# Special Program of Differentiation Expressed in Keratinocytes of Human Haarscheiben: An Analysis of Individual Cytokeratin Polypeptides

Ingrid Moll, Sergey M. Troyanovsky, and Roland Moll

Department of Dermatology (IM), Mannheim Medical School, University of Heidelberg, Mannheim; Institute of Cell and Tumor Biology (SMT), German Cancer Research Center, Heidelberg; and Institute of Pathology (RM), University of Mainz, Mainz, Germany

Human haarscheiben, epidermal Merkel cell–rich sensory organs of hairy skin, were studied for the expression of various cytokeratin (CK) polypeptides and other epithelial and neuronal differentiation markers by applying immunoperoxidase and immunofluorescence microscopy to frozen sections and by two-dimensional gel electrophoresis. The basal clusters of Merkel cells were specifically detected by antibodies against CK 20. Haarscheiben keratinocytes were unique mainly by the prominent expression of CK 17 in the lower and middle layers. Further differences as compared to keratinocytes of usual epidermis included the enlargement of the basal compartment, characterized by the expression of CK 5 and the absence of the maturation-associated CKs 1/10/11, and the reduction of CK 15, which is a constituent of normal basal cells. Using CK 17 as a highly sensitive Haarscheibe marker in skin tissue sections, variabilities in the spatial relationship of the haarscheibe and the corresponding hair follicle were recorded. The results show that haarscheibe keratinocytes express a special program of differentiation that may be important for optimal stimulus perception. Immunohistochemical stainings for CK 17 will facilitate further studies on the distribution and biology of haarscheibe. *J Invest Dermatol 100:69–76, 1993*

In 1902, F. Pinkus published the first description of isolated epidermal discs that he referred to as haarscheiben along with their innervation in humans and other mammals [1]. Within these discs he observed arrays of Merkel cells *(Tastzellen)* accumulated at the base of the epidermis, which led him to the conclusion that haarscheiben are sensory organs of the hairy skin. He further noted the similarity between these structures and Römer's tubercle in the echidna [2]. Several decades later, Winkelmann published a study concerning the haarscheiben of cats and hairless mice [3]. Straile [4,5] found that, in most mammals, this epidermal disc is a part of the tylotrich hair follicle, and he also postulated that haarscheiben are important sensory organs because of the thick myelinated nerve fibers that terminate near them. In humans, these structures appear as irregularly shaped, rounded elevations that, in contrast to animals, are only inconsistently associated with hair follicles. On average, one haarscheibe is present per five hair follicles [6,7]. Their distribution in hairy skin is not uniform, a roughly estimated average being one per 1–2 $cm^2$ of skin, with a particularly high density occurring in the skin of the neck, abdomen, and arms [2,8].

Light microscopy and later ultrastructural studies demonstrated unequivocally the presence of Merkel cells—the neuroendocrine cells of the epidermis—clustered at the tips of all rete ridges within the haarscheibe [8,9]. Each Merkel cell is in a synapse-like contact to a neurite [10,11] and has small, thin desmosomes extending to neighboring keratinocytes [12]. The myelin of these neurites only extends a few microns below the epidermis, after which the neurites penetrate the basement membrane, to end as disc-shaped terminals in the immediate vicinity of a Merkel cell. Further studies revealed that, in humans, the epidermis of the haarscheibe is thicker than normal epidermis owing to the presence of one or two basal layers of columnar pseudostratified cells oriented perpendicular to the skin surface bordering the basement membrane. Furthermore, the dermo-epidermal junction is more highly convoluted, and hemidesmosomes are more numerous in haarscheiben epidermis [8].

Physiologic features of the haarscheibe were first presented and discussed by Frankenhäuser [13], who investigated slowly adapting receptors with a low mechanical threshold in rabbits. Later, a similar mechanoreceptor was described in other mammals [14]. Iggo *et al* were able to demonstrate that the haarscheibe is a modality-specific, slowly adapting, sensitive touch receptor (SA I) innervated by a large myelinated sensory nerve fiber that branches into unmyelinated terminals that are in contact with a Merkel cell [15,16]. The stimulus-response properties of the haarscheibe were later demonstrated using microelectrode techniques [17], from which it emerged that such structures are sensitive to surface indentations of only 1 $\mu$m and produce a sensory response to such indentations.

Such a highly sensitive response is indicative not only of a sensitive organ but also of a stable and elastic cytoskeleton of keratinocytes that constitute the backbone of the haarscheibe. The aim of the

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Reprint requests to: Dr. Ingrid Moll, Hautklinik, Klinikum der Stadt Mannheim, Theodor-Kutzer-Ufer, 6800 Mannheim 1, Germany.

Abbreviations:
CK: cytokeratin
DAB: diaminobenzidine
IF: intermediate filament
NEPHG: non-equilibrium pH-gradient electrophoresis
SA I: slowly adapting type I
SDS-PAGE: sodium dodecylsulfate–polyacrylamide gel electrophoresis

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**Table I.** List of Antibodies Used

| Antibody | Specifity | Reference | Source |
|---|---|---|---|
| MoAb $K_k$8.60 | Cytokeratins 1 (weakly) and 10/11 | [19] | Bio-Makor, Rehovot, Israel |
| GP-1 | Cytokeratin 1 | [20] | Kindly provided by Drs. T. Achtstätter and W.W. Franke, Heidelberg, Germany |
| MoAb 6B10 | Cytokeratin 4 | [21] | Euro-Diagnostics, Apeldoorn, The Netherlands |
| MoAb AE14 | Cytokeratin 5 | [22] | Kindly provided by Dr. T.-T. Sun, New York, USA |
| MoAb CK-7 | Cytokeratin 7 | [23] | Boehringer, Mannheim, Germany |
| MoAb RCK105 | Cytokeratin 7 | [24] | Euro-Diagnostics, Apeldoorn, The Netherlands |
| MoAb CAM 5.2 | Cytokeratin 8 | [25] | Becton-Dickinson, Neckargemünd, Germany |
| GP 11 | Cytokeratins 8 and 18 | [18] | Progen, Heidelberg, Germany |
| MoAb 1C7 | Cytokeratin 13 | [21] | Euro-Diagnostics, Apeldoorn, The Netherlands |
| MoAb $K_s$8.12 | Cytokeratins 13, 15, and 16 | [19] | Sigma, Deisenhofen, Germany |
| MoAb LL001 | Cytokeratin 14 | [26] | Kindly provided by Dr. E.B. Lane, London, UK |
| GP CK 15 | Cytokeratin 15 | [27] | Kindly provided by Drs. R. Leube, H.W. Heid, and W.W. Franke, German Cancer Research Center, Heidelberg, Germany |
| MoAb E3 | Cytokeratin 17 | [28] | Dr. S.M. Troyanovsky |
| MoAb $K_s$18.174 | Cytokeratin 18 | [29] | Progen Biotechnics, Heidelberg, Germany |
| MoAb $K_s$19.2.105 | Cytokeratin 19, trichocytic cytokeratin Hax | [30] | Progen Biotechnics, Heidelberg, Germany |
| MoAb IT-$K_s$20.1-6 | Cytokeratin 20 | [31] | Progen Biotechnics, Heidelberg, Germany |
| MoAb VIM-3B4 | Vimentin | [30] | Progen Biotechnics, Heidelberg, Germany |
| MoAb NR4 | Neurofilament protein NF-L | [32] | Boehringer, Mannheim, Germany |
| MoAb G-A-5 | Glial Filament Protein (GFP) | [32] | Boehringer, Mannheim, Germany |
| MoAb DP1&2-215 | Desmoplakins I and II | [33] | Boehringer, Mannheim, Germany |
| MoAb AKH 1 | Filaggrin | [34] | Paesel + Lorei, Frankfurt, Germany |
| MoAb SY 38 | Synaptophysin | [35] | Boehringer, Mannheim, Germany |
| MoAb MOC-1 | Neuroendocrine antigen | [36] | Euro-Diagnostics, Apeldoorn, The Netherlands |
| MoAb OKT 6 | T-6 surface membrane antigen | [37] | Ortho Diagnostic Systems, Neckargemünd, Germany |

present study was to establish the particular features of the cytoskeleton of human haarscheiben, notably the various cytokeratin (CK) polypeptides [18] and associated structures present in keratinocytes.

## MATERIALS AND METHODS

**Tissues** Skin specimens containing Pinkus haarscheiben were obtained from 10 adult patients aged 38 to 88 years (cases 1–10) and one child aged 15 months (case 11). The skin specimens studied were from the upper arm (cases 3, 4, 6, and 7), the leg (case 5), and the trunk (cases 1, 2, 8, 9, and 10), and were obtained during the surgical removal of various skin tumors or of tattoos or, in one case (case 9), from an autopsy. All of the samples were frozen in liquid nitrogen immediately after removal and stored at −80°C.

**Immunohistochemistry** From the frozen skin samples, cryostat sections (4–5 μm thick) were cut, air-dried, fixed in acetone at −20°C, and then air-dried again. The primary antibodies used for immunohistochemistry are listed in Table I. Indirect immunoperoxidase staining was performed using peroxidase-coupled goat antibodies against guinea pig or mouse immunoglobulins (Dakopatts, Hamburg, Germany) as the secondary antibodies, with 3,3′-diaminobenzidine (DAB) and $H_2O_2$ being employed for the staining reactions (for details, see [38]). In some experiments, double immunofluorescence microscopy was performed as described previously [39].

**Gel Electrophoresis** Cytoskeletal material from Pinkus' haarscheiben (case 9) was prepared from 20-μm–thick cryostat sections after microdissection of an epidermal region corresponding to a Pinkus' haarscheibe using a stereomicroscope equipped with dark-field illumination (Wild/Leica, Bensheim, Germany). In these unstained sections, the haarscheibe region was identified by comparing them with parallel cryostat sections immunostained for CK 17. As a control, non-haarscheibe epidermis was also prepared from the same sections. Subsequent extraction was performed using a high-salt, detergent-containing buffer [18].

The insoluble cytoskeletal residues were analyzed using two-dimensional gel electrophoresis, employing non-equilibrium pH gradient (NEPHG) electrophoresis for the first dimension and sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) for the second dimension. The gels were stained using a previously described silver staining method [40].

## RESULTS

Using antibodies against CK 18 or CK 20, Merkel cells could be specifically identified in frozen samples from various body sites by immunoperoxidase microscopy. Pinkus' haarscheiben were considered to be present when one or more Merkel cells were found at the tips of four to eight neighboring epidermal ridges close to a hair follicle (Figs 1*a*, 2*a*, and 3*a*). The keratinocytes of the basal and first suprabasal layer were often vertically elongated and had oval nuclei. The average diameter of these structures was 0.45 mm (range 0.25–0.72 mm) for adults and about 0.25 mm for the child. In one specimen (case 9), we were able to estimate the distance between two neighboring haarscheiben and found it to be 4.8 mm. Another distinctive feature of haarscheiben is the presence of many large capillaries and myelinated nerve fibers in the upper dermis. The dermal nerve fibers that terminally branch and contact each Merkel cell of the haarscheiben were demonstrable using a neurofilament antibody (Fig 1*b*). In one case, double immunofluorescence microscopy clearly revealed the close association of a Merkel cell and a fine nerve cell process (Fig 1).

Serial sections of epidermis containing an identified haarscheibe (see above) were subjected to immunohistochemical procedures using a battery of antibodies against intermediate-filament (IF) proteins, such as CKs, desmosomal proteins, and other cellular antigens (see Table I). Figure 2 shows the immunostaining patterns of an adult haarscheibe, while Fig 3 shows those observed in a haarscheibe of a 15-month–old child. No age-related differences in the staining pattern were detectable. The results are summarized in Table II.

The CK antibodies revealed a series of differences in the distribution of CK polypeptides in haarscheibe and non-haarscheibe epidermis. The most striking finding was the specific decoration of keratinocytes in the haarscheibe epidermis by antibody E3, which is specific for CK 17 (Figs 2*b* and 3*b*). The basal and lower suprabasal layers were uniformly CK-17 positive (except for the Merkel cells), whereas the middle spinous cells exhibited more heterogeneous mosaic-like staining, with increasing frequency of negative cells

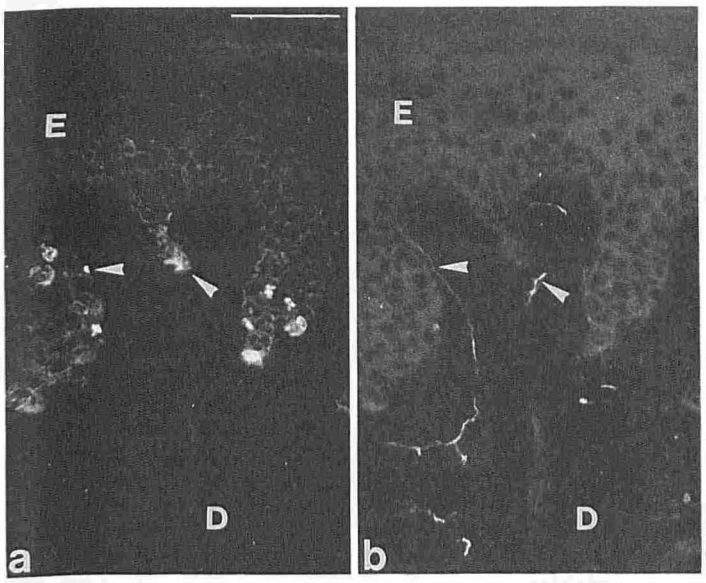


**Figure 1.** Double-label immunofluorescence microscopy of an adult human haarscheibe (central portion) using guinea pig antiserum GP 11 specific for CKs 8 and 18 staining the Merkel cells (*a*) and the neurofilament antibody NR4 decorating the nerve fibers in longitudinal and cross sections (*b*). Note the close contacts between nerve fibers and Merkel cells (*arrowheads*). E, epidermis; D, dermis; *bar*, 50 μm.

towards the epidermal surface. The cells of the uppermost stratum spinosum, the stratum granulosum, and the stratum corneum, were negative. CK 17 was completely absent in non-haarscheibe epidermis (Figs 2*b* and 3*b*, *margins*). Antibody AE 14 against CK 5, which typically stains basal keratinocytes, was found to decorate not only the basal layer of haarscheibe epidermis, but also to produce heterogeneous staining in a further one or two suprabasal layers (Fig 2*c*). Antibody LL 001 (specific for CK 14) stained the majority of keratinocytes present in both normal and haarscheibe epidermis, producing a heterogeneous pattern in the uppermost spinous layers (Fig 2*d*). A surprising observation was that CK 15, which is specifically expressed in the basal keratinocytes of normal epidermis, was detectable only in basal cells at the periphery of the haarscheibe area, the central portion being entirely negative for this CK (Figs 2*e*, 3*c*). Also, antibody $K_S$8.12 against CK 13, CK 15, and CK 16, which weakly and heterogeneously stains basal epidermal cells, produced a negative reaction in the haarscheibe epidermis, although somewhat stronger staining than normal was visible in the basal cell layer of the epidermis immediately adjacent (Fig 2*f*). The keratinocytes of the "true" stratum spinosum (i.e., from about the third cell layer on) and the stratum granulosum of haarscheibe epidermis were positive for CKs 10/11, and the keratinocytes of the lowest layer stained often exhibited thin cell processes extending in a basal direction (Fig 2*g*). In general, CKs 10/11 were absent in the basal and the first suprabasal layer of haarscheibe epidermis, whereas in normal epidermis, only the basal layer is negative for these CKs (Fig 2*g*). The antiserum against CK 1 produced an almost identical staining pattern (Fig 2*h*). Evaluation of serial sections of the same specimen showed that CK 17 (Fig 2*b,g,h*) was co-expressed together with CK 1 and CKs 10/11 in several, particularly lower, spinous cell layers, whereas CK 5 was only rarely co-expressed with CK 1 and CKs 10/11 (Fig 2*c,g,h*).

As expected, Merkel cells were stained exclusively by antibodies directed against the simple-epithelial CK 8, CK 18, CK 19, and CK 20 (Figs 1*a*, 2*a*, 3*a*) and were negative for all other CKs. In addition, Merkel cells were weakly positive for the neuroendocrine antigen, MOC-1, with immunoreactivity being observable in at least some of these cells (not shown). The nerve endings in contact with Merkel cells were also decorated by the neurofilament antibody, NR4 (Fig 1*b*), as well as the MOC-1 antibody (not shown). In these nerve endings (including Schwann cells), the reactions for glial filament protein were negative (not shown).

The differentiation marker, filaggrin, was detected in the granular and horny layers of haarscheibe epidermis, similar to normal epidermis, but in the case of the child the filaggrin staining appeared to be slightly reduced as compared to normal epidermis (Fig 3*d*). Scattered vimentin-positive dendritic cells were present in the basal and suprabasal layers of haarscheibe epidermis (Fig 3*e*); at least some of these were Langerhans cells, as they were also OKT-6 positive (Fig 3*f*).

CK 4, CK 7, and CK 13 were detected neither in haarscheiben nor normal epidermis. In haarscheiben, desmoplakins I and II produced a punctate staining pattern at the cell border like that seen in normal epidermis (not shown).

The CK-17–positive epidermal compartment, which delimits the haarscheibe, exhibited a relatively sharply defined border with the adjacent negative epidermis (Fig 2*b*, 3*b*) and the infundibular epithelium of the neighboring hair follicle (Fig 4*b*). In a few cases, a variable short segment of normal (CK-17 negative) epidermis was interposed between the haarscheibe and the infundibulum of the corresponding hair follicle (Fig 4*a*); in other cases, though, the CK-17–positive haarscheibe area extended a short way into the infundibular region (Fig 4*c*). That this infundibular segment is actually a part of the haarscheibe itself was evidenced by its containing Merkel cells. In the hair follicle itself, antibody E3 stained the outer root sheath below the opening of the sebaceous gland (not shown).

Two-dimensional gel electrophoresis of microdissected epidermal tissue revealed the presence of CK polypeptides CK 1, CK 5, CK 11, and CK 14 in both the haarscheibe and normal epidermis (Fig 5*a,b*). The former additionally contained a marked amount of CK 17 (Fig 5*a*); this represents biochemical evidence confirming our immunohistochemical finding of the specific expression of CK 17 in haarscheibe epidermis. No other CK was detected in the haarscheibe preparation. As expected, CK 15 was only demonstrable in the gel prepared from normal epidermis (Fig 5*b*).

## DISCUSSION

The haarscheibe, a slowly adapting mechanoreceptor (SA I) of the skin, has mainly been studied in various mammalian animal species [14,41], investigations of these structures in humans being comparatively rare [1,2,6,7,8,17]. It is generally assumed that haarscheiben are distributed irregularly throughout human (hairy) body skin [1,2]. In the present study, the distance between two of these structures was found to be only 4.8 mm, thus indicating a higher density than previously believed [10]. The most conspicuous features of these haarscheiben are the neuroendocrine Merkel cells and the thickening of the epidermis due to the presence of layers of columnar-pseudostratified cells [10]. Because of the morphologic differences between haarscheibe and normal epidermis, we studied the IF cytoskeleton of the cells that comprise the haarscheibe. We were easily able to localize Merkel cells accumulated at the tips of the rete ridges of the haarscheibe epidermis on the basis of their specific expression of CK 20 [42], as well as to confirm their close association to nerve fibers, which is considered to be synapse-like [10].

The CK expression of the keratinocytes of the haarscheibe turned out to be strikingly different from that found in normal epidermis. The expression of the "basal-type" CK 5 not only in the basal cell layer but also in one or two suprabasal layers, along with the corresponding absence in the first suprabasal layer of the "suprabasal maturation" CKs 1/10/11, reflects the quantitative expansion of the basal cell compartment for at least one layer. This may be important for the proper harboring of the Merkel cell clusters. Of even greater interest are the qualitative alterations in CK expression in haarscheiben as compared to normal epidermis. The most striking is the prominent amount of CK 17 present in the (expanded) basal cell compartment and even in more suprabasal (CK 1/10/11–expressing) maturing keratinocytes. This CK is essentially absent in normal adult epidermis but is prominent in fetal epidermis, i.e., up to week 13, after which its level gradually decreases [43]. It is con-

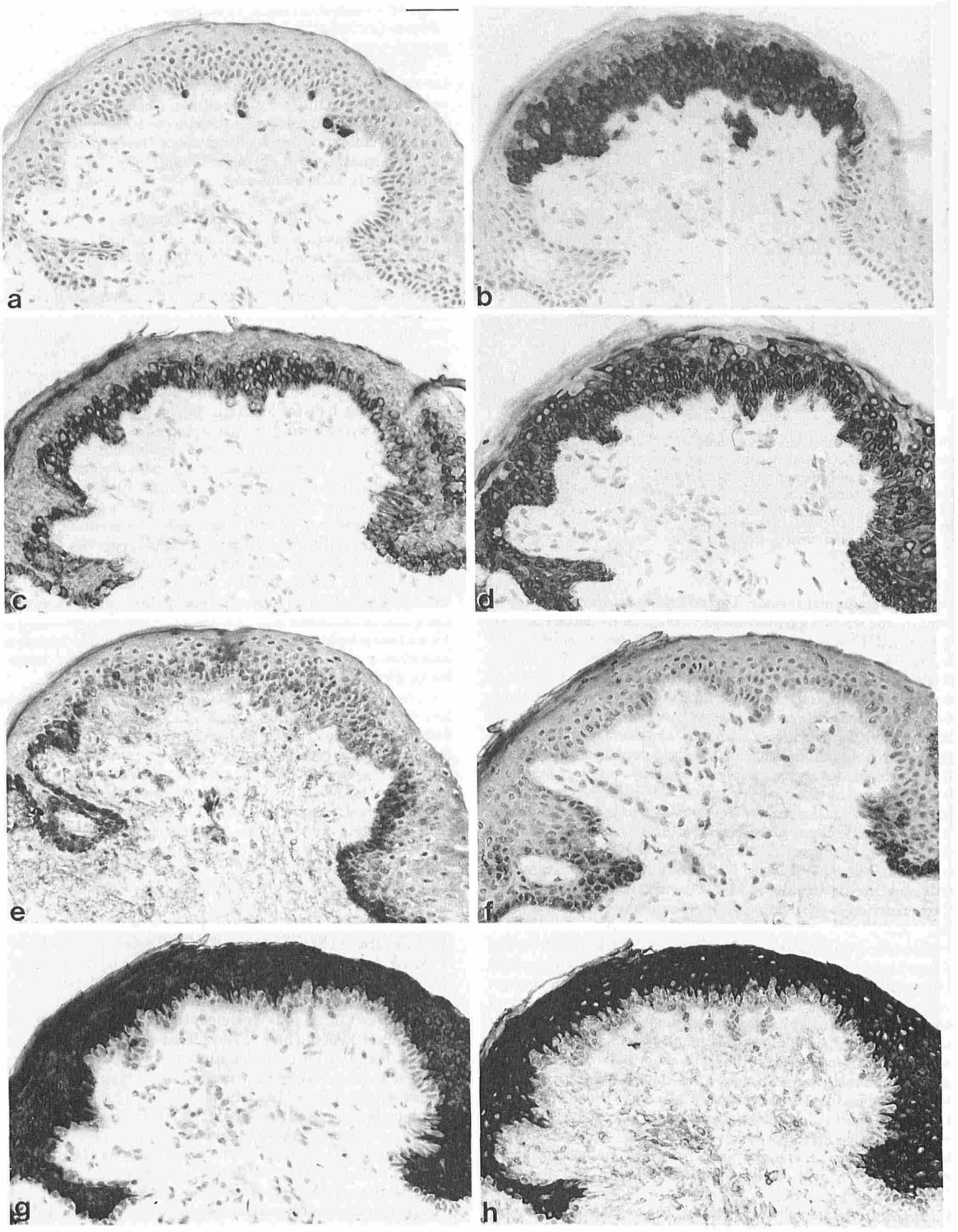


**Figure 2.** Immunoperoxidase staining of serial sections through adult human haarscheibe (case 9) using antibodies against various CKs. *a*, MoAb IT-K$_2$0.3 (against CK 20) specifically decorates the Merkel cells present at the tips of the epidermal ridges. *b*, MoAb E3 (against CK 17) clearly marks the specialized haarscheibe epidermis, whereas the surrounding usual epidermis is totally negative. *c*, MoAb AE14 (against CK 5) decorates in the haarscheibe area not only the basal layer but also one to two suprabasal layers. *d*, MoAb LL001 (against CK 14) stains both haarscheibe and usual epidermis extendedly. *e*, *f*, guinea pig antibodies against CK 15 (*e*) and MoAb $k_5$ 8.12 (against CKs 13, 15, 16; *f*) are sparse or negative in haarscheibe epidermis. *g*, *h*, MoAb $K_k$ 8.60 (mainly against CKs 10/11; *g*) and guinea pig antiserum GP-1 (against CK 1; *h*) both stain the maturating compartment of haarscheibe epidermis from approximately the third layer on. *Bar* (in *a*), 50 $\mu$m.

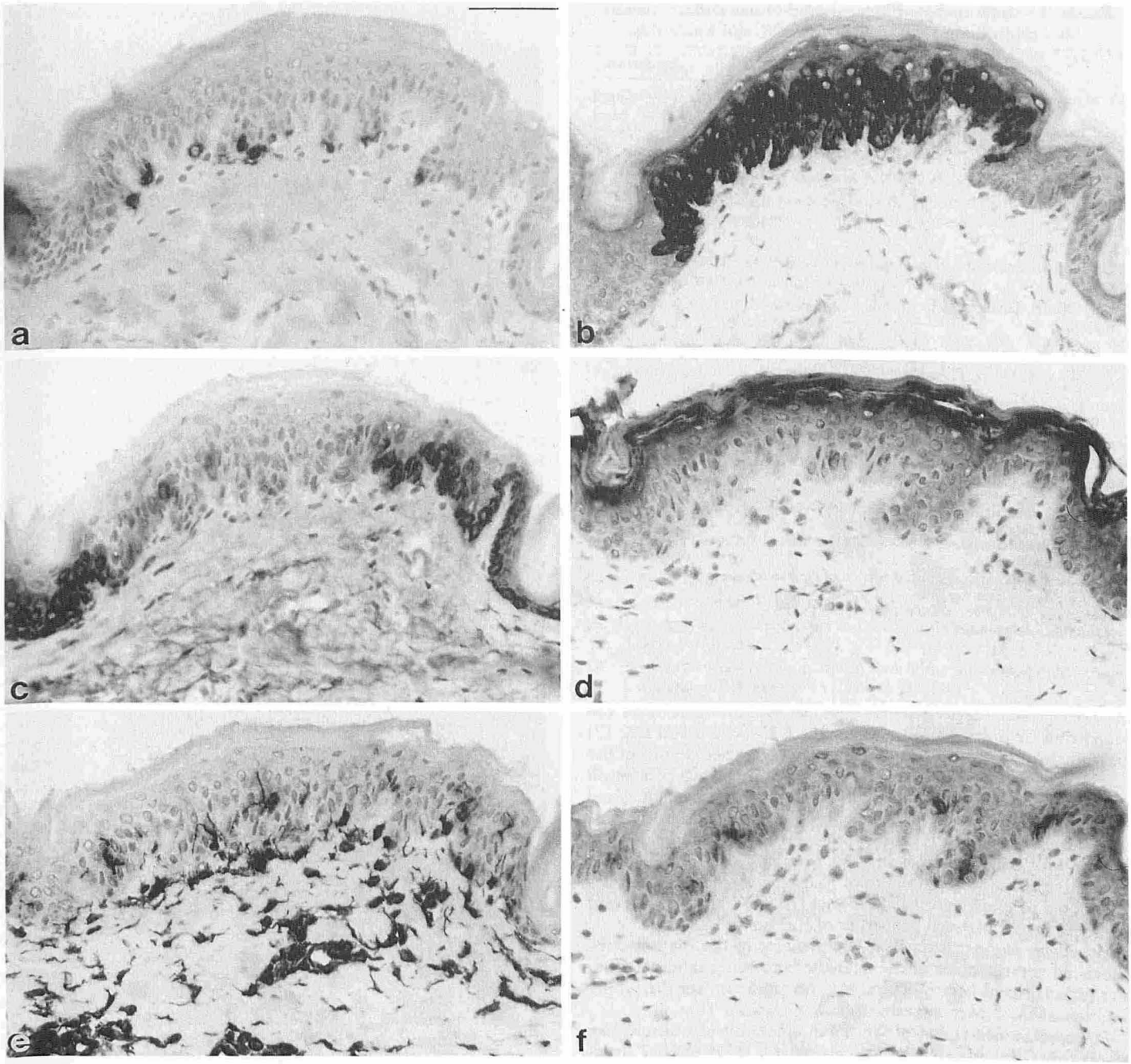


**Figure 3.** Immunoperoxidase staining of serial section through a haarscheibe of a child (15 months old) using antibodies against different CK (*a–c*), filaggrin (*d*), vimentin (*e*), and T6 antigen (*f*). *a,* MoAb IT-K$_s$20.3 (against CK 20) identifies the Merkel cells. *b,* MoAb E3 (against CK 17) selectively stains the haarscheibe epidermis. *c,* guinea pig antibodies against CK 15 produce the usual basal layer pattern only in the peripheral zone of the haarscheibe epidermis. *d,* filaggrin is detected in granular and horny cells of haarscheibe epidermis. Note that the staining appears to be more irregular (*i.e.,* interrupted) than in normal epidermis (visible at the right margin). *e,* MoAb VIM-3B4 against vimentin decorates scattered dendritic basal and suprabasal cells, probably representing melanocytes and Langerhans cells, respectively, as well as mesenchymal cells (including, fibroblasts and blood vessel cells) in the dermis. *f,* MoAb OKT6 identifies several Langerhans cells. *Bar* (in *a*), 50 $\mu$m.

ceivable that in haarscheibe epidermis, the fetal level of CK 17 is maintained, whereas in the rest of the epidermis this CK disappears during development. Furthermore, we noted a sharp reduction in CK-15 expression in haarscheibe epidermis. CK 15 is specifically and consistently expressed in the epidermal basal cells of hairy skin (H. W. Heid *et al,* manuscript in preparation). The completely negative reaction for antibody K$_s$8.12 in haarscheiben in contrast to the variable staining of normal epidermal basal cells would seem to suggest the absence not only of CK 15 but also of CK 16 in these disc-like structures. Together with the apparent absence of CK 6 (as revealed by gel electrophoresis) it can be concluded that the hyperproliferation-associated CK pair, CK 6/CK 16 [44], is not expressed in haarscheibe epidermis, and that this specialized epidermis is — even though thickened — not in a state of hyperproliferation.

Our results concerning CK expression indicate that the tonofilaments of haarscheibe keratinocytes must have a different molecular composition, at least in the lower and intermediate epidermal layers of these structures. This leads to the conclusion that the haarscheibe epidermis may have mechanical properties distinct from those of

**Table II.** Intermediate Filaments and Other Differentiation Markers in Pinkus' Haarscheiben and Usual Epidermis

| | Haarscheibe | | | Epidermis | |
|---|---|---|---|---|---|
| | Basal | Supra-basal | Merkel Cells | Basal | Supra-basal |
| CK 5 | +[a] | ±[b] | − | + | − |
| CK 14 | + | +[c] | − | + | +[c] |
| CK 15 | ∓[d] | − | − | + | − |
| CK 17 | + | +[c] | − | − | − |
| CK 13/15/16 | − | − | − | (+) | − |
| CK 13, CK 4 | − | − | − | − | − |
| CK 1, CK 10/11 | − | +[c] | − | − | + |
| CK 7 | − | − | − | − | − |
| CK 8, CK 18, CK 19, CK 20 | − | − | + | − | − |
| DP 1&2-215 | + | + | + | + | + |
| Filaggrin | − | +[f] | − | − | +[f] |
| Vimentin | −[g] | −[g] | − | −[g] | −[g] |
| Neurofilament protein NF-L | − | − | − | − | − |
| Synaptophysin | − | − | (+) | − | − |
| MOC-1 | − | − | (+) | − | − |
| OKT6 | − | −[g] | − | − | −[g] |

[a] +, homogenous staining; (+), weak staining; −, negative.
[b] 1–2 lower suprabasal layers positive.
[c] Upper suprabasal layers showed variable and heterogenous staining (see text for further details).
[d] Only basal cells of the peripheral portion of the haarscheibe area positive.
[e] First suprabasal layer negative.
[f] Staining of stratum granulosum and corneum.
[g] Dendritic cells positive.

the normal epidermis. It seems possible that the increase in the proportion of lower-molecular-weight CKs (CK 5 and CK 17) may impart more softness and/or elasticity to the epidermis of the haarscheibe region. This may be important for optimal perception and transduction of mechanical forces to the associated nerves, and may thus account for the extremely high mechanical sensitivity of the haarscheibe, which is capable of generating nerve signals upon contact with indentations as small as 1 μm in depth [17]. Also, the especially thin stratum corneum of haarscheibe epidermis that has been noted in some mammalian species [10,44,45] may be relevant to the particular physical properties of this tissue.

Moreover, the particular CK complement of the Merkel cell–associated keratinocytes in the haarscheiben, being different from that of usual basal keratinocytes, may be important for the proper function of these Merkel cells.

The specific expression of CK 17 in epidermis of haarscheiben makes this CK a highly sensitive marker for these sensory structures. Available data also suggest a high degree of specificity: CK 17 appears to be absent—except for haarscheiben areas—in human normal adult epidermis of hairy skin of varicus localization, including breast, face, arm, back, abdomen, and leg. However, in glabrous skin (foot sole), we have previously found this CK in rare epidermal basal cell clusters [46] but in this type of skin haarscheiben do not exist [2]. Although we did not detect CK 17 in adult foreskin epidermis *in situ* (unpublished data) it is a prominent CK in cultured cells derived from newborn foreskin epidermis [44].

In humans, the spatial relationship between the hair follicle and the associated haarscheibe is variable, ranging from very close to somewhat distant [10]. Our results obtained using CK 17 as a "haarscheibe marker" confirmed these earlier morphologic data and, in addition, showed that, in some cases, the region of CK-17 positivity (and hence the haarscheibe area) extends into the upper infundibular epithelium. The presence of typically clustered Merkel cells, as detected by specific CK antibodies, provides further evidence that this infundibular portion must be regarded as a part of the haarscheibe. Outside of the haarscheibe area, CK 17 is absent in the infundibular epithelium, whereas it is regularly expressed in deeper

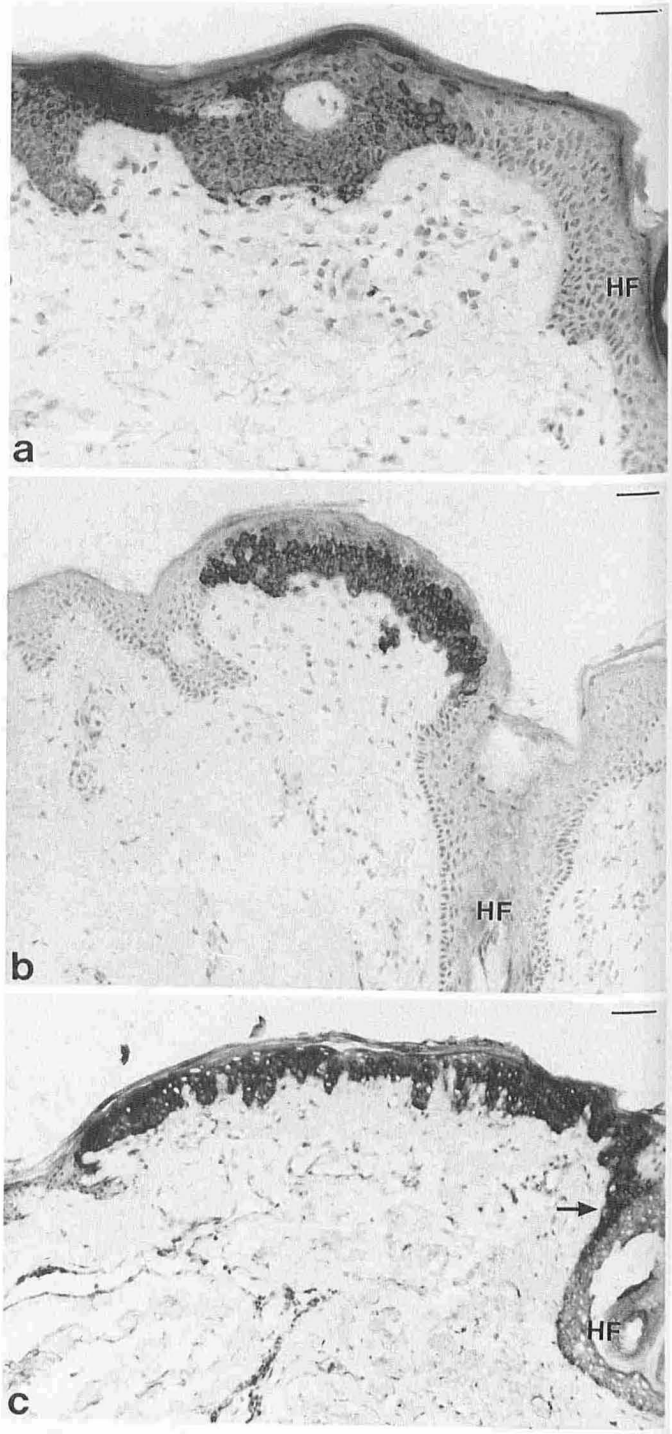


**Figure 4.** Different topographical relationships between haarscheiben and corresponding hair follicles (HF) as demonstrated by immunoperoxidase staining using CK 17 (MoAb E3) as a marker for haarscheiben (*a*, case 2; *b*, case 9; *c*, case 10). The haarscheibe may be separated from the follicle ostium by a short segment of CK 17-negative usual epidermis (*a*), may border immediately at the ostium (*b*), or may even extend a short distance into the infundibular epithelium (*c*, *arrow*). *Bars*, 50 μm.

portions of the hair follicle, *i.e.*, in the outer root sheath below the opening of the sebaceous duct [28,47]. Clearly, there is no continuity between the haarscheibe epidermis and the outer root sheath of the hair follicle, or any other relationship between these two distinct structures.

The development of haarscheiben is still unclear. In cats at birth,

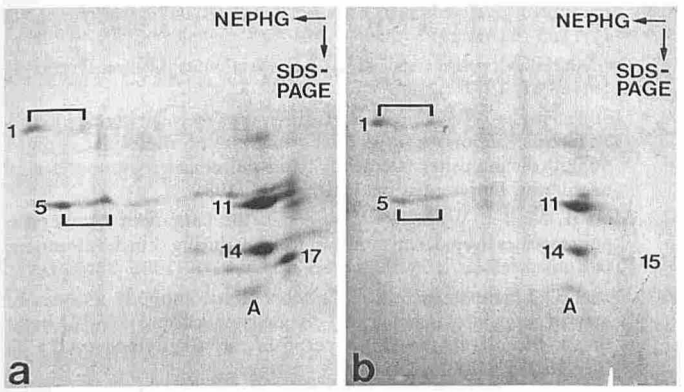


**Figure 5.** Two-dimensional gel electrophoresis of cytoskeletal proteins of microdissected haarscheibe epidermis (*a*) and usual epidermis (*b*, case 9). First dimension, NEPHG electrophoresis; second dimension, SDS-PAGE. CK polypeptides present have been identified and denoted by Arabic numerals according to [18]. (Of the closely related CKs 10/11, only CK 11—as defined by gel electrophoretic coordinates—has been identified in these particular gels derived from the same individuum). Note the presence of major amounts of CK 17 in the haarscheibe preparation (*a*). A, endogenous actin.

haarscheiben structures are present but contain few or no Merkel cells [45]. In contrast, in rats, Merkel cells are detectable in the skin before the haarscheiben primordia become elevated and before hair follicles develop [41]. This development has not yet been studied in humans, but such investigations will now be much easier using CKs as markers. Our present results already suggest that, at least at the age of 15 months, the development of human haarscheiben is complete. Another interesting question that can now be more easily dealt with is whether, in humans, adult haarscheiben have the capability to regenerate after trauma. The regeneration of haarscheiben does seem possible in adult cats: some investigators have shown the disappearance of epidermal thickening and a decrease in the number of Merkel cells in haarscheiben following denervation, as well as their complete regeneration after nerve sprouting [48–51]. The localization of these regenerated haarscheiben often coincided with old sites, but these structures also developed at new locations and even on scars [48,50].

In conclusion, the expression patterns of the CK polypeptides of human haarscheibe epidermis established in the present study demonstrate the distinct and specific phenotype of haarscheibe keratinocytes. Moreover, CK 17 may be applied as molecular marker for haarscheiben, and this may help in future studies to elucidate their distribution, development, and regeneration.

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