Formation of Epidermal and Dermal Merkel Cells During Human Fetal Skin Development

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The origin of Merkel cells is still a matter of debate, specifically the question of whether they are derived from epithelial cells of the epidermis or from immigrated neural crest cells. As an argument for the latter hypothesis the occurrence of dermal, nerve-associated Merkel cells in human fetal skin has often been mentioned. Therefore, we analyzed the distribution of Merkel cells in epidermis and dermis of plantar skin of human embryos and fetuses, ranging in gestational age between 7 and 17 weeks. Merkel cells were identified by immunocytochemistry on frozen sections using antibodies against simple epithelium-type cytokeratins and by electron microscopy. In the 17-week-old fetus, 17% of the total cutaneous (epidermal and dermal) Merkel cells were located in the upper dermal compartment, whereas in the 14-week-old fetus only 3.9% of the Merkel cells were dermal, including some cells that seemed to be in the process of traversing the dermal-epidermal junction. Thirteen-week-old fetuses showed even fewer dermal Merkel cells. Twelve-week-old fetuses exhibited 660 epidermal Merkel cells per 100 mm total section length, but none in the upper or deep dermis. In 7- to 9-week embryos, no Merkel cells were recognized. However, at this stage, but not in later stages, the basal cells of the plantar epidermis expressed certain simple epithelium-type cytokeratin polypeptides. These results speak against an invasion of Merkel cells or putative neural crest-derived precursor cells into the epidermis via a dermal passage. They suggest that in plantar skin Merkel cells arise, between weeks 8–12, from precursor stages of epithelial cells of the early fetal epidermis which still express simple epithelium-type cytokeratins. The results further suggest that in subsequent stages of skin development some epidermal Merkel cells detach from the epithelium and migrate into the upper dermis where some of them may associate with small nerves. *J Invest Dermatol 87:779–787, 1986*

The origin of Merkel cells, the neuroendocrine cells of the epidermis, and the hair follicles during fetal skin development, is still a matter of debate. The 2 major hypotheses discussed are, on the one hand, the origin from epithelial cells of the epidermis and, on the other hand, an immigration of cells derived from the neural crest (for reviews see [1,2]). The finding, in certain species, of forms that appear to be "intermediate" in morphology between keratinocytes and Merkel cells [3–6], the detection of cytokeratins, i.e., the epithelial type of intermediate-sized filament (IF) proteins [7–12], and various other experimental findings (e.g. [13,14]) seem to support the notion that Merkel cells derive from epithelial cells present in the fetal epidermis.

However, the cytokeratin filaments of Merkel cells are composed of polypeptides different from those constituting the tonofilaments of keratinocytes [7] and thus their composition cannot be considered evidence for a histogenic relationship between these two cell types. The theory of the derivation of Merkel cells from the neural crest and their immigration along peripheral nerves into the epidermis and the outer root sheath of the hair follicle has been mainly supported by reports of the occurrence, in early fetal dermis, of Merkel cells that seemed to be free and were assumed to be "on their way" from the neural crest to the epidermis [1,15–19]. By immunohistochemistry using antibodies specific for cytokeratins of Merkel cells, we have recently demonstrated abundant Merkel cells in the upper dermis of fetal plantar skin [7] that were closely associated with nerve fibers, as well as some Merkel cells that apparently were in the process of penetrating the dermal-epidermal boundary. In fetal week 17, the earliest stage of that study, approximately 83% of the Merkel cells were located within the epidermis whereas 17% appeared to be located in the dermis. While these findings would be compatible with the "immigration hypothesis," they do not exclude a migration of epidermally derived Merkel cells in the opposite direction, i.e., from the epidermis into the dermis.

The present study has been undertaken to obtain further insight into Merkel cell formation by analyzing the Merkel cell distribution in epidermis and dermis of earlier development stages.

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Abbreviations:
FITC: fluorescein isothiocyanate
IF: intermediate-sized filaments
NF-L: small neurofilament polypeptide
NF-M: medium-sized neurofilament polypeptide
TRITC: tetramethylrhodamine isothiocyanate

MATERIALS AND METHODS

Tissues Fourteen normal human embryos and fetuses were obtained during iatrogenic abortions performed for medical and

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nonmedical reasons. The gestational age, as estimated by the size of the uterus, as well as the crown–rump length, the heel–toe length standards [20], and the menstrual age, ranged between week 7–17 of gestation (7 weeks, 1 case; 8 weeks, 2 cases; 9 weeks, 1 case; 12 weeks, 1 case; 13 weeks, 5 cases; 14 weeks, 1 case; 15 weeks, 2 cases; 17 weeks, 1 case). Tissue samples from foot soles were excised immediately after removal and frozen in isopentane that had been precooled in liquid nitrogen; in some cases, samples were also fixed in glutaraldehyde for electron microscopy.

Immunofluorescence Microscopy Indirect immunofluorescence microscopy was carried out on approximately 5 μm-thick cryostat sections cut vertical to the epidermal surface and fixed in acetone −20°C [21]; 8 μm- or 10 μm-thick cryostat sections were used for counting of Merkel cells. The following primary antibodies were used: (1) monoclonal antibody CK 2 specific for the simple epithelium-type cytokeratin no. 18 [22] of the human cytokeratin catalog [23] which in the epidermis selectively labels Merkel cells [7]; (2) monoclonal murine antibody RGE 53 also specific for cytokeratin no. 18 [24]; (3) a guinea pig antiserum reacting with cytokeratin no. 18 and "IT-protein" [25] but not with other cytokeratin polypeptides (R. Moll, S. Winter, and W. W. Franke, unpublished data); (4) monoclonal murine antibody K_K 8.60 which is specific for cytokeratin polypeptides nos. 10/11 occurring in terminally differentiating keratinocytes [26]; (5) murine monoclonal antibody KA 1 specific for cytokeratin filaments of stratified squamous epithelial and myoepithelial cells [27]; (6) murine monoclonal antibody PKK 1 reacting with several cytokeratin polypeptides including nos. 8, 18, and 19 [28]; (7) murine monoclonal antibody K_s 8.1 specific for the simple epithelium-type cytokeratin no. 8 (R. Hazan and W. W. Franke, unpublished data); (8) monoclonal murine antibody A 53-B/A 2 specific for cytokeratin no. 19 which occurs in various simple and some stratified epithelia [29]; (9) rabbit antibodies against the small neurofilament polypeptide NF-L [30]; (10) rabbit antibodies against the medium-sized neurofilament polypeptide NF-M [31]; and (11) monoclonal murine antibodies NR-4 [32] and 2F11 [33] specific for the small neurofilament polypeptide NF-L.

As secondary antibodies, fluorescein isothiocyanate (FITC)-coupled, tetramethylrhodamine isothiocyanate (TRITC)-coupled, or Texas Red-coupled goat antibodies to total immunoglobulins of mouse, guinea pig, or rabbit were used (obtained from Medac or Dianova, Hamburg, F.R.G.). Double label immunofluorescence microscopy was performed by simultaneously applying both primary antibodies and subsequently, after washing, FITC-coupled secondary antibodies against immunoglobulins of the species of the first antibodies, together with TRITC-coupled or Texas Red-coupled secondary antibodies directed against immunoglobulins of the other species.

Electron Microscopy For electron microscopy of ultrathin sections, small samples of plantar skin were fixed in buffered glutaraldehyde, dehydrated, embedded in epoxy resin, and sectioned as previously described [7,34]. Three fetuses of weeks 7–8, 13, and 15 were studied. From each fetus 3 different samples taken at random were fixed, embedded, and sectioned at intervals >10 μm, totaling an average epidermal length of ~500 μm per block, i.e., 1.5 mm cumulative epidermal length per fetal stage.

Morphometric Analyses Numerical analyses of Merkel cells were performed on immunofluorescence microscopy slides made from serial 8–10 μm-thick frozen sections vertical to the surface, which were incubated with antibodies against cytokeratin no. 18 (see also [7]). For the specimens of the 17-week-old fetus in which glandular ridges were already developed, the sections were approximately vertical to these ridges. Every third section of a section series was used for counting of Merkel cells. Cells positive for cytokeratin no. 18 were registered when the nucleus-containing cell body was identified in the section, whereas sections through the cell processes or partial cross sections with areas smaller than half of the nuclear diameter were disregarded. Epidermal and dermal Merkel cells were counted separately. The epidermis and the dermis were identified in the sections using phase contrast microscopy. The length of the epidermis taken for the counting of Merkel cells was measured at the microscope using a graded ocular eyepiece.

RESULTS

In epidermis of plantar skin of *13- to 15-week-old fetuses* only some inconspicuous thickenings of the epidermal basal cell layer were recognized by phase contrast microscopy that might correspond to developing glandular ridges (Fig 1). In immunofluorescence microscopy, antibodies to cytokeratin no. 18 stained individual cells that were scattered throughout the basal cell layers (Fig 1*a–d*). Often they were located at a position just above the basalmost cell layer, but in other places they reached the dermal-epidermal junction (Fig 1*a–d*). Such cytokeratin no. 18-positive cells had previously been identified as neuroendocrine, i.e., Merkel cells [7] (see also Fig. 3 of the present article). The Merkel cells were characterized by roundish or ellipsoid cell bodies and often exhibited slender, sometimes branched cell processes that were also decorated by antibodies to cytokeratin no. 18 and sometimes made contact with neighboring Merkel cells or the dermal-epidermal junction (Fig 1*a,b*; see also Fig 2*j*). In cross sections these cell processes appeared as small dots (e.g., Fig 1*g*).

In fetuses of this stage, some Merkel cells of the basal layer, which usually were devoid of processes, seemed to protrude somewhat toward the dermis (Fig 1*e–h*). Moreover, some Merkel cells were noted the cell bodies of which seemed to be located partly in the epidermis and partly in the dermis (Fig 1*i,j*). Finally, some Merkel cells appeared to be located free in the upper dermis (Fig 1*k*) and often seemed to be associated with dermal nerve fibers, as revealed by double immunofluorescence microscopy using neurofilament antibodies (Fig 1*l*). In a 14-week-old fetus, 129 mm of skin section length were quantitatively evaluated. Among 930 Merkel cells counted, 3.7% were located within the dermis (Table I). The 13-week-old fetuses also exhibited some dermal Merkel cells but considerably fewer than were found in the 14- and 15-week-old fetuses. In later stages, i.e., 17- to 24-week-old fetuses, which have been described in detail in a preceding study [7], the glandular ridges were more clearly developed, the Merkel cells were concentrated in the ridges, and dermal Merkel cells were clearly more abundant (Table I) [7].

In foot-sole skin of a *12-week-old fetus,* Merkel cells stained with antibodies against cytokeratin no. 18 were relatively frequent in— or just above—the basal layer of the epidermis which was straight and did not show any ridges (Fig 2*a–c*). In survey pictures, a certain spatial pattern suggestive of a segmental distribution of the Merkel cells was apparent (Fig 2*a*). All 860 Merkel cells counted were epidermal, and neither dermal Merkel cells nor Merkel cells crossing the dermal-epidermal junction were observed (Table I).

When antibodies against the neurofilament polypeptides NF-L and NF-M were applied in single or double label immunofluorescence microscopy, together with antibodies against cytokeratin no. 18, the Merkel cells from all fetal stages examined were found to be negative for neurofilaments (Fig 2*c,d*). In contrast, dermal nerve fibers were positive and some of them were in contact with the dermal-epidermal junction (Fig 2*d*).

The monoclonal antibody K_K 8.60 against cytokeratins nos. 10/11 stained the suprabasal epidermal keratinocytes (Fig 2*f*) whereas the Merkel cells, as identified by staining of antibodies against cytokeratin no. 18, were negative, as were the basal keratinocytes (Fig 2*e,f*). Monoclonal antibody KA1 stained the whole epidermis, including the basal layer, except for the Merkel cells (Fig 2*g,h*). In contrast, the Merkel cells were selectively decorated by monoclonal antibodies K_s 8.1 and A53-B/A 2 against the simple epithelium-type cytokeratins nos. 8 and 19, respectively (Fig 2*i,j*).

Electron microscopy confirmed the identification of Merkel cells in the basal compartment of the fetal epidermis (Fig 3). As best recognized by immunofluorescence microscopy, cell bodies

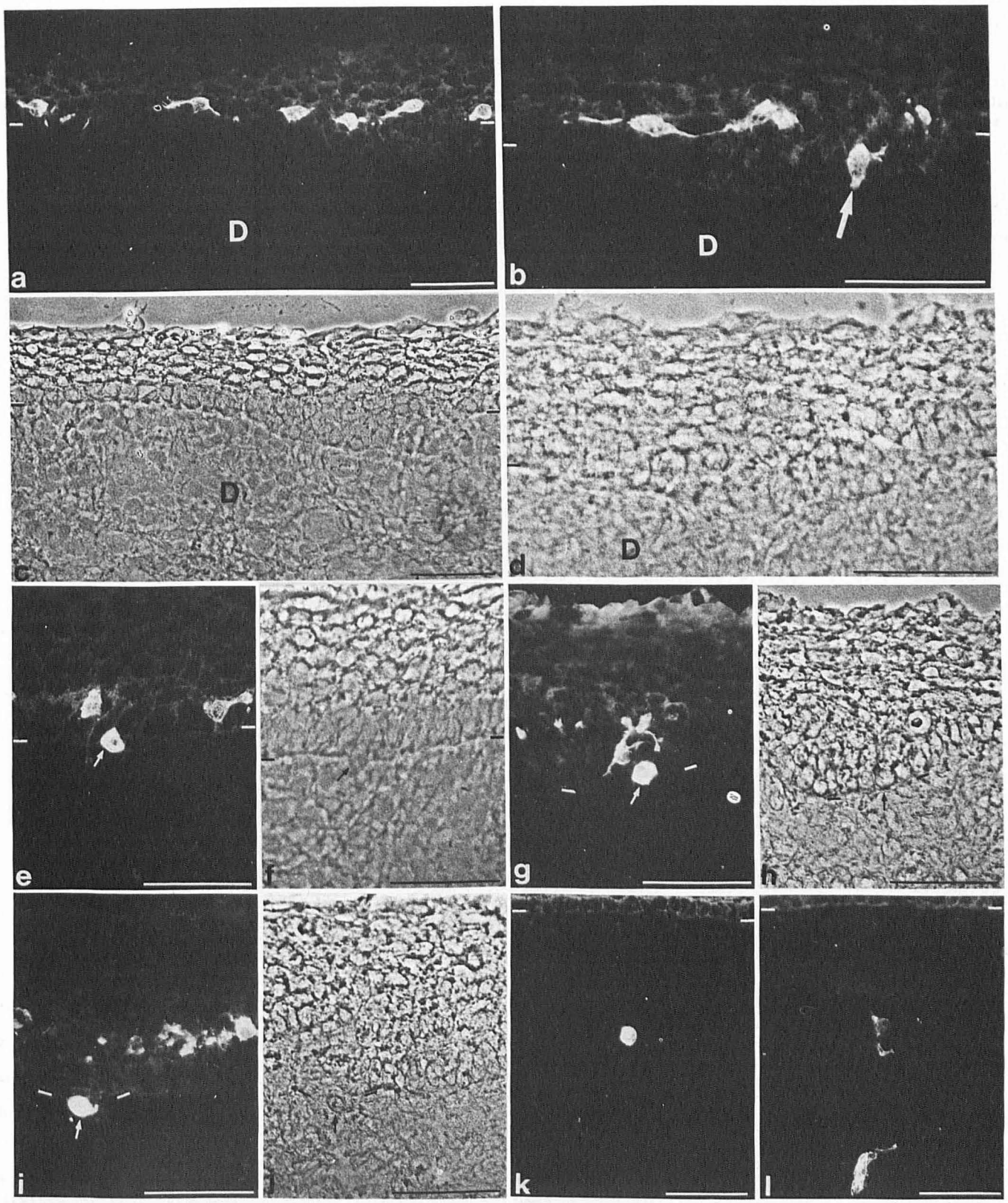

Figure 1. Immunofluorescence microscopy of plantar skin of 14-week-old human fetus. *a*, Monoclonal antibody RGE 53 against cytokeratin no. 18 stains several Merkel cells situated in or above the basal cell layer. *b*, Guinea pig antibodies against cytokeratin no. 18 showing decoration of Merkel cells in suprabasal position extending thin processes. Only one Merkel cell (*arrow*) has contact to the basal lamina. *c and d*, Phase contrast pictures corresponding to (*a*) and (*b*), respectively. *e through h*, Antibodies against cytokeratin no. 18 (*e*, RGE 53; *g*, guinea pig antibodies) showing Merkel cells in basal position protruding toward the dermis (*arrows*; *f* and *h* are phase contrast pictures corresponding to *e* and *g*, respectively). *i and j*, Guinea pig antibodies against cytokeratin no. 18, showing a Merkel cell positioned half in the epidermis and half in the dermis (*arrow*; *j* is the phase contrast picture to *i*). *k and l*, Double immunofluorescence microscopy showing, in the upper dermis, a solitary Merkel cell positive for antibody RGE 53 against cytokeratin no. 18 (*k*) but negative for the rabbit antibodies against neurofilament protein NF-L (*l*); the neurofilament antibodies stain, however, nerve fibers closely associated with the dermal Merkel cell. *Small bars* denote the dermal-epidermal junction. *D* = dermis. *Bars* represent 50 μm.

Table I. Quantitative Aspects of Merkel Cells in Human Fetal Plantar Skin

Fetal Age (weeks)	Length of Epidermis Analyzed in Sections (mm)	Numbers of Epidermal Merkel Cells[a]	Numbers of Dermal Merkel Cells[a]	Numbers of Merkel Cells per Millimeter Epidermis in Section	Percentage of Dermal Merkel Cells
8	12.5	0	0	0.0	0.0
12	130.0	860	0	6.6	0.0
14	129.0	895	35	6.9	3.7
17	20.7	140	29	6.8	17.2

[a]As detected by immunofluorescence microscopy using antibodies against cytokeratin no. 18.

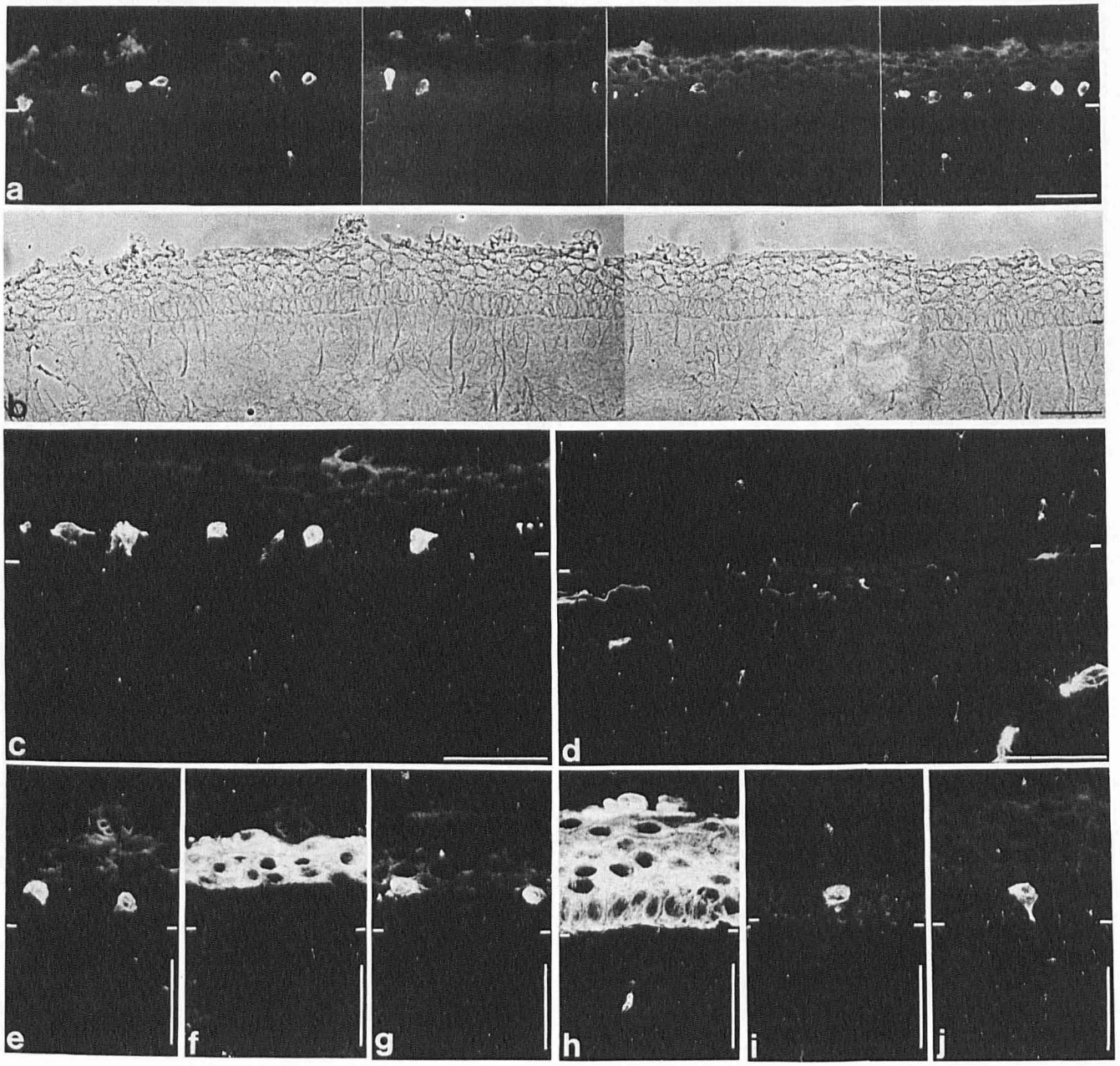

Figure 2. Immunofluorescence microscopy of plantar skin of 12-week-old human fetus. *a*, Survey micrograph showing positive staining with antibody CK 2 against cytokeratin no. 18 of Merkel cells exclusively within the epidermis. *b*, Phase contrast picture corresponding to (*a*). *c and d*, Double immunofluorescence microscopy using antibody CK 2 against cytokeratin no. 18, which stains epidermal Merkel cells (*c*), counterstained with rabbit antibodies against the neurofilament polypeptide NF-M, which stains dermal nerve fibers (*d*). *e and f*, Double immunofluorescence microscopy, demonstrating that Merkel cells identified by guinea pig antibodies against cytokeratin no. 18 (*e*) are negative with antibody K_K 8.60, which stains suprabasal keratinocytes (*f*). *g and h*, Double immunofluorescence microscopy, showing that Merkel cells positive for guinea pig antibodies against cytokeratin no. 18 (*g*) are not decorated by antibody KA1, which stains all keratinocytes (*h*). *i*, A Merkel cell selectively stained by antibody K_s 8.1 against cytokeratin no. 8. *j*, A Merkel cell selectively positive for antibody A53-B/A2 specific for cytokeratin no. 19. *Small bars* denote the dermal-epidermal junction. *Bars* represent 50 μm.

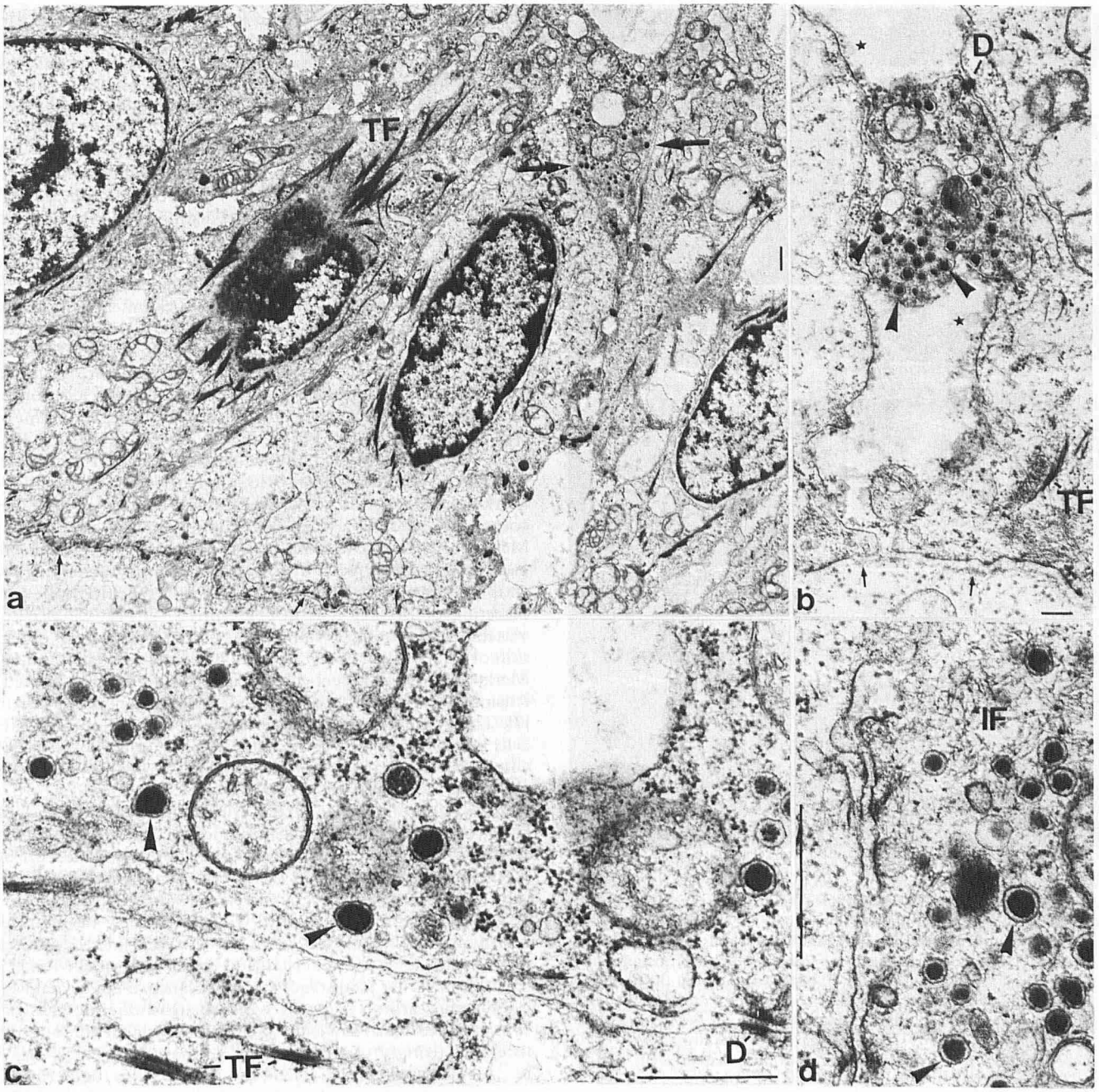

Figure 3. Electron microscopy of plantar epidermis of 13-week-old human fetus. *a*, Survey electron micrograph showing a small section of a Merkel cell (*arrows*) containing dense-cored granules, situated above the layer of the tonofibril (*TF*)-containing basal keratinocytes. *Small arrows* denote the basal lamina. *b*, Small cytoplasmic process of a Merkel cell containing numerous dense-cored neurosecretory granules (*arrowheads*) situated in a widened space between 2 keratinocytes which appears to be lined by a mucus-like substance. *D* = desmosome connecting the process with the adjacent keratinocyte. *TF* = tonofibril. *Small arrows* denote the basal lamina. *c*, Detail of a Merkel cell containing neurosecretory granules (*arrowheads*) of variable size (65–150 nm diameter). A small desmosome (*D*) connects this cell with the adjacent basal keratinocyte. *TF* = tonofibrils. *d*, Merkel cell with neurosecretory granules (*arrowheads*) and intermediate filaments (*IF*) arranged in loose and random pattern. *Bars* represent 0.5 μm.

containing the nucleus (not shown) as well as cytoplasmic processes of Merkel cells (Fig 3*a,b*) were often seen at positions immediately above the basal keratinocyte layer (Fig 3*a*). Higher magnification revealed numerous dense-cored neurosecretory granules in the cytoplasm of Merkel cell bodies and processes (Fig 3*b–d*). Small, although typical desmosomes connected the Merkel cells with the adjacent keratinocytes which were also identified by their densely packed bundles of tonofilaments (Fig 3*b,c*). In contrast to the keratinocytes, the Merkel cells did not show densely fasciated tonofilament bundles but their IF appeared in rather loose and often random arrangements (Fig 3*d*). We did not observe cells containing both tonofilaments and neurosecretory granules, i.e., cells that would correspond to "transitional cells" [6,14].

In plantar skin of 7- *to 9-week-old fetuses,* the epidermis was relatively straight and consisted of 2–3 cell layers. The upper cell layer contained some partly rounded, probulging cells typical of the periderm (Fig 4*a–d*). In immunoflourescence microscopy, both the basal epidermal cell layer and the periderm layer were stained by the cytokeratin antibody PKK 1 (Fig 4*a*). In contrast, the antibodies against cytokeratin no. 18 used in this study, i.e., CK2 and RGE 53, selectively stained the upper periderm layer, while the basal layer was negative (Fig 4*b*). As another cytokeratin no. 18 recognizing monoclonal antibody (K_s 18.18) did react with

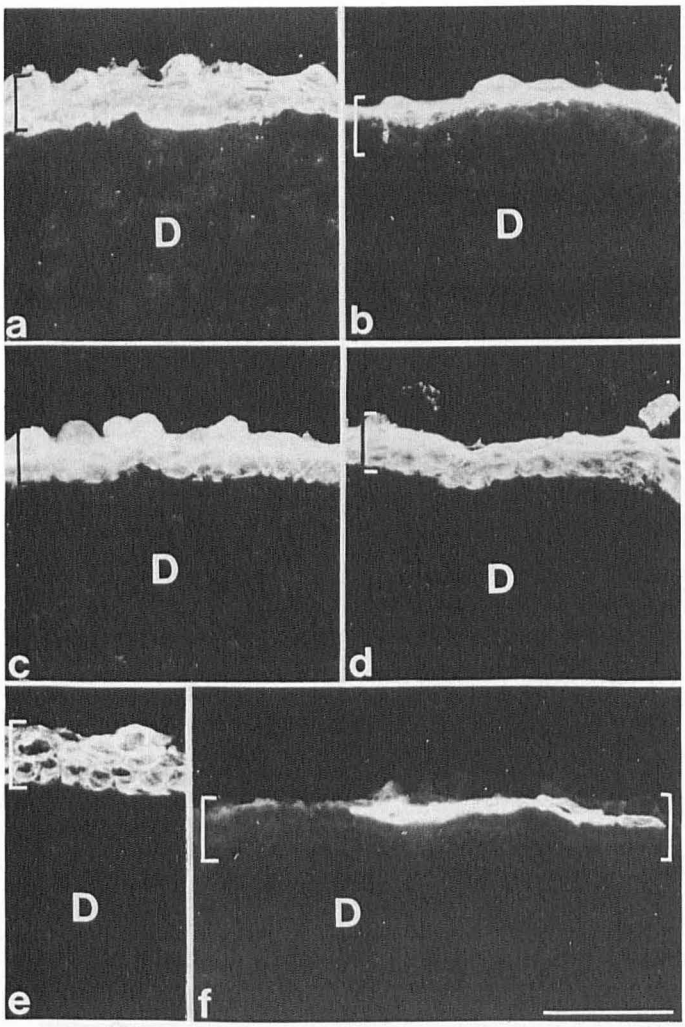

Figure 4. Immunofluorescence microscopy of plantar epidermis of 8-week-old human fetus. *a*, Antibody PKK1 staining the whole epidermis. *b*, Antibody CK2 against cytokeratin no. 18 staining the upper (periderm) layer, while the basal layer is negative and Merkel cells are not detected therein. *c and d*, Antibody K_s 8.1 against cytokeratin no. 8 (*c*) and antibody A53-B/A2 against cytokeratin no. 19 (*d*), both staining both the periderm and the basal cell layer. *e*, Antibody KA1 specific for stratified squamous epithelial and myoepithelial cells also decorates both the periderm and the basal cell layer. *f*, Antibody K_K 8.60 against cytokeratins nos. 10/11, selectively staining foci of developing flat intermediate cells. *D* = dermis. The *brackets* denote the thickness of the epidermis. *Bars* represent 50 μm.

these basal cells, the negative reaction of CK 2 and RGE 53 is probably due to a different arrangement of the IF and/or the epitope, respectively. In none of the specimens of this stage, could Merkel cells be selectively identified with the antibodies against cytokeratin no. 18 (Table I). Similarly, no Merkel cells were detected by electron microscopic examination of plantar skin of a *7-week-old fetus* with a total epidermal length of ~1.5 mm. Antibodies against the simple epithelium-type cytokeratins nos. 8 (Fig 4*c*) and 19 (Fig 4*d*) stained, as expected, the periderm layer but also the cells of the basal cell layer. Both periderm cells and the basal cell layer were positive with antibody KA1 (Fig 4*e*) which is specific for cytokeratin filaments of stratified squamous epithelia and myoepithelial cells. Flattened cells with a heterogeneous, patchlike distribution in an intermediate layer position showed a positive reaction with antibody K_K 8.60 against the epidermal cytokeratins nos. 10/11 (Fig 4*f*).

When deeper regions of the fetal plantar skin of the various stages were examined by immunofluorescence microscopy with antibodies against cytokeratin no. 18, positive cells could not be detected in any of the specimens, while thick bundles of neurofilament-positive nerve fibers were present in this tissue (not shown).

DISCUSSION

We have previously shown that, in plantar skin of human fetuses and adults, Merkel cells are relatively frequent and are immunocytochemically recognized—and distinguished from keratinocytes—by antibodies specific for cytokeratin no. 18 [7]. Similar immunocytochemical results have been reported for Merkel cells in various other locations and in other species [8,9,11,12]. This is confirmed by our present immunocytochemical demonstration that in early fetal epidermis individual cells that are selectively positive for cytokeratin no. 18 correspond to typical Merkel cells as recognized by electron microscopy. Characteristic morphologic features of these cells include their near-basal location, slender cytoplasmic processes, and dense-core neuroendocrine vesicles.

Electron microscopic findings of single Merkel cells situated in the dermis of human fetal skin, together with observations of Merkel cells that seem to pass the dermal-epidermal junction, are commonly taken as evidence in support of the hypothesis that Merkel cells originate from neural crest-derived cells which invade the epidermis [1,15–19]. Using immunocytochemistry with antibodies against cytokeratin no. 18 as a specific probe for the detection of Merkel cells in epidermis [7–9,11], we also found variable proportions of Merkel cells in the upper dermis of plantar skin of human fetuses of 17 weeks and older as well as individual Merkel cells at the level of the basal lamina which showed extensions into both the dermal and the epidermal compartment [7]. These findings suggest that during fetal development Merkel cells migrate from one compartment to the other but they do not allow a decision on the direction of this migration. Therefore, we have studied plantar skin of earlier fetal stages.

Our results show that dermal Merkel cells and Merkel cells in transit between the dermal and epidermal compartments exist rarely, if at all, in early stages of skin development, i.e., up to week 12. From week 13 on, a detectable proportion of the Merkel cells was located in the upper dermis, and this proportion appeared to increase, at least until week 17. If these dermal Merkel cells would be on their way from the neural crest to the epidermis, one would expect more dermal and fewer epidermal Merkel cells to occur in earlier fetal stages and an absence of epidermal Merkel cells in the earliest stages in which neuroendocrine cells of the skin are detected. However, our data show that the opposite is true and strongly suggest that the dermal Merkel cells originate in the epidermal compartment and migrate into the dermis. The frequency of mitotic divisions of epidermal and dermal Merkel cells during the developmental period studied is not known. However, the occurrence and frequency of such divisions in either compartment would not bear on the histogenic pathway proposed.

Our findings are also in agreement with several electron microscopic observations that Merkel cells are not seen in the dermis prior to week 12 [1,15,18,35] and with cytoskeletal protein characterizations of Merkel cells. Their epithelial nature is demonstrated by the presence of desmosomes and desmosomal proteins as well as cytokeratin filaments [7–9,11,12]. On the other hand, cytoskeletal neural markers such as neurofilaments and glial filaments are absent [this study, 7–9,12,36,37] (for the occurrence of neurofilaments in some forms of the putatively Merkel cell-derived neuroendocrine tumors of the skin see [10,38,39]).

Further support for the epidermal derivation of Merkel cells is the observation of so-called transitional cells in fetal, newborn, and adult mammals of various species. These cells show features of both keratinocytes and Merkel cells and, therefore, are thought to be developing Merkel cells [40]. However, there are arguments

against this interpretation such as reports of transitional cells in adult but not in embryonic epidermis of birds [41]. Perhaps the most conclusive argument supporting the epidermal histogenesis of neuroendocrine cells of the skin is provided by results from experimental studies in animals. In amphibia, for example, the development of epidermal neuroendocrine cells, i.e., cells equivalent to Merkel cells, takes place even after removal of the neural crest in earlier stages of embryogenesis [13]. Moreover, in regenerating stratified squamous epithelium of the labial mucosa of adult rabbits, Merkel cells can appear as transitional cells located within the epithelium, although undifferentiated neuroectodermal tissue is not present in the adult animals [14].

Although the cytokeratin content clearly identifies the Merkel cells as epithelial, their complement of cytokeratin polypeptides expressed (for details see [7]) does not indicate a derivation from differentiated keratinocytes which have a very different cytokeratin polypeptide composition [cf. 7,42–45]. Therefore, a direct histogenic relationship between basal keratinocytes and Merkel cells would have to involve a complete switch in cytokeratin polypeptide expression from stratified to simple epithelium-type components, which we consider unlikely. Our examinations of earlier fetal stages offer an alternative explanation [7; see also 11,42]. Cells of the basal epidermal layer of 7- to 9-week embryos in which Merkel cells are not yet detectable, express the simple epithelium-type cytokeratins nos. 8 and 19 [42,46] and probably also minor amounts of cytokeratin no. 18. Most likely these basal cells also express some stratified epithelium-type cytokeratins because polypeptides nos. 5 and 6 have been found in early fetal skin of other body sites from the 2-layer stage on [42] and basal cells of embryonic and fetal plantar epidermis are positive with the cytokeratin antibody KA1 which recognizes cytokeratins of this group [27]. Therefore, the apparent simultaneous expression of stratified and simple epithelium-type cytokeratins in the epidermal basal cell layer of 7- to 9-week-old fetuses suggests that these basal cells include multipotent epithelial stem cells that might alternatively give rise to true keratinocytes, expressing stratified epithelium-type cytokeratins, and to Merkel cells, expressing only simple epithelium-type cytokeratins. A complex cytokeratin polypeptide pattern similar to that probably present in the early basal cells has also been found in an embryologically and topologically related epithelium, the amnion epithelium [47].

This hypothesis of Merkel cell origin would imply that basal cells of 7- to 9-week-old fetuses may begin to produce neurosecretory granules and also lose their tonofilaments consisting of stratified epithelium-type cytokeratins. It is not yet clear whether the expression of neuroendocrine features occurs synchronously with the cytoskeletal change to the simple epithelial cytokeratin pattern, or whether the cytoskeletal changes precede the onset of expression of neuroendocrine features. Conversely, the possible existence of precursor cells that might simultaneously express some stratified-type cytokeratins as well as the simple epithelial ones, together with neuroendocrine elements, i.e., cells that would correspond to the transitional cells described in other species [6,14,40,41,48–50], will have to be examined in future immunocytochemical experiments.

Our conclusion that Merkel cells develop from certain epithelial cells within the fetal epidermis, and not from cells immigrated from the neural crest, puts the neuroendocrine cells of the epidermis into one line with other epithelial neuroendocrine cells such as the enterochromaffin and other APUD cells of the gastrointestinal tract as well as the pancreatic islet cells for which a neural crest derivation has been excluded (for review see [51]). Thus, at present the only bona fide epithelial cell type, expressing cytokeratins, that has been proved to originate from the neuroectoderm is the calcitonin-producing ("C") cell of the thyroid gland [52; for literature see 51].

The biologic functions of epidermal and dermal Merkel cells in fetal development of mammals, as well as in postnatal life are not clear. A popular concept is that these cells represent mechanoreceptors, and this is primarily based on morphologic similarities of mammalian Merkel cells with cells present in dermal sensory corpuscles of some lower vertebrates (for review see [2]). The frequent and close association of dermal Merkel cells with dermal nerves may also suggest an analogy to the dermal sensory corpuscles found in a number of non-mammalian vertebrates such as the Grandry corpuscles of the upper dermis of the avian hard palate, which apparently function as sensory organs in the adult animals [48,49]. The interpretation of the dermal Merkel cell–nerve complexes as rudimentary Grandry corpuscles in the sense of phylogenic relicts would explain their only transient existence in fetal life. However, Saxod [41] has suggested that the avian dermal Merkel cells might differ in histogenesis from the epidermal Merkel cells of other species. Unfortunately, the cell type relationship of mammalian Merkel cells to the dermal Merkel cells of birds has not yet been demonstrated with adequate cell biologic criteria, including epithelial markers. An alternative hypothesis of Merkel cell function is that these cells, as well as the neuroendocrine epithelial cells of other epithelia (for references see [10]), produce and secrete [53] tissue hormones which induce and/or promote the local formation, growth, and differentiation of special skin structures such as, depending on the specific body site, hair follicles, eccrine sweat glands, and nerves. The products of Merkel and other neuroendocrine epithelial cells could then serve a similar function in local cell proliferation and morphogenesis as, for example, the neuropeptides described as "head activators" in lower metazoa such as in *Hydra* [54]. The concepts of a paracrine function of Merkel cells would also be in line with the idea that Merkel cells, specifically the epidermal ones, can act as "target structures" for outgrowing nerve fibers [55], and the disappearance of dermal Merkel cells during later fetal development [7] might then merely reflect the loss of such functions in histogenesis. A considerable reduction in the number of neuroendocrine cells during the later stages of fetal life has also been reported for the neuroendocrine ("Kutschinsky") cells of the bronchial epithelium [56] which express, among other components, bombesin, a molecule for which paracrine and autocrine functions have been reported [57].

It is still unsettled whether the neuroendocrine tumors of the skin, the so-called Merkel cell tumors, indeed developed from Merkel cells (for review see [10]). These tumors appear to develop in the dermis without a direct spatial connection to the epithelia of the epidermis and outer root sheath. The assumption that the dermal Merkel cell might be the cell of origin of Merkel cell tumors would easily overcome this apparent discrepancy. However, in adults in whom these tumors develop, dermal Merkel cells have not yet been convincingly shown, although occasional residual Merkel cells may exist in the postnatal dermis and provide a reservoir for potentially proliferative cells. At least our observation that Merkel cells, in principle, are able to migrate into the dermis may offer a new possible relationship between Merkel cells and the neuroendocrine tumors of the skin, i.e., Merkel cell tumors.

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REFERENCES

1. Breathnach AS: The mammalian and avian Merkel cell, The Skin of Vertebrates. Edited by RIC Spearman, PA Riley. Dorchester, Dorset, Henry Ling, Dorset Press, Linnean Society Series no. 9, 1979, pp 283–291
2. Hartschuh W, Weihe E, Reinecke M: The Merkel cell, Biology of the Integument, vol. 2, Vertebrates. Edited by J Bereiter-Hahn, AG Maltoltsy, KS Richards. Berlin/Heidelberg/New York, Springer-Verlag, 1986, pp 605–617
3. Munger B: The intra-epidermal innervation of the snout skin of the oppossum. A light and electron microscope study with observation on the nature of Merkel's Tastzelle. J Cell Biol 26:79–97, 1965
4. Kurosumi K, Kurosumi U, Inoue K: Morphological and morphometric studies with the electron microscope on the Merkel cells and associated nerve terminals of normal and denervated skin. Arch Histol Jpn 42:243–261, 1979
5. Smith KR: The ultrastructure of the human Haarscheibe and Merkel cell. J Invest Dermatol 54:150–159, 1970
6. English KB: Morphogenesis of Haarscheiben in rats. J Invest Dermatol 69:58–67, 1977
7. Moll R, Moll I, Franke WW: Identification of Merkel cells in human skin by specific cytokeratin antibodies: changes of cell density and distribution in fetal and adult plantar epidermis. Differentiation 28:136–154, 1984
8. Saurat JH, Didierjean L: The epidermal Merkel cell is an epithelial cell. Dermatologica 169:117–120, 1984
9. Saurat JH, Didierjean L, Skalli O, Siegenthaler G, Gabbiani G: The intermediate filament proteins of normal epidermal rabbit Merkel cells are cytokeratins. J Invest Dermatol 83:431–435, 1984
10. Gould VE, Moll R, Moll I, Lee I, Franke WW: Neuroendocrine (Merkel) cells of the skin: hyperplasias, dysplasias, and neoplasms. Lab Invest 52:334–353, 1985
11. Lane EB, Bártek J, Purkis PE, Leigh IM: Keratin antigens in differentiating skin, Intermediate Filaments. Edited by E Wang, D Fischman, RKH Liem, TT Sun. Ann NY Acad Sci 455:241–258, 1985
12. Ortonne JP, Darmon M: Merkel cells express desmosomal proteins and cytokeratins. Acta Derm Venerol (Stockh) 65:161–164, 1985
13. Tweedle CD: Ultrastructure of Merkel development in aneurogenic and control amphibian larvae (*Ambystoma*). Neuroscience 3:481–486, 1978
14. Tachibana T, Ishizeki K: Merkel cell development in the wound healing in the labial mucosa of adult rabbits. Arch Histol Jpn 44:151–165, 1981
15. Hashimoto K: The ultrastructure of human embryos. X. Merkel tactile cells in the finger and nail. J Anat 111:99–120, 1972
16. Winkelmann RK: The Merkel cell system and a comparison between it and the neurosecretory or APUD cell system. J Invest Dermatol 69:41–46, 1977
17. Winkelmann RK, Breathnach AS: The Merkel cell. J Invest Dermatol 60:2–15, 1973
18. Breathnach AS: Embryology of human skin: a review of ultrastructural studies. J Invest Dermatol 57:133–143, 1971
19. Breathnach AS: Ultrastructure of embryonic skin. Curr Probl Dermatol 9:1–28, 1981
20. Streeter GL: Weight, sitting height, head size, foot length, and menstrual age of the human embryo. Contributions to Embryology 11:143–170, 1920
21. Franke WW, Schmid E, Freudenstein C, Appelhans B, Osborn M, Weber K, Keenan TW: Intermediate-sized filaments of the prekeratin type in myoepithelial cells. J Cell Biol 84:633–654, 1980
22. Debus E, Weber K, Osborn M: Monoclonal cytokeratin antibodies that distinguish simple from stratified squamous epithelial: characterization on human tissues. EMBO J 1:1641–1647, 1982
23. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R: The catalog of human cytokeratin polypeptides: patterns of expression of specific cytokeratins in normal epithelial, tumors and cultured cell. Cell 31:11–24, 1982
24. Ramaekers F, Hysmans A, Moesker O, Kant A, Jap P, Herman C, Vooijs P: Monoclonal antibody to keratin filaments specific for glandular epithelia and their tumors. Lab Invest 49:353–361, 1983
25. Moll R, Franke WW: Cytoskeletal differences between human neuroendocrine tumors: a cytoskeletal protein of molecular weight 46,000 distinguishes cutaneous from pulmonary neuroendocrine neoplasms. Differentiation 30:165–175, 1985
26. Huszar M, Gigi-Leitner O, Moll R, Franke WW, Geiger B: Polypeptide-specific monoclonal cytokeratin antibodies in the differential diagnosis of squamous carcinomas and adenocarcinomas. Differentiation, in press
27. Nagle BR, Moll R, Weidauer H, Nemetschek H, Franke WW: Different patterns of cytokeratin expression in the normal epithelia of the upper respiratory tract. Differentiation 30:130–140, 1985
28. Miettinen M, Clark R, Lehto V-P, Virtanen I, Damjanov I: Intermediate filament proteins in parathyroid glands and parathyroid adenomas. Arch Pathol Lab Med 109:986–989, 1985
29. Karsten U, Papsdorf G, Roloff G, Stolley P, Abel H, Walther I, Weiss H: Monoclonal anti-cytokeratin antibody from a hybridoma clone generated by electrofusion. Eur J Cancer Clin Oncol 21:733–740, 1985
30. Shaw G, Weber K: Differential expression of neurofilament triplet protein in brain development. Nature 298:227–229, 1982
31. Shaw G, Weber K: The distribution of the neurofilament triplet proteins within individual neurones. Exp Cell Res 136:119–125, 1981
32. Debus E, Weber K, Osborn M: Monoclonal antibodies specific for glial fibrillary acidic (GFA) protein and for each of the neurofilament triplet polypeptides. Differentiation 25:193–203, 1983
33. Van Muijen GNP, Ruiter DJ, Van Leeuwen C: Cytokeratin and neurofilaments in lung carcinomas. Am J Pathol 116:363–369, 1984
34. Franke WW, Lüder MR, Kartenbeck J, Zerban H, Keenan TW: Involvement of vesicle coat material in casein secretion and surface regeneration. J Cell Biol 69:173–195, 1976
35. Breathnach AS, Robins J: Ultrastructural observations on Merkel cells in human fetal skin. J Anat 106:411–419, 1970
36. Saurat HJ, Didierjean L, Dahl D: Normal rabbit Merkel cells (MC) do not express the intermediate filament protein of glial or neuronal cells (abstr). J Invest Dermatol 82:424, 1984
37. Saurat JH, Merot Y, Didierjean L, Dahl D: Normal rabbit Merkel cells do not express neurofilament proteins. J Invest Dermatol 82:641–642, 1984
38. Miettinen M, Lehto V-P, Asko-Seljavaara S, Pitkänen J, Dahl D: Neuroendocrine carcinoma of the skin (Merkel cell carcinoma): ultrastructural and immunohistochemical demonstration of neurofilaments. Ultrastruct Pathol 4:219–225, 1983
39. Hoefler H, Kerl H, Rauch H-J, Denk H: New immunocytochemical observations with diagnostic significance in cutaneous neuroendocrine carcinoma. Am J Dermatopathol 6:525–530, 1984
40. Tachibana T, Nawa T: Merkel cell differentiation in the labial mucous epithelium of the rabbit. J Anat 131:145–155, 1979
41. Saxod R: Development of Merkel corpuscles in the chicken beak. The problem of their origin and identity. Biol Cell 37:61–66, 1980
42. Moll R, Moll I, Wiest W: Changes in the pattern of cytokeratin polypeptides in epidermis and hair follicles during skin development in human fetuses. Differentiation 23:170–178, 1982
43. Baden HP, Lee LD: Fibrous protein of human epidermis. J Invest Dermatol 71:148–151, 1978
44. Fuchs E, Green H: Changes in keratin gene expression during terminal differentiation of the keratinocyte. Cell 19:1033–1042, 1980
45. Woodcock-Mitchell J, Eichner R, Nelson WG, Sun T-T: Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. J Cell Biol 95:580–588, 1982
46. Dale BA, Holbrook KA, Kimball JR, Hoff M, Sun T-T: Expression of epidermal keratins and filaggrin during human fetal skin development. J Cell Biol 101:1257–1269, 1985
47. Regauer S, Franke WW, Virtanen J: Intermediate filament cytoskeleton of amnion epithelium and cultured amnion epithelial cells: expression of epidermal cytokeratins in cells of a simple epithelium. J Cell Biol 100:997–1009, 1985
48. Anderson AE, Nafstad PHJ: An electron microscopic investigation of the sensory organs in the hard palate region of the hen (*Gallus domesticus*). Z Zellforsch Mikroskop Anat 91:391–401, 1968
49. Saxod R: Etude au microscope électronique de l'histogènese du cor-

puscule sensoriel cutané de Grandry chez le canard. J Ultrastruct Res 32:477–496, 1970

50. Garant PR, Feldman J, Cho MI, Cullen MR: Ultrastructure in the hard palate of the squirrel monkey (*Saimiri sciureus*). Am J Anat 157:155–167, 1980

51. Le Douarin N: The Neural Crest. Developmental and Cell Biology Series. Edited by PW Barlow, PB Green, CC Wylie. Cambridge/London/New York/New Rockalle/Melbourne/Sydney, Cambridge Univ Press, 1982, pp 91–107

52. Pearse AGE, Polak J: Immunocytochemical localization of substance P in mammalian intestine. Histochemistry 41:373–375, 1975

53. Tachibana T, Ishizeki K, Sakakura Y, Nawa T: Ultrastructural evidence for a possible secretory function of Merkel cells in the barbels of a teleost fish, *Cyprinus carpio*. Cell Tissue Res 235:695–698, 1984

54. Kemmner W, Schaller HC: Actions of head activator and head inhibitor during regeneration in hydra. Differentiation 26:91–96, 1984

55. Diamond I: The regulation of nerve sprouting by extrinsic influences, The Neurosciences: Fourth Study Program. Edited by FO Schwitt, FC Worden. Boston, Massachusetts Institute of Technology Press, 1979, pp 937–955

56. Gould VE, Linnoila RI, Memoli VA, Warren WH: Neuroendocrine components of the bronchopulmonary tract: hyperplasias, dysplasias, and neoplasms. Lab Invest 49:519–537, 1983

57. Cuttitta F, Carney DN, Mulshine J, Moody TW, Fedorko J, Fischler A, Minna JD: Bombesin-like peptides can function as autocrine growth factors in human small cell lung cancer. Nature 316:823–826, 1985