Cytokeratin No. 9, an Epidermal Type I Keratin Characteristic of a Special Program of Keratinocyte Differentiation Displaying Body Site Specificity

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Abstract. Plantar epidermis of the bovine heel pad as well as human plantar and palmar epidermis contain large amounts of an acidic (type I) keratin polypeptide (No. 9) of Mr 64,000 which so far has not been found in epidermis of other sites of the body. We present evidence for the keratinous nature of this protein, including its ability to form cytokeratin complexes and intermediate-sized filaments in vitro. We have isolated RNA from plantar epidermis of both species and show, using translation in vitro, that these polypeptides are genuine products of distinct mRNAs. Using immunofluorescence microscopy with specific antibodies against this protein, we demonstrate its location in most cells of suprabasal layers of plantar epidermis as well as in sparse keratinocytes which occur, individually or in small clusters, in upper layers of epidermis of other body locations. We conclude that cytokeratin No. 9 is characteristic of a special program of keratinocyte differentiation which during morphogenesis is expressed in most epidermal keratinocytes of soles and palms but only in a few keratinocytes at other body sites. This example of cell type-specific expression of a member of a multigene family in relation to a body site-related program of tissue differentiation raises important biological questions concerning the regulation of keratinocyte differentiation and morphogenesis as well as the function of such topological heterogeneity within a given type of tissue.

Cell type-specific expression of a certain protein of a multigene family is a widespread phenomenon in cell differentiation. A particularly impressive example is found in the large family of cytoskeletal-karyoskeletal proteins that includes the proteins forming the intermediate-sized filaments (IFs) of the cytoplasm (23, 40, 64, 71) and the proteins that constitute the nuclear lamina (6, 44). The IF proteins are usually classified according to their expression in certain pathways of cell differentiation: cytokeratins occur in all epithelia, neurofilaments in neurons and in some other neurosecretory cells, glial filament protein in astrocytes and certain non-glial cells, desmin in myogenic cells, and vimentin in mesenchymally derived cells as well as in various other kinds of cells in which it can co-exist with any of the other kinds of IF proteins (for reviews see 23, 40, 53, 71).

The cytokeratins display a further refined principle of cell type-specific expression, in that the IFs of different epithelia are formed by different sets of cytokeratin polypeptides (13, 19–21, 23, 72, 75). For example, in human tissues and tumors, 19 different cytokeratin polypeptides have been distinguished which are expressed in sets characteristic of a specific type of epithelium (9, 23, 45, 48, 55, 67, 68), and similar complexities of cytokeratin polypeptide expression in different epithelia have been reported for bovine and rodent tissues (9, 19–21, 23, 57). The epithelial cytokeratins, like sheep wool keratins (10), can be grouped into two subfamilies, the usually somewhat smaller acidic (type I) cytokeratins and the mostly larger, more basic (type II) polypeptides (30, 33, 57, 64).

Unlike the other types of IF proteins, the cytokeratins always appear to be expressed as “pairs” of polypeptides; i.e., one representative from both the basic and the acidic subfamily. These pairs form four-chain heterotypic complexes which represent the architectural subunits of cytokeratin IFs in which the polypeptides of the two subfamilies are held together by hydrogen bonds of varying strengths depending on the pair in question (24, 54, 74). It has been emphasized by some authors (9, 64, 67) that within each pair the basic (type I) partner exceeds the acidic (type II) polypeptide by Mr, ~8,000. Certain cells contain only one type of keratin pair (20, 23, 25, 45, 54, 55) whereas others possess more, with up to 11 different cytokeratin polypeptides existing in a single cell (25, 45, 46). While in vitro re-assembly experiments show that most, probably all, cytokeratin polypeptides are able to
form complexes with any partner of the other subfamily (34), random expression and pairing has not been observed in vivo.

Biochemical analyses of IF proteins from a wide range of human tissues and tumors and immunolocalization studies using antibodies specific for individual cytokeratins have shown that certain cytokeratins are typical of simple epithelia whereas others occur in stratified epithelia (1, 9, 12, 23, 25, 45, 50, 70). Among the various stratified epithelia, epidermis contains a particularly rich complement of cytokeratin polypeptides (3, 4, 7, 15, 20, 23, 27-29, 34, 45, 46, 49, 65-67), and fetal epidermis can be even more complex than neonatal and adult epidermis (5, 11, 47, 50, 58). Moreover, the different epithelia present in skin, such as hair follicles and their root sheaths, sweat glands, and sebaceous glands differ qualitatively in their cytokeratin composition from the keratinocytes of the interfollicular epidermis (46), despite their common embryological origin from epidermis. Furthermore, different sets of cytokeratins are synthesized in different epidermal layers, indicating the existence of a vertical program of changes of cytokeratin expression during keratinocyte differentiation and maturation (3, 4, 27-29, 32, 47, 60, 62, 73).

In addition to these vertical differences, differences of cytokeratin composition between epidermal tissue samples taken from different body sites have been reported, in particular the specific occurrence, in cow and human, of an unusually large (Mr 63,000-64,000), acidic (type I) cytokeratin which has been found only in human callus-forming epidermis (51) such as in foot soles or palms (45, 46, 49, 50; see also 7, 28, 32, 65) and in the corresponding bovine tissue (for anatomy, see reference 76); i.e., the heel pad on the posterior side of the hoof (cf. 14, 17, 41, 63, 69). However, apparently because of the unusual properties of this polypeptide and in view of various reports describing the formation of several epidermal keratins from precursor molecules by proteolytic and other modifications (8, 27, 28, 32), the significance of this sole- and palm-specific epidermal cytokeratin polypeptide, which has been designated "cytokeratin No. 9" (45, 57), has been questioned. In particular, Sun and colleagues (9, 66, 67) have expressed doubts as to whether this component represents an "intact, genuine keratin species." Therefore, we have examined this question in greater detail. Our present study provides evidence that cytokeratin No. 9 is a true cytokeratin encoded by a discrete mRNA that is abundantly expressed in the palmar and plantar epidermis but can also occur, in sparser cells, in epidermis of other body locations.

Materials and Methods

Preparation of Cytoskeletal Material and Keratins for Gel Electrophoresis

Epidermal tissue from bovine heel pads, muzzle epidermis, and body skin from various sites (14, 17, 20, 56) was obtained from cows, calves, and late fetuses by sectioning with a scalpel parallel to the surface. Tissue sections were either used directly or frozen in liquid nitrogen and stored at -80°C. Alternatively, for extraction of cytokeratins the tissue sections were either used directly or frozen in liquid nitrogen and stored at -80°C.

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including antisera raised against individual bovine heel pad cytokeratin polypeptides (17–21, 26, 46, 56), and identification of the antibodies bound by [125I]-protein A were performed as described (31).

**Antibodies to Cytokeratin No. 9 and Immunofluorescence Microscopy**

Antisera to purified cytokeratin No. 9 raised in guinea pigs (17), and antibodies were immunofluorescently purified on cytokeratin No. 9 bound to nitrocellulose strips, using, however, Poncova-S instead of Amido black staining, and 3 M KSCN for elution (39). Possible traces of residual cross-reactivity with other epidermal cytokeratins were removed by additional immunoadsorption on keratins from dissected middle and lower layers of bovine snout epidermis which were separated by SDS PAGE and blotted on nitrocellulose paper. For comparison, monoclonal murine antibody Kc, 8.13, which shows broad cytokeratin specificity (31) was used. Indirect single- and double-label immunofluorescence microscopy was performed on cryostat sections essentially as described (50). The secondary antibodies, fluorescein isothiocyanate–coupled goat antibodies to guinea pig or mouse Ig's and Texas Red–labeled goat antibodies to murine Ig's were obtained from Di-anova (Hamburg, FRG).

**Results**

**Identification of Bovine Cytokeratin No. 9 as Translation Product**

To examine whether bovine cytokeratin polypeptide No. 9 is a genuine product of translation of a tissue-specific mRNA, we isolated RNA from the total epidermis of the heel pad of the hoof (for histology see 41, 63) and used it for translation in vitro in the rabbit reticulocyte lysate system. For comparison, RNA samples isolated from epidermis of other body sites such as the mouth and normal hair follicle–containing skin of the cheek region were used in parallel. As an example, Fig. 1, a–c, presents the major cytokeratin polypeptides of bovine muzzle epidermis and the corresponding in vitro translation products, identified by co-electrophoresis. Cytokeratins designated Ia–c, III, IV, VIa, b and VII appeared as the prominent polypeptides of both the tissue (a, b) and in vitro translation (c). In addition, we consistently noted two minor basic (type II) cytokeratin polypeptides, designated No. 4 and 5 in previous reports (36, 38; see also 20). Most of the mRNAs encoding these components have recently been cloned, partially sequenced, and identified as distinct gene products (35–37, 42). The same components have also been identified among the in vitro translation products of other regions of the cow’s head and rump which contained the hair follicle α-keratins as additional components (these authors, unpublished findings).

The heel pad epidermis displayed a different pattern of cytokeratins and of translation products in vitro (Fig. 1, d–f). In this tissue, polypeptides corresponding in size and electrical charge to cytokeratins Ia–c (Nos. 1–3 of the bovine catalog of reference 57) were not detected. Instead, the cytokeratin polypeptide No. 5 appeared as a major cytokeratin, and the proportion of polypeptide No. 4 was greater than in the epidermal samples from other body locations. The relative amounts of epidermal keratins III and IV were also higher. The most remarkable difference, however, was found in the complement of acidic (type I) cytokeratins which comprised, besides keratins VIa, b and VII, a third major polypeptide of Mr, 64,000. This cytokeratin (No. 9) was often resolved into a series of at least three isoelectric variants in isoelectric focusing (Fig. 1 e), probably representing different degrees of phosphorylation, and seemed to correspond to the upper intense band in the range of Mr, 60,000–64,000 described in previous one-dimensional gel electrophoretic separations reported by us and other authors studying bovine hoof epidermis (14, 17, 41, 63). In addition, we frequently noted in such cytoskeletal preparations a minor acidic polypeptide of an isoelectric point similar to that of cytokeratin VII but slightly faster in electrophoresis in the presence of SDS (arrows in Fig. 1, d and f). Probably this latter polypeptide, whose cytokeratinous nature is not fully clear, corresponds to the Mr, 48,000 component designated epidermal keratin band VIII in our previous one-dimensional analyses (17, 26). Clearly and most importantly, the in vitro translation of bovine sole epidermal RNA (Fig. 1 f) provided evidence that both acidic components, cytokeratin No. 9 and the small Mr, 48,000 polypeptide, are products of distinct RNAs.

These findings are schematically summarized in Fig. 2 which shows the common bovine epidermal keratin and the body site–specific ones.

**Criteria for Classification of Bovine Epidermal Polypeptide No. 9 as a Cytokeratin**

Epidermal polypeptide No. 9 has previously been classified as a cytokeratin because of its resistance to extractions with low and high salt buffers and various detergents, its solubility in high concentrations of urea and citric acid, its inclusion—among several other keratins—in reconstituted IFs produced from total hoof-sole epidermis, and its cross-reactivity with several guinea pig antisera raised against bovine muzzle epidermal cytokeratins (e.g., 17, 18, 26; see also 14, 41, 63). On the other hand, it does not significantly react with subfamily I–specific monoclonal antibodies such as AE1 (cf. 68). In the present study we have used additional and more stringent criteria to examine the cytokeratinous nature of this polypeptide. In Fig. 3, we show the progressive “melting” of cytokeratin complexes in increasing concentrations of urea (24). When bovine heel pad cytokeratins were denatured in buffer containing 9.5 M urea and then dialyzed against various concentrations of urea, polypeptide No. 9 was seen at 5 M and 6 M urea (Fig. 3, a and b) in the same intermediate isoelectric position as the other cytokeratins present, indicative of its inclusion in a typical cytokeratin complex. At concentrations of 7 M and above, polypeptide No. 9 separated from the other cytokeratins and migrated to its typical position at pH 5.4 (Fig. 3, c–e). This melting behavior is typical of cytokeratin complexes of an intermediate melting point (Umr) between 6 and 7 M urea (24, 25, 34). A similar complex formation and melting behavior was observed when purified polypeptide No. 9 was mixed with bovine epidermal keratins III and IV and the non-epidermal cytokeratin No. 8 (not shown).

We also examined the ability of purified bovine cytokeratin No. 9 to form IFs in vitro by mixing it, in a molar ratio of 1:1, with various basic (type II) cytokeratins such as epidermal components III and IV and the non-epidermal cytokeratin No. 8 isolated from bovine bladder. All these combinations resulted in the formation of typical protofilaments and IFs as judged from electron microscopy. As an example we show in Fig. 4, the IFs formed in vitro from the combination of cytokeratins 8 and 9, which are not co-expressed in any
Figure 1. Two-dimensional gel electrophoresis of cytokeratins isolated from bovine muzzle and heel pad epidermis and the in vitro translation products of mRNA extracted from these tissues. Non-equilibrium pH gradient (NEPHG; a-d and f) electrophoresis or isoelectric focusing (IEF; e) was used in the first dimension. SDS, direction of the electrophoresis in the second dimension, in the presence of SDS. (a) Coomassie Blue staining of a gel showing all cytokeratins of bovine muzzle epidermis, with basic components Iα-c and III/IV particularly well resolved. Note cytokeratin Nos. 4 and 5 as minor components. (b and c) Co-electrophoresis of muzzle epidermis keratins and the in vitro translation products of muzzle epidermal mRNA: (b) Coomassie Blue staining; (c) fluorograph of the same gel, showing the $[^{35}S]$methionine-labeled proteins synthesized in vitro. (d) Coomassie Blue staining of cytokeratins extracted from bovine heel pad epidermis. (e) Same preparation as in d but using IEF in the first dimension to resolve the isoelectric variants of the acidic cytokeratin polypeptides, notably No. 9. (f) Fluorograph of the same gel as shown in d, presenting the co-electrophoresed $[^{35}S]$methionine-labeled in vitro translation products of mRNA extracted from heel pad epidermis. Note that component Nos. 4, 5, and 9 are among the major translation products.
tissue in vivo, demonstrating the type I cytokeratin character of polypeptide No. 9.

**Identification of Human Cytokeratin No. 9 as a Translation Product**

We also compared the cytokeratins of human epidermis from various body sites (e.g., breast, face), including tumors and fetal epidermis (20, 45–50) with those present in epidermis of foot soles and palms. The typical complement of cytokeratins characteristic of normal human interfollicular epidermis has been amply documented in the literature, including analyses of products of in vitro translation of epidermal RNA (e.g., 3, 7–9, 11, 15, 20, 27–30, 32, 45, 46, 49, 65–67). The data obtained in the course of the present study confirm the literature data, notably those of Fuchs and Green (27, 28; see also 32). Therefore, in this paper we present only the data referring to human foot-sole epidermis. Cytoskeletal preparations from epidermal tissue obtained from amputated feet or legs contain, with somewhat variable frequencies and degrees of preservation, a relatively large amount of a rather acidic (isoelectric in 9.5 M urea at pH 5.4) polyepitope of approximately $M_r$, 64,000 designated cytokeratin No. 9 (Fig. 5, a and b; cf. 45–47). Criteria for its keratinous nature have been given in previous papers, including the ability of the polyepitope purified by anion-exchange chromatography and subsequent reverse-phase high performance liquid chromatography to form complexes and IFs with various purified epidermal and non-epidermal type II cytokeratin polypeptides (34, 45, 46).

Fig. 5, c and d, shows the $[^{35}S]$methionine-labeled products of in vitro translation of mRNA isolated from human foot sole epidermis. Cytokeratin polypeptides Nos. 1, 5, 6, 9, 10/11, 14, and 16 are clearly identified among the major mRNA products of this tissue, which include considerable amounts of actin. This demonstrates that cytokeratin No. 9 is a genuine epidermal polyepitope which, however, is detectable with this method only in specific body sites such as plantar epidermis. Moreover, the lack of translation products of a similar size and electrical charge as cytokeratin No. 9 in analyses of epidermal RNAs from various other parts of the body (data not shown; see also refs. 27, 28, 32) indicates that translatable mRNA coding for this protein is not synthesized in appreciable amounts in epidermis of these regions.

**Similarities and Differences of Human and Bovine Cytokeratin No. 9**

Although the cytokeratin polypeptides designated No. 9 in both the bovine (57) and human (45) catalog exhibit several similarities such as in size, isoelectric pH, and the selectively abundant expression in keratinocytes of plantar epidermis, the polyepitope differs in the two species in the specific two-dimensional peptide maps (Fig. 6, a and b). Clearly, the peptide maps of both polypeptides exclude a basic (type II) cytokeratin character (cf. 45, 57). On the other hand, this species difference in peptide maps, which has also been noted for other acidic (type I) cytokeratins (46, 57) suggests considerable number of exchanges in the amino acid sequences of the two polypeptides, a prediction that is currently under investigation in our laboratory using cDNA clones.

When guinea pig antibodies raised against purified cyto-
keratin No. 9 from bovine hoof pad is examined by immuno-
blotting analysis, specific reactivity with only cytokeratin No. 9 is noted in both species, bovine and human (Fig. 7, d–c'). As human palmar cytokeratin No. 9 is a widespread laboratory contaminant, which can present special problems in immunoblot studies when very sensitive antibodies reacting with this and/or other cytokeratins are used (for details see references 1, 52), we have also confirmed the nature of the specific Coomassie Blue-stained antigens as bovine and human cytokeratin No. 9 by their species-specific peptide map pattern (data not shown). The cross-reactivity of

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Arabic and Roman numerals denote the different bovine epidermal keratin polypeptides (cf. reference 57). Bovine serum albumin (B), phosphoglycerokinase (P), and $\alpha$-actin ($\alpha$) were added for co-electrophoresis as reference polypeptides. A ($\beta$, $\gamma$) denotes the position of the labeled bovine $\beta$ and $\gamma$ actin, synthesized in vitro from the mRNAs used in these experiments. The arrows in $d$ and $f$ point to a bovine polyepitope of $M_r$, 48,000 which is a minor component, probably a cytokeratin, of the heel pad epidermis cytoskeleton (previously described as component VIII in references 17 and 26). The triangle in $e$ denotes a trace component in heel pad epidermis which may represent a minor specific cytokeratin (No. 10 of the bovine catalog of reference 57). The arrowheads show residual complexes of certain basic and acidic cytokeratins which resist the highly denaturing conditions (9.5 M urea) used to dissociate and denature the proteins before electrophoresis (cf. reference 24). Note that cytokeratin No. 9 is detected only in the heel pad epidermis and is a genuine in vitro translation product.
Electron micrograph showing a negatively stained preparation of IFs re-assembled from purified bovine cytokeratins No. 8 from bladder and No. 9 from heel pad epidermis. Purified polypeptides were mixed in 9.5 M urea and dialyzed to 50 mM Tris-HCl buffer (for details see text). Typical compact IFs as well as loosely packed protofilamentous structures are seen. Bar, 0.2 µm.

Immunolocalization of Cytokeratin No. 9

When antibodies specifically recognizing cytokeratin No. 9 were used for immunofluorescence microscopy on bovine hoof pad epidermis, intense staining was seen in columns of suprabasal keratinocytes (Fig. 7, d and e). The first signs of cytokeratin No. 9 positivity were usually apparent in the fourth or fifth cell layer. In upper layers of this epidermis, most keratinocytes were strongly stained although still individual cells were noted that were negative for cytokeratin No. 9 but positive for other cytokeratins (Fig. 7f). Columns of cytokeratin No. 9-positive cells were also prominent in human foot-soles reacted with the antibodies against bovine cytokeratin No. 9 (data not shown).

Unexpectedly, we found that keratinocytes expressing cytokeratin No. 9 were not restricted to plantar and palmar epidermis. Immunofluorescence microscopy of cryostat sections of skin from various body sites showed sparse cytokeratin No. 9-positive cells also in other locations where they occurred as individual cells or piles of cells. As an example, double label immunofluorescence microscopy of bovine snout epidermis is presented in Fig. 8, a and b. While "broad spectrum" cytokeratin antibodies such as K8.13 stain all keratinocytes of all layers (Fig. 8 a) the few cytokeratin No. 9-positive cells are restricted to certain regions where they often form clustered or columnar arrays (Fig. 8 b). In addition, cytokeratin No. 9-positive cells were seen around certain glandular ducts present in this tissue. The presence of cytokeratin No. 9 in some cells of the uppermost layers was confirmed by immunoblotting of cytoskeletal proteins of such tissue regions microdissected from cryostat sections (not shown; for technique see reference 50).

Sparsely distributed piles of cytokeratin No. 9-positive cells were also found in some non-palmar, non-plantar hu-
man body sites such as the neck epidermis (not shown). A detailed dermatological study on the histological and anatomical distribution of these cytokeratin No. 9-expressing keratinocytes will be presented elsewhere (Moll, I., H. Heid, T. Achtstätter, N. Zaidi, W. W. Franke, and R. Moll, manuscript in preparation).

Discussion

The results of this study confirm the existence of an acidic polypeptide of Mr 64,000 with cytokeratinous features which occurs as an abundant protein in the epidermis of human foot-soles and palms as well as in the bovine equivalent (i.e., the heel pad of the hoof), but so far had not been found in epidermal tissue of other body sites (cf. 7, 14, 17, 28, 32, 41, 45, 46, 49, 63). Our demonstration that in both species this polypeptide is a major in vitro translation product provides definitive evidence that it is an intact, genuine cytokeratin encoded by a specific mRNA. The development of antibodies specific for cytokeratin No. 9 has now also allowed the identification of sparse cells expressing this protein in the epidermis of other (i.e., non-palmar, non-plantar) body sites which have escaped detection by the relatively crude integral biochemical methods used previously.

We conclude, in agreement with data of other authors (7, 28, 32), that cytokeratin No. 9 is expressed during terminal differentiation of a certain subtype of keratinocytes which is very rare at most body sites but is abundantly present in plantar and palmar epidermis. From our findings that cytokeratin No. 9 is present in calf fetuses and in relatively early stages of human fetal development (for a demonstration in 20-wk fetuses see reference 50), we also conclude that cytokeratin No. 9 is not a "stress protein" induced by external influence in palms and soles and suggest that its enhanced expression in these tissues is related to the morphogenesis of these parts of the body.

als denote the human cytokeratin polypeptides according to Moll et al. (45). Other symbols are as in Fig. 1. (a) Coomassie Blue staining of a gel showing the major cytokeratins of microdissected foot-sole epidermis; arrowheads denote residual amounts of the very stable complex of cytokeratin Nos. 5 and 14 (cf. reference 34). (b) Coomassie Blue staining of the acidic cytokeratins separated by isoelectric focusing, resolving the individual isoelectric variants. (c) Fluorograph showing the major in vitro translation products of total RNA from foot sole epidermis as revealed after short exposure (1.5 d). (d) Longer (8 d) exposure fluorograph of the gel shown in c, revealing significant amounts of polypeptide Nos. 1, 9, and 10/11.
Figure 7. Characterization of affinity-purified guinea-pig antibodies against bovine cytokeratin No. 9 by immunoblotting and immunofluorescence microscopy. (a–c’) Gel electrophoresis and immunoblotting of cytoskeletal polypeptides from various tissues and culture cells. (a) SDS PAGE showing major cytokeratins Coomassie Blue staining. (Lane 1) Proteins from middle and lower layers of bovine snout epidermis; (lane 2) proteins from human foot-sole; (lane 3) bovine heel pad epidermis; (lane 4) proteins from cultured human mammary adenocarcinoma cells of line MCF-7 (for details see reference 45). (a’) Autoradiography of nitrocellulose paper blot of an SDS PAGE in parallel to that shown in a after reaction with antibodies against cytokeratin No. 9, followed by incubation with 125I-labeled protein A. Significant immunoreaction is seen only with a polypeptide of M, ~64,000, i.e. cytokeratin No. 9, in human foot-sole (lane 2’) as well as bovine heel pad (lane 3’). In contrast, cytokeratins of middle and lower layers of bovine snout epidermis (lane 1’) and MCF-7 cells (lane 4’) are negative. (b and b’) Two-dimensional gel electrophoresis (symbols as in Fig. 1; in this example an extended pH range of ampholytes was used; cf. the Journal of Cell Biology, Volume 103, 1986).
Figure 8. Double-label immunofluorescence microscopy of a frozen section through bovine snout epidermis with monoclonal murine antibody K8.13 (a) and affinity-purified pig guinea antibodies against cytokeratin No. 9 (b). While the broad range monoclonal cytokeratin antibody reacts with keratinocytes of all layers of snout epidermis, only individual cells or small clusters of cells located in upper layers of epidermis reveal positive staining for cytokeratin No. 9 (some keratinocytes that are positive with both antibodies are indicated by arrows). CT, connective tissue. Bar, 100 μm.

Our results allow us to define at least two different types of keratinocytes distinguishable by their patterns of terminal differentiation, one type that synthesizes cytokeratin No. 9 and one type that does not. At present it is not clear whether the cytokeratin No. 9–positive keratinocytes also express the other large type I cytokeratin(s) characteristic of suprabasal differentiation (i.e., cytokeratin Nos. 10 and 11 [cf. 2, 9, 28, 32, 45, 66, 67]), or whether the expression of cytokeratin No. 9, on the one hand, and Nos. 10/11, on the other, is mutually exclusive. The reason for the selectively high frequency of the cytokeratin No. 9–expressing type of keratinocytes in certain regions of epidermis but not in others is not known. It will be exciting to identify the factors that induce the expression of this protein so specifically in one subtype of keratinocytes. We also cannot say at the moment whether the specific synthesis of cytokeratin No. 9 is also related to special functions of this protein in the keratinocytes of the callus-forming epidermis of the palms and the sole and/or contributes to the formation of special morphological features of this epidermis such as ridges and sulci (for histology see 41, 50, 51, 69). In this context, a close spatial relationship to the terminal (“straight”) portions of sweat gland ducts of human foot-soles is striking.

At present we also do not know whether the expression of the anatomical site-specific cytokeratin No. 9 is maintained in keratinocytes growing in culture and whether its synthesis is influenced by vitamin A and its analogs as it has been described for other epidermal cytokeratins (29, 32). It will also be important to find out whether the expression of this cytokeratin is switched off after grafting of palmar or plantar keratinocytes to other regions of the body and whether it represents an intrinsic or an extrinsic program of regulation (for discussion see reference 13).

The observations of this study as well as those of previous reference 57) of cytoskeletal polypeptides from bovine heel pad. (b) Coomassie blue–stained gel; (b') corresponding immunoblot reaction on nitrocellulose paper. Positive immunoreaction is detected only with cytokeratin No. 9 (c and c') Two-dimensional gel electrophoresis of cytoskeletal polypeptides of human foot-sole (conditions as in b). (c) Coomassie Blue staining (arrows denote yet unidentified stratum corneum polypeptides; cf. reference 49); (c') corresponding immunoblot reaction with antibodies to cytokeratin No. 9, showing the specificity for this cytokeratin. (d–f) Immunofluorescence microscopy of cryostat sections of bovine heel pad epidermis, using broad range monoclonal murine antibody K8.13 (d) and affinity-purified pig guinea antibodies against cytokeratin No. 9 (e and f). All epidermal cells are positive with antibody K8.13 (d), whereas the antibodies to cytokeratin No. 9 do not show significant reaction with the basal cell layer and the first suprabasal cell layers (demarcated by brackets in e) and react only with certain columns and clusters of cells of suprabasal layers. The frequency of cytokeratin No. 9–positive cells increases in upper layers (f shows a region near the apical surface). Note, however, that even in these upper layers not all cells are positively stained. CT, connective tissue. Bars, 100 μm.
papers (14, 17, 41, 63) further indicate that there are some other site-specifically expressed epidermal cytokeratin polypeptides, at least in the bovine heel pad, such as the Mr 48,000 component designated VIII (No. 19 of the bovine catalog of reference 57). The phenomenon of anatomical site-determined expression of certain cytokeratins may not be restricted to higher mammals and feet and hands. Candidates for such a body site-specific synthesis have been described, for example, for the epidermis of mouse tails and ears (59).

Our results, taken together with those of a preceding study (34), indicate that the human acidic (type I) cytokeratin No. 9 of Mr 64,000 forms complexes with the basic (type II) cytokeratin No. 1 (Mr 68,000) and No. 2 (Mr 65,500) and probably also No. 5 (Mr 58,000), and that bovine cytokeratin No. 9 forms complexes with bovine cytokeratins No. 4 (Mr 59,000), No. 5 (Mr 58,000) and No. 6 (i.e., epidermal component III; Mr 56,000). This demonstrates that the size difference of Mr <8,000 observed between the specific basic and acidic cytokeratin polypeptide partners of most "pairs" (9, 64, 67) is not a necessary requirement of cytokeratin complex and IF formation.

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