacting specifically with CK polypeptide no. 18 in man and with a CK polypeptide of molecular weight 49 Kd in guinea pig [22].
4. Monoclonal anti-CK KS 8.58 specific for human polypeptides nos. 13 and 16, which are present in stratified epithelia [11, 16] and to CK polypeptides with apparent molecular weights of 53 Kd, 46 Kd, and 50 Kd in guinea pig [22].
5. Monoclonal antibody DK 80.20, which reacts specifically with desmosomes (Leitner and Geiger, unpublished results).
6. Monoclonal antibodies specific for vimentin, desmin, the 68-Kd neurofilament polypeptide, and GFAP.
7. Polyclonal antibodies specific for desmin and neurofilaments.
Most of the above-mentioned antibodies were obtained or are now available from BioMakor, Rehovot, Israel.

Secondary antibodies were affinity-purified antibodies to mouse Fab′, or to rabbit IgG, raised in goat, and conjugated to lissamine rhodamine sulfonyl chloride prepared as previously described [5, 10].

Electron microscopy and detergent extraction

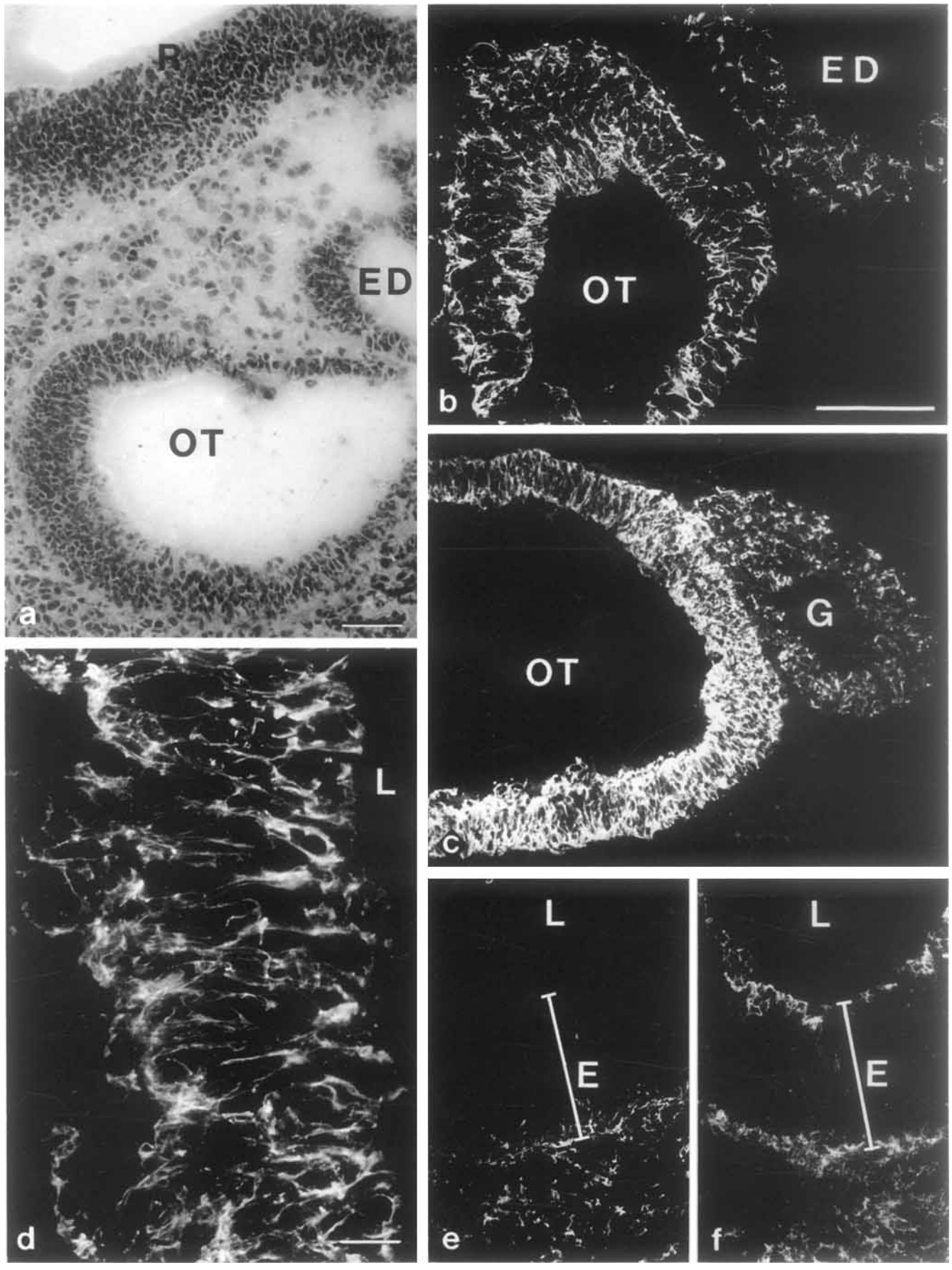
Freshly dissected cochleas were immersed in a solution containing 0.5% Triton X-100 in 50 m*M* 2(N-Morpholino)ethanesulfonic acid (MES) buffer, pH 6.5, for 5 min. After rinsing, the tissue samples were fixed for 2 h in 2% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.3, containing 0.5 m*M* $CaCl_2$. Tissues were postfixed in osmium tetroxide, en-block stained with 2% uranyl acetate, dehydrated in alcohol, and embedded in epon (Polybed 812, Polysciences, USA). Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a Philips 410T Electron Microscope at 80 KV.

Results

Cytokeratin distribution in the otocyst

Immunofluorescent labelling of the early guinea pig otocyst epithelium with CK-specific antibodies (Fig. 1) revealed a nearly homogeneous distribution of CK throughout the epithelial layer, while the surrounding mesenchyme (Fig. 1a)

Fig. 1a–f. Cryosections of embryonic inner ears at the otocyst stage in guinea pig. The sections were stained with hematoxylin-eosin (**a**) or immunofluorescently labelled (**b–f**). Staining with broad-spectrum monoclonal antibody to cytokeratin (CK), KG 8.13 (**b–d**) is positive in the epithelium of the otocyst (*OT*) and in the endolymphatic duct (*ED*), but not in the surrounding mesenchyme or the brain (*R*), seen in **a**. Intense CK labelling appears near the lumen (*L*). Note that CK-positive cells in **c** are associated with the acoustic ganglion (*G*). The ganglion in these sections was identified by its positive reactivity with antibody directed against neurofilament protein, the morphology in phase contrast, and the position relative to the otocyst, endolymphatic duct, and rhombencephalon. **e** Antibodies to vimentin stain the mesenchyme outside the epithelium (*E*). **f** Labelling for actin with fluoresceinated phalloidin; *bars* represent 50 μ in **a–c, e** and **f** and 10 μ in **d**


P
ED
OT
a
ED
OT
b
G
OT
c
L
d
L
E
e
L
E
f

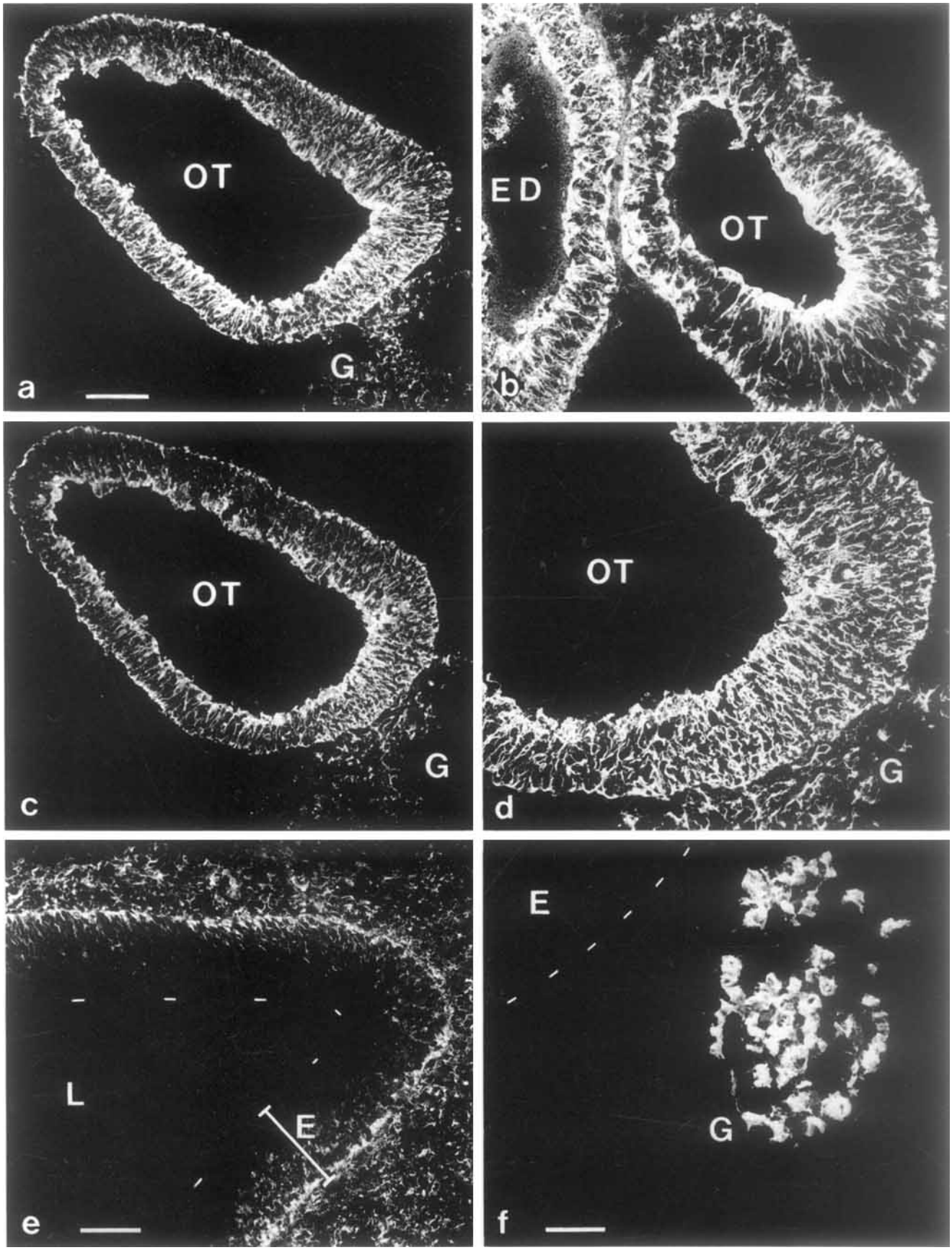


Fig. 2a–f. Cryosections of human otocyst immunofluorescently labelled with antibodies specific for various CK polypeptides (**a–d**), vimentin (**e**), and neurofilament protein (**f**). **b** Labelling with the broad-spectrum KG 8.13, monoclonal antibody to CK, shows staining in otocyst (*OT*) and adjacent ganglion (*G*) (**a**) and primordial endolymphatic duct (*ED*). The distribution of CKs nos. 18 and 19 in both the otocyst and the ganglion is revealed by antibodies Ks B.18 (**c**) and KS 4.62 (**d**). **e** Labelling of vimentin in the mesenchyme surrounding the otocyst (*E*). **f** Staining for neurofilament protein is confined to the ganglion (*G*). *Bars* represent 50 μ in **a–c**, 25 μ in **d, e**, and 10 μ in **f**

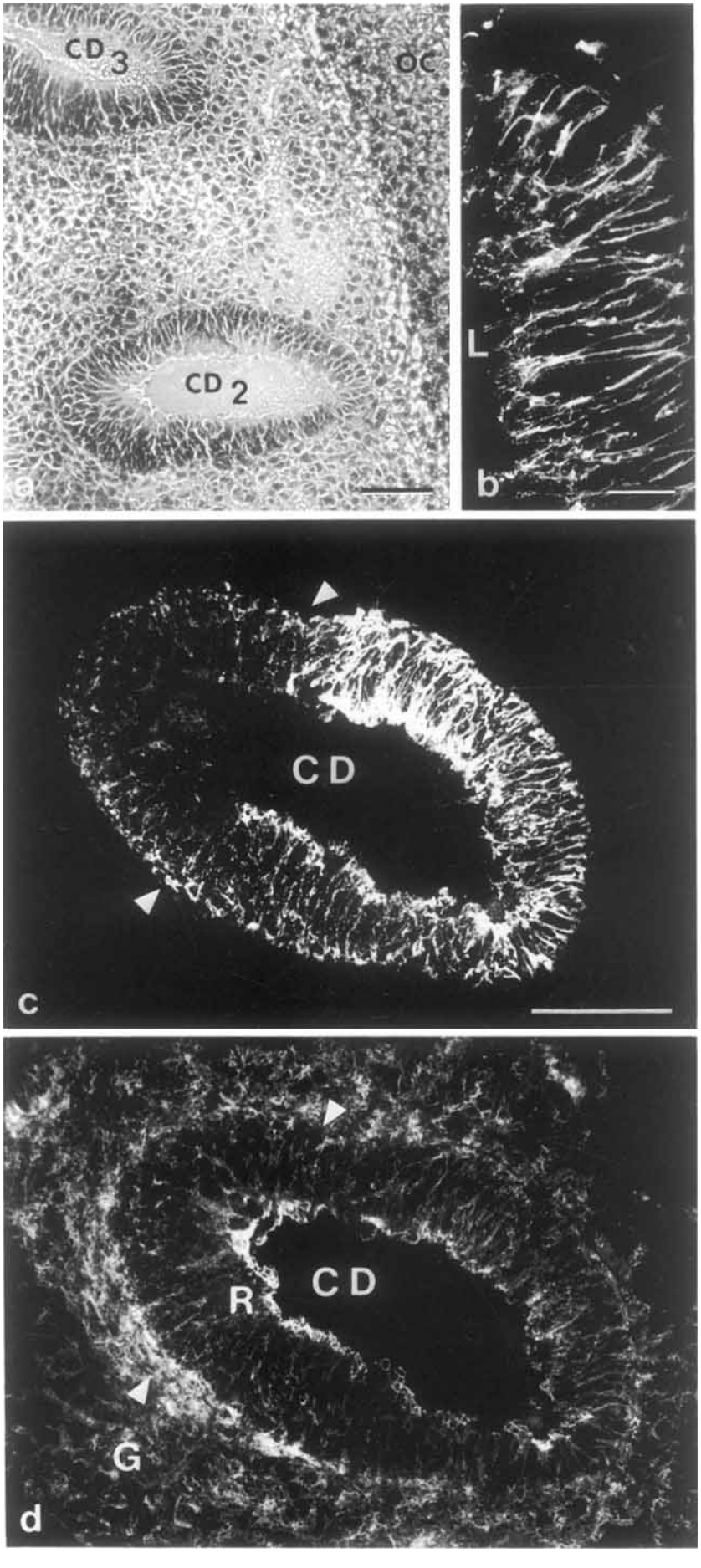


Fig. 3a–d. Cryosections of cochlear ducts in 3-week-old guinea pig, stained with hematoxylin-eosin (**a**) or immunofluorescently labelled for CK (**b–c**) or actin (**d**). The coiled membranous labyrinth (*CD2* and *CD3* indicate the second and third cochlear coils) is ovally shaped and is surrounded by undifferentiated mesenchyme. **b** Antibody KG 8.13 demonstrated intense labelling of CK in the epithelium from the basement membrane to the lumen (*L*). **c–d** Section through the cochlear duct, double-labelled with KG 8.13 (**c**) and phalloidin (**d**). Note the uneven distribution of CK and actin, showing reduced CK-staining where epithelial ridges (*R*) start to rise (*area left of arrowheads* in **c**). The actin concentration in the apical area of these cells is apparently increased. *Bars* represent 50 μ in **a** and **c** and 10 μ in **b**

was essentially CK negative (Fig. 1b, c). The guinea-pig otocyst was stained by the broad-spectrum monoclonal antibody KG 8.13. The human otocyst of a similar developmental stage was labelled with several polypeptide-specific antibodies to CK, including KG 8.13 (Fig. 2a, b), Ks B.18 (Fig. 2c) and KM 4.62 (Fig. 2d). Immunofluorescent labelling of relatively thin sections indicated that most CK filaments were aligned longitudinally along the basal-apical axis, displaying intense staining both in the subapical region and along the basal membrane. The anlage of the endolymphatic duct, which extends rostromedially from the otocyst at this stage (Figs. 1b and 2b), expressed CK immunoreactivity indistinguishable from that of the otocyst. The otocysts of both species were not stained with antibody KS 8.58. This antibody, which reacts with stratified epithelia, is specific for human CKs nos. 13 and 16 and guinea-pig CK polypeptides with apparent molecular weights of 53 Kd, 50 Kd, and 46 Kd. The mesenchyme surrounding the otocyst, which did not stain with any of the CK antibodies, showed intense labelling with vimentin-specific antibodies in both species (Figs. 1e and 2e). Weak staining of mesenchymal processes was also detected along the base of the otocyst epithelium.

The acoustic ganglion, in the ventromedial aspect of the otocysts, was identified by either phase-contrast microscopy or labelling of cryosections with an antibody to neurofilament protein, (see Fig. 2f). Interestingly, many cells throughout the ganglion displayed intense and specific CK immunoreactivity with the same antibodies which stained the otocyst (Figs. 1c and 2a, c, d). Although this immunostaining extended toward ventral and medial areas of the ganglion, it was particularly marked in the region immediately adjacent to the basement membrane of the otocyst. Immunolabelling with an antibody to neurofilaments revealed expression of this IF in the brain (results not shown), as well as in cells located in the central areas of the developing acoustic ganglion (Fig. 2f), but not in the otocyst. Filaments of the other two classes of IF, namely desmin and GFAP, were not detected in either the otocyst or the acoustic ganglion. Actin was abundant in the otocyst and the structures associated with it, and showed maximal labelling near the lumen and adjacent to the basement membrane (Fig. 1f).

Cytokeratin distribution in the undifferentiated cochlear duct

The epithelia of the vestibular and cochlear ducts and sensory organs are formed by an elaborate process of spatially coordinated cell proliferation. The mammalian cochlea is an outgrowth of the saccule, which is derived from the ventral wall of the otocyst [30, 32]. Sections of early guinea pig cochleas stained with hematoxylin-eosin (Fig. 3a) showed that the duct was surrounded by mesenchyme, which become involved in the formation of the bony otic capsule, and that the epithelium appeared largely homogeneous. Nevertheless, sections immunolabelled for CK and actin (Fig. 3c, d), revealed the first signs of differentiation in the forming epithelial ridges, where increased actin concentration was observed in the presumptive organ of Corti. Staining with antibody KG 8.13 showed that the distribution pattern of CK in the early cochlear epithelium is generally similar to that found in the otocyst (Fig. 3b), with longitudinal filaments radiating from the luminal aspect toward the basement membrane. Antibody KS 8.58 specific to stratified epithelium did not stain the epithelial ridge or any other part of the cochlear duct epithelium.

Cytokeratin distribution in epithelium of differentiating cochlear duct

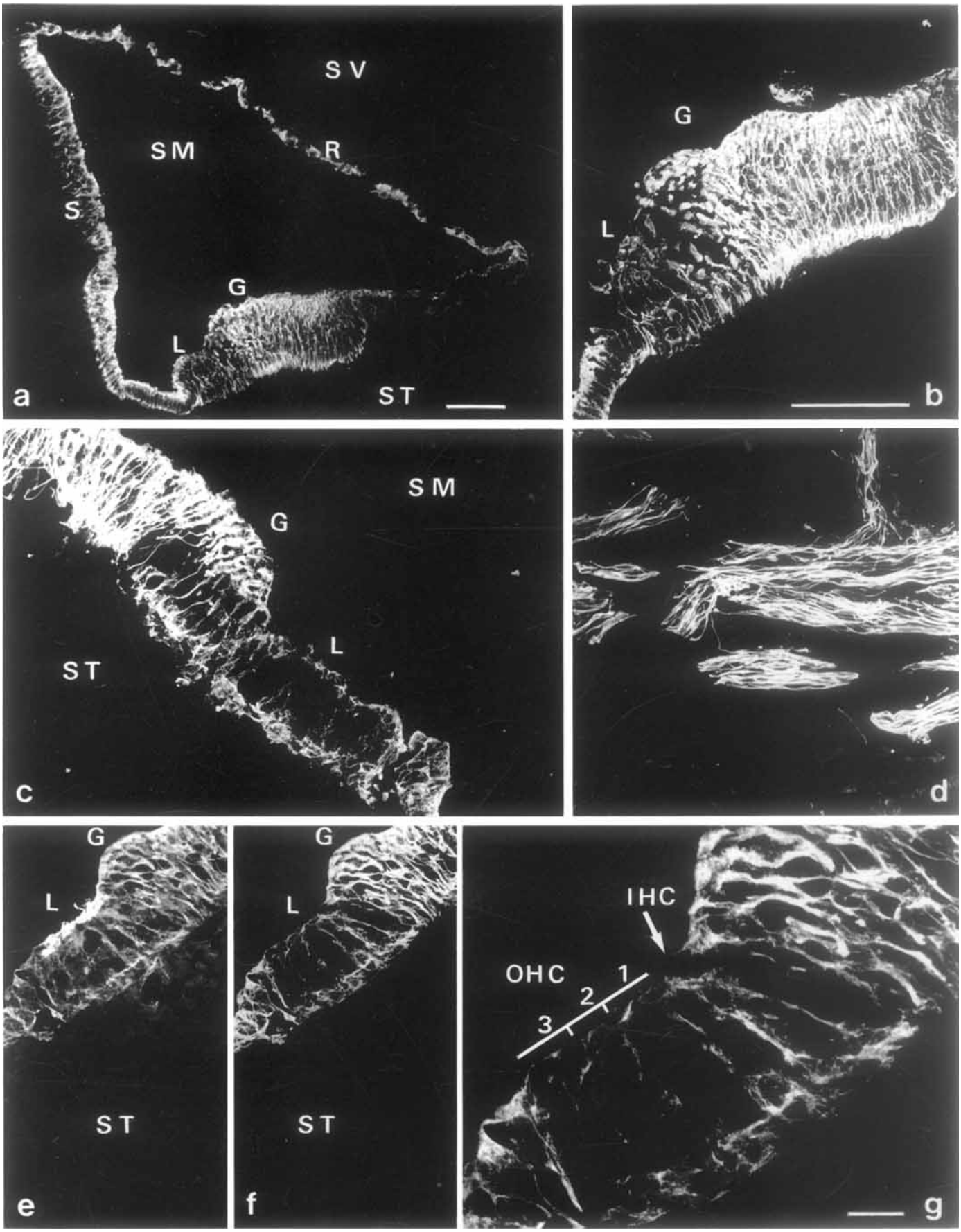
Following the intense cell proliferation in the developing cochlear duct, the nearly homogeneous, immature cochlear epithelium starts to differentiate, giving rise to diverse cell types with different structures and functions. The underlying mesenchymally derived components start to form the perilymphatic spaces, and the oval configuration of the membranous labyrinth acquires the triangular shape which is characteristic of the adult cochlear duct (Fig. 4a, cf. 3a).

At this stage, cells in the epithelium of the membranous labyrinth, exhibit varying degrees of CK immunoreactivity. The otocyst-derived epithelium of the stria vascularis and the membrane of Reissner showed uniform staining for CK, while the adjacent mesenchymal cells were not stained with CK (Fig. 4a). The greater and lesser epithelial ridges revealed a nonuniform pattern of CK staining. Notably, the presumptive sites of inner and outer hair cells in the greater and lesser ridges (respectively) showed decreased staining (Fig. 4b). As differentiation proceeded, less CK could be seen in the hair-cell region, until CK staining was confined to the most apical part of the epithelium, namely the reticular lamina (Fig. 4c, and at higher magnification, Fig. 4g). Although the organ of Corti is not fully mature at this stage, the presence of the cuticular plate and the bundle of stereocilia could be seen by colabelling for actin and CK (Fig. 4e, f), thus facilitating localization of the hair cells.

Cytokeratin distribution in mature cochlear epithelium

In guinea pig, labelling for IF in the nearly mature cochlea (Fig. 5), and also in the fully differentiated membranous labyrinth (Fig. 6), revealed CK expression in most epithelial cells, with the striking exception of the hair cells (Fig. 5a). The latter could easily be recognized within the same sections by the bright labelling for actin in their cuticular plate and stereocilia (Fig. 5b). In order to visualize the distribution of CK along the reticular lamina better, whole-mounts of mature guinea-pig cochleas were prepared and double-stained with anti CK (KG 8.13) and fluoresceinated phalloidin (Fig. 6a and b, respectively). Comparison of the two

Fig. 4a–g. Cryosections of 14-week (**a, b**) and 19-week (**c–g**) human embryos, immunofluorescently labelled with various antibodies. KG 8.13 (**a**) shows intense labelling in the membranous labyrinth, in which the perilymphatic spaces are present; *SM*, scala media; *SV*, scala vestibuli; *ST*, scala tympani. Note the CK distribution in marginal cells of the stria vescularis (*S*) with processes protruding into adjacent mesenchymal intermediate cells; *R*, epithelial layer of Reissner's membrane, *G*, greater epithelial ridge; *L*, lesser epithelial ridge. **b** Higher magnification of the greater- and lesser-ridge areas, showing the differentiating organ of Corti with reduced CK staining in the hair-cell region. **c** KM 4.62 labelling of the epithelial ridges, revealing CK staining confined to the reticular lamina. **d** With antineurofilament, labelling is confined to the nerves. **e, f** Double labelling for actin (**e**) and CK (**f**) permits precise localization of hair cells. **g** Higher magnification of the hair-cell region shown in **f**; there is no labelling of the inner hair cells (*IHC*) or the three rows of outer hair cells (*OHC 1, 2, 3*). *Bars* in **a–f** represent 50 μ; in **g**, 10 μ


SV
SM
R
S
G
L
ST
a
G
L
b
SM
G
L
ST
c
d
G
L
ST
e
G
L
ST
f
IHC
OHC
1
2
3
g

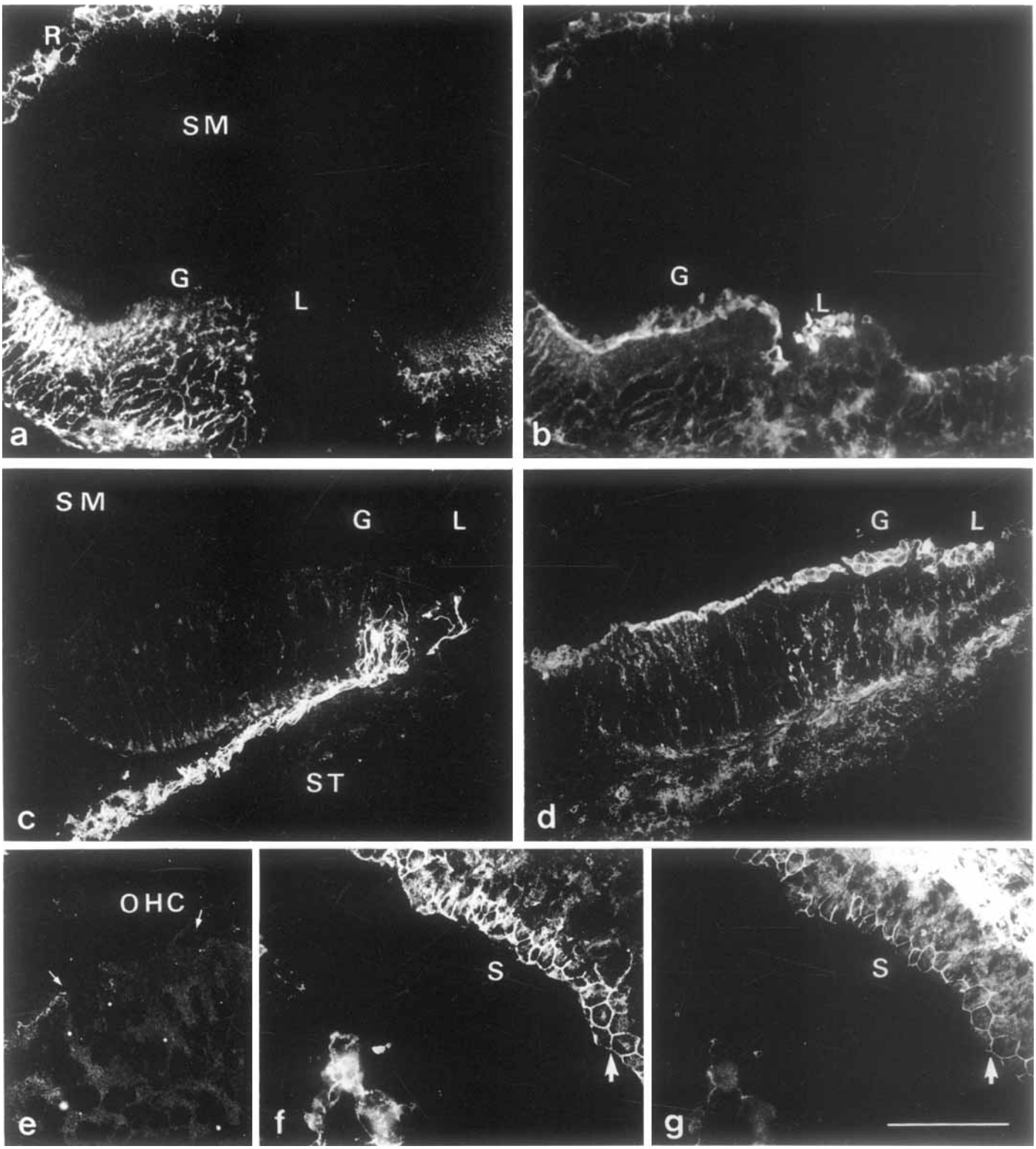


Fig. 5a–g. Immunofluorescence labelling of cryosections of nearly mature cochlear epithelium in guinea pig. **a, b** Double labelling for CK (**a**) and actin (**b**). Note pronounced staining for actin and absence of CK in hair cells. Mesenchymal cells in Reissner's membrane (*R*) are CK negative. **c, d** A section double-stained for neurofilaments (**c**) and actin (**d**) showing neurofilaments in nerves penetrating the organ of Corti, but not in hair cells. **e** Staining with desmosome-specific monoclonal antibody DK 80.20. Note the absence of desmosomes in the outer part of the hair-cell region (*area between arrows*) and positive labelling in adjacent supporting cells. **f, g** A section double-stained with phalloidin (**g**) and KG 8.13 (**f**) showing stria vascularis (*S*) with CKs restricted to the marginal cells. Note the abundance of actin in marginal cell junctions (*arrows* in **f** and **g** indicate the same area); *bar*, 50 μ

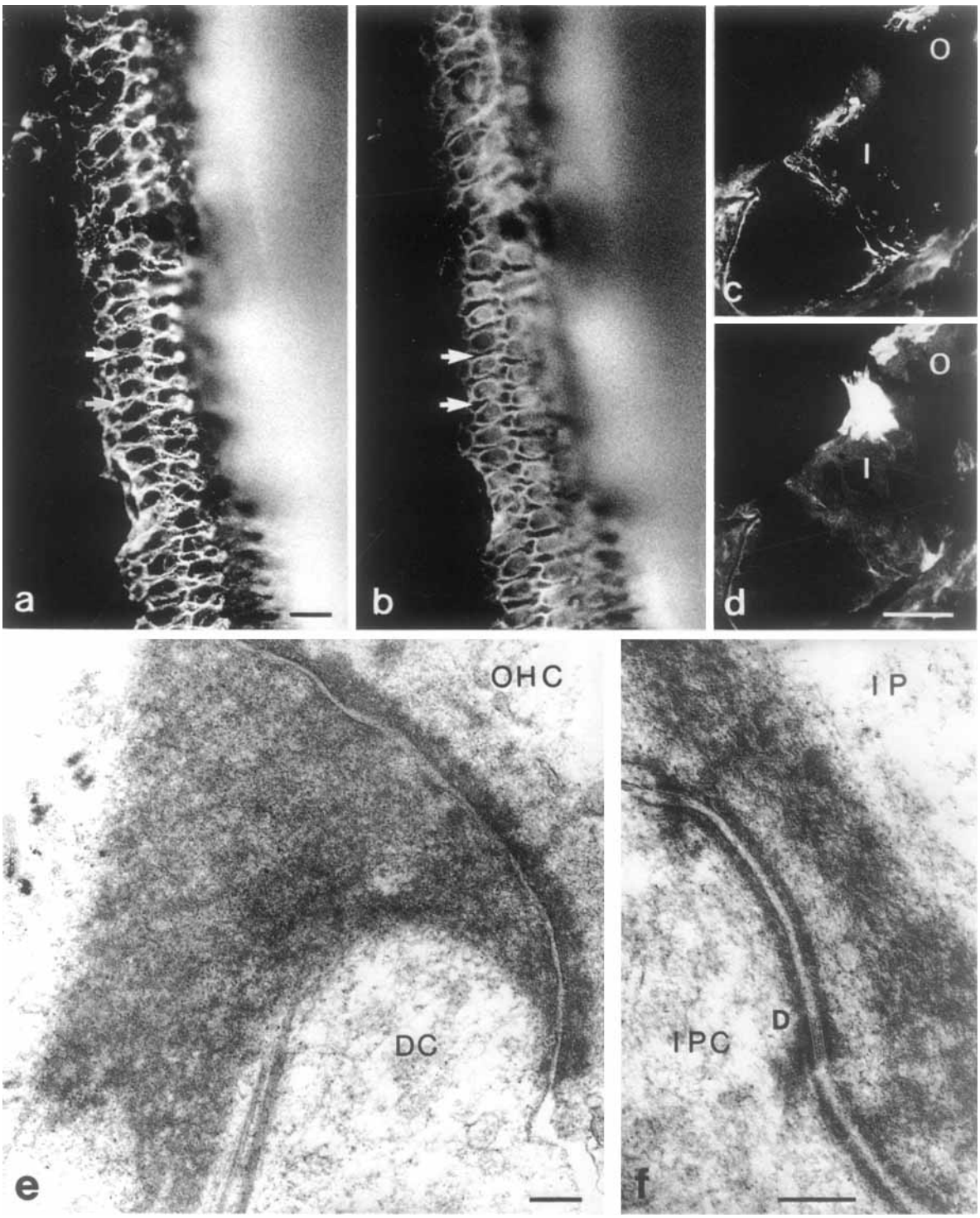


Fig. 6a–f. Surface preparation (**a, b**), cyrosection (**c, d**), and transmission electron photomicrographs of detergent-extracted organ of Corti (**e, f**) from mature cochlea in guinea pig. **a, b** Double fluorescent labelling with KG 8.13 antibody to CK, and fluoresceinated phalloidin. CK labelling is restricted to the support cells in between the actin-rich hair cells. **c, d** Section of neonatal-guinea-pig cochlea double-labelled for CK (**c**) and actin (**d**). Note lack of CK in inner (*I*) and outer (*O*) hair cells. **e** Electron photomicrograph of the asymmetric heterotypic junction between an outer hair cell (*OHC*) and the head process of a phalangeal (Deiter's) cell (*DC*) in the reticular lamina. **f** Intercellular junctions between inner pillar cells (*IPC*) and inner phalangeal cells (*IP*), with desmosomes (*D*) and intermediate filaments anchored to them. *Bars* represent 10 μ in **a**, 25 μ in **d**, and 0.2 μ in **e** and **f**

indicated that their patterns of labelling were largely complementary, with CK-specific fluorescence apparently restricted to the heads of the support-cell processes which are interposed between the hair cells.

Using antibodies to other types of IF, it was found that antineurofilament staining (Fig. 5c, d) was confined to the neurons which innervated the hair cells. Vimentin was ubiquitously expressed in the stroma surrounding the epithelium and in cellular processes adjacent to the basilar membrane, but could not be detected in the epithelial ele-

ments. Neither GFAP nor desmin were detected in labyrinth tissue. Sections of neonatal guinea-pig cochleas (Fig. 6c, d) supported the observation that CK immunoreactivity in the area of the hair cells was confined to the support cells at the reticular lamina.

To further determine whether the cochlear hair cells are indeed devoid of IF, we isolated the organ of Corti and briefly extracted it with Triton X-100, then fixed it and examined it by transmission electron microscopy. Careful analysis of such preparations failed to disclose any IF or IF-bound desmosomes in the cytoplasm or on the membrane of the hair cells (Fig. 6e). Consistent with previous electron microscopic studies [14, 17], the absence of desmosomes from hair cells was also confirmed by immunolabelling with the antibody to desmosomes, DK 80.20 (Fig. 5e). The support cells, in contrast, contained IF-bound desmosomes, which were most prominent in support cell-support cell (homotypic) junctions (Fig. 6f).

Discussion

The present study aimed to characterize the spatial and temporal modulation of IF expression in the developing and mature inner ear. The membranous labyrinth is composed of epithelial cells, all of which develop from the apparently homogeneous epithelium of the otocyst, yet at maturity they display a remarkable heterogeneity of structure and function [17, 21, 26, 29, 32]. We have shown here that the otocyst expressed IFs of the CK class and that the apparently uniform expression of these filaments persisted throughout the proliferative stage during which the cochlear duct was formed. However, as soon as the first signs of differentiation were detectable in the area of the presumptive sensory cells, CK expression gradually diminished, until it became essentially undetectable in the fully differentiated hair cells. Since neither neurofilaments, nor any other type of IF, were detected in the cochlear hair cells, it appears that the auditory neuroepithelium is comprised of an unique type of cell, devoid of IFs. Based on the fact that similar immunohistochemical findings were obtained in both man and guinea pig, and supported by parallel electron microscopic examination, it appears that this phenomenon may be common to auditory sensory cells in mammals.

Consistent with previous reports [32, 34] the early otocyst consists of a pseudostratified epithelium, limited basally by a basement membrane and apically by a lumen. Our immunohistochemical results using antibodies to CK, indicated that CK polypeptides, which are characteristic of simple epithelia, were present in all the cellular elements of this epithelium. Vimentin was present in the surrounding mesenchyme, and neurofilaments in the brain and the acoustic ganglion. Other IFs, including desmin and GFAP, were apparently absent from the developing and mature inner ears.

Of particular interest, at the otocyst stage, was the detection of CK in the acoustic ganglion, indicating that ganglionic cells may be derived from the otocyst epithelium. This finding directly supports previous suggestions based on histological studies in human [27] and avian [19] embryos. The fact that IFs of the neurofilament class were detected even at this early stage indicates that differentiation of the neural elements commenced very early in the development of the inner ear. Since CK expression is later shut off in all ganglion cells, it appears likely that cells of the acoustic ganglion, which are derived from the otocyst epithelium, can switch from epithelial (CK-positive) to neuronal (neurofilament-positive) differentiation, and may thus serve as an interesting model for epithelial-neuronal transition and for the reciprocity of CK and neurofilament expression.

The uniform distribution of CK in the epithelium of the undifferentiated cochlear duct resembled that of the otocyst. The early structural signs of cochlear differentiation were the acquisition of the triangular configuration in cross section and the formation of mesenchymally derived accessory elements, including the perilymphatic spaces and the bony otic capsule. In the cochlear epithelium, differentiation generated cellular diversification which was accompanied by modulation of CK expression. Cells which formed the marginal layer of the stria vascularis and the epithelium of Reissner's membrane, albeit different in function and ultrastructure, maintained their simple epithelial profile of CK expression.

A different pattern of distribution was seen in the emerging epithelial ridges, which give rise to the organ of Corti. Here it became apparent that during differentiation cochlear hair cells switched off CK expression completely. It seemed that, in the neighboring support cells, rearrangement of the cytoplasmic CK took place, leading to marked CK immunoreactivity in the head processes of the phalangeal cells which are integrated in the reticular membrane. The absence of labelling from the stalk region of these cells may be attributed either to an actual absence of CK or to the small dimensions of the stalk relative to the head process. It is notable that the apparent absence of CK from cochlear hair cells at the level of the reticular lamina was previously reported, using a polyclonal antibody to prekeratin [7]. A recent publication regarding IFs in the differentiating (14–18 weeks of gestation) human cochlea [4] presented similar results concerning the lack of CKs in hair cells. These authors did not describe results concerning CK expression and distribution in earlier developmental stages or in the mature inner ear. In contrast to our findings, however, vimentin was detected in various areas of the epithelium, including the hair cells. Furthermore, the same group has reported the presence of vimentin in several epithelial elements of the newborn mouse cochlea [3]. The basis for the discrepancy is not clear at present.

Our conclusions are based on immunohistochemical labelling, and not on direct biochemical analysis, which does not seem readily feasible in view of the mosaic nature of this tissue. Yet, the absence of labelling in cochlear hair cells with all IF antibodies tested, including various monoclonal and polyclonal antibodies, strongly supports the notion that cochlear hair cells are indeed devoid of IFs.

Modulation of CK expression in the acoustic ganglion and the organ of Corti are two aspects that merit some further consideration. As pointed out above, the loss of CK expression in the hair cells occurred concomitantly with the differentiation of these cells. While it is common for differentiating epithelia to alter the sets of CK polypeptides they express [9, 25, 28], the phenomenon of complete disappearance of CK from cells which remain within a confluent epithelial layer is unusual and intriguing. Like olfactory epithelium [36], cochlear hair cells undergo an epithelial-to-neural transition, which appears to be associated with the loss of CKs, the only IF initially expressed by these cells. The hair cells, however, do not apparently complete the

neuronal transition, and neurofilament expression is not detected in them. Such changes in CK expression are reminiscent of the ones described here for early epithelial cells of the acoustic ganglion, which were shown to undergo a transition from CK expression to the expression of neurofilaments. We could not directly determine whether the same cells which initially contained CK later expressed neurofilament-type polypeptides, but the loss of CK from these cells seems to be largely similar to the one described above for the hair cells.

In the absence of detailed direct information regarding the cellular functions of IFs in general, it may not be useful to speculate here on the functional significance of the loss of IF expression in hair cells. Apparently, previous experiments in which IF expression was seriously impaired by microinjection of specific antibodies into cells [18] failed to indicate specific roles played by IFs in specific cell functions. We may nevertheless indicate that the absence of IF from hair cells appears to be compatible with the unique mechanical properties of the cells, their polarity, and their specialized subcellular structural organization. It is also compatible with the formation and maintenance of the special junctional complexes formed by hair cells and neighboring support cells in the organ of Corti. Evidently, mammalian mature hair cells do not form homotypic junctions [14] and also lack structurally recognizable desmosomal junctions.

Further studies will be needed to elucidate the fate of the ganglion cells that express CK, and the temporal sequence and dynamic nature in which CK is eliminated from hair cells in the developing organ of Corti.

Acknowledgements. We would like to thank Prof. L. Nebel, Head of the Department of Embryology and Teratology, Sackler Faculty of Medicine, Tel-Aviv University, for his support and encouragement. This study was supported by grants from the Rockefeller-Weizmann Foundation and the Pasteur-Weizmann Foundation. B.G. is an E. Neter Professor in Cell and Tumor Biology.

References

1. Ades HW, Engstrom H (1974) Anatomy of the inner ear. In: Keidel WD, Neff WD (eds) Handbook of sensory Physiology. Springer, Berlin Heidelberg New York, Vol. 5/1 pp 125–158
2. Anderton BH (1981) Intermediate filaments: a family of homologous structures. J Muscle Res Cell Motil 2:141–166
3. Anniko M, Thornell L-E, Gustafsson H, Virtanen I (1986) Intermediate filaments in the newborn inner ear of the mouse. ORL 48:98–106
4. Anniko M, Thornell L-E, Virtanen I (1987) Cytoskeletal organization of the human inner ear. II. Characterization of intermediate filaments in the cochlea. Acta Otolaryngol Suppl 437:29–54
5. Brandtzaeg P (1973) Conjugates of immunoglobulin G with different fluorophores. I. Characterization by anionic exchange chromatography. Scand J Immunol 2:273–290
6. Chondynicki S (1968) Embryogenesis of the auditory part of the inner ear in the guinea pig. Acta Theriol 13:219–260
7. Flock A, Bretscher A, Weber K (1982) Immunohistochemical localization of several cytoskeletal proteins in inner ear sensory and supporting cells. Hear Res 6:75–89
8. Franke WW, Appelhans B, Schmid E, Freudenstein C, Osborn M, Weber K (1979) Identification and characterization of epithelial cells in mammalian tissues by immunofluorescence microscopy using antibody to prekeratin. Differentiation 15:7–25
9. Franke WW, Schmid E, Schiller DL, Winter S, Jarasch ED, Moll R, Denk H, Jackson BW, Illmensee K (1982) Differentiation-related expression of proteins of intermediate-size filaments in tissues and cultured cells. Cold Spring Harbor Symp Quant Biol 46:431–453
10. Geiger B, Singer SJ (1979) Participation of α-actinin in the capping of membrane components. Cell 16:213–222
11. Geiger S, Geiger B, Leitner O, Marshak G (1987) Cytokeratin polypeptide expression in different epithelial elements of human salivary glands. Virchows Arch 410:403–414
12. Gigi O, Geiger B, Eshhar Z, Moll R, Schmid E, Winter S, Schiller DL, Franke WW (1982) Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody. EMBO J 1:1429–1437
13. Gigi-Leitner O, Geiger B (1986) Antigenic interrelationship between 40 Kd cytokeratin polypeptide and desmoplakins. Cell Motil 6:628–639
14. Gulley RL, Reese TS (1976) Intercellular junctions in the reticular lamina of the organ of Corti. J Neurocytol 5:479–507
15. Hamilton WJ, Boyd JD, Mossman HW (1972) Human embryology. Williams and Wilkins Comp. Baltimore
16. Huszar M, Gigi-Leitner O, Moll R, Franke WW, Geiger B (1986) Monoclonal antibodies to various acidic (type I) cytokeratins of stratified epithelia. Selective markers for stratification and squamous cell carcinomas. Differentiation 31:141–153
17. Kimura RS (1984) Sensory and accessory epithelia of the cochlea. In: Friedmann I, Ballantyne J (eds) Ultrastructural atlas of the inner ear. Butterworth, London, U.K. pp 101–132
18. Klymkowsky MW, Miller RH, Lane EB (1983) Morphology, behavior and interaction of cultured epithelial cells after the antibody-induced disruption of keratin filament organization. J Cell Biol 96:494–509
19. Knowlton VY (1967) Correlation of the development of membranous and bony labyrinths, acoustic ganglia, nerves, and brain centers of the chick embryo. J Morphol 121:179–208
20. Lazarides E (1982) Intermediate filaments: A chemically heterogeneous, developmentally regulated class of proteins. Annu Rev Biochem 51:219–250
21. Lim D (1986) Functional structure of the organ of Corti: a review. Hear Res 22:117–146
22. Marshak G, Leitner O, Geiger B (1987) Cytokeratin polypeptide expression during the histogenesis of guinea pig submandibular salivary gland. Development, in press
23. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalogue of human cytokeratins. Patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24
24. Osborn M, Weber K (1983) Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. Lab Invest 48:372–394
25. Osborn M, Geisler N, Shaw G, Sharp G, Weber K (1982) Intermediate filaments. Cold Spring Harbor Symp Quant Biol 46:413–429
26. Pickles JO (1985) Recent advances in cochlear physiology. Prog Neurobiol 24:1–42
27. Politzer G (1956) Die Entstehung des Ganglions acusticum beim Menschen. Acta Anat 26:1–13
28. Quinlan RA, Schiller DL, Hatzfeld M, Achtstatter T, Moll R, Jorcano JL, Magin TM, Franke WW (1986) Patterns of expression and organization of cytokeratin intermediate filaments. In: Wang E, Fischman D, Liem RKH, Sun TT (eds) Intermediate filaments. Ann NY Acad Sci 455:282–306
29. Romand R (1983) Development of the Cochlea. In: Romand R (ed) Development of auditory and vestibular systems. Academic Press, New York, USA, pp 47–88
30. Rubel EW (1978) Ontogeny of Structure and Function in the Vertebrate Auditory System. In: Jacobson M (ed) Handbook of sensory physiology, Vol. IX, Development of sensory systems. Springer, Berlin Heidelberg New York, pp 135–237
31. Schliwa M (1986) The cytoskeleton: An introductory survey. Springer, Berlin Heidelberg New York Tokyo
32. Sher AE (1971) The embryonic and postnatal development of the inner ear of the mouse. Acta Otolaryngol Suppl 285:1–77

33. Thorn L (1975) Die Entwicklung des cortischen Organs beim Meerschweinchen. Advan Anat Embryol Cell Biol 51 (6):1–97
34. Van de Water TR, Ruben RJ (1971) Organ culture of the mammalian inner ear. Acta Otolaryngol 71:303–312
35. Virtanen I, Miettinen M, Lehto V-P, Kariniemi A-L, Paasivuo R (1985) In: Wang E, Fischman D, Liem RKH, Sun T-T (eds) Intermediate filaments. Application of monoclonal antibodies to intermediate filaments. Annals of the New York Academy of Science, Vol. 455, pp 635–648
36. Vollrath M, Altmannsberger M, Weber K, Osborn M (1985) An ultrastructural and immunohistological study of the rat olfactory epithelium: Unique properties of olfactory sensory cells. Differentiation 29:243–253

Received April 1987 / Accepted July 16, 1987