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Distribution of Caspase-14 in Epidermis and Hair Follicles Is Evolutionarily Conserved Among Mammals

LORENZO ALIBARDI,[1*] ERWIN TSCHACHLER,[2] AND LEOPOLD ECKHART[2]
[1]Department of Biology, University of Bologna, Bologna, Italy
[2]Department of Dermatology, Medical University of Vienna, Vienna, Austria

ABSTRACT

Caspase-14, a member of the caspase family of cysteine proteases, is almost exclusively expressed in the epidermis. Studies on human and mouse cells and tissues have implicated caspase-14 in terminal differentiation of epidermal keratinocytes and in the formation of the stratum corneum. Here we investigated evolutionary aspects of the role of caspase-14 by analyzing its distribution in the epidermis and hair follicles of representative species of placental mammals, marsupials, and monotremes. Immunocytochemical staining showed that caspase-14 is consistently expressed in the granular and corneous layer of the epidermis of all mammalian species investigated. Ultrastructural analysis using gold-labeled anti-caspase-14 antibodies revealed that caspase-14 is associated preferentially with keratin bundles and amorphous material of keratohyalin granules, but is also present in nuclei of transitional cells of the granular layer and in corneocytes. In hair follicles, caspase-14 was diffusely present in cornifying cells of the outer root sheath, in the companion layer, and, most abundantly, in the inner root sheath of all mammalian species here analyzed. In Henle and Huxley layers of the inner root sheath, labeling was seen in nuclei and, more diffusely, among trichohyalin granules of cornifying cells. In summary, the tissue expression pattern and the intracellular localization of caspase-14 are highly conserved among diverse mammalian species, suggesting that this enzyme is involved in a molecular process that appeared early in the evolution of mammalian skin. The association of caspase-14 with keratohyalin and trichohyalin granules may indicate a specific role of caspase-14 in the maturation of these keratinocyte-specific structures.

Key words: epidermis; hair; caspase-14; ultrastructural immunocytochemistry; mammals

The epidermis of mammals consists of layers of progressively differentiating keratinocytes that ultimately convert into enucleate cornified envelopes (Fuchs, 1990; Ishida-Yamamoto et al., 2000; Menon and Norlen, 2002). The latter build up the outermost barrier of the body, i.e., the stratum corneum. The production of differentiation-associated proteins such as keratins and transglutaminases as well as their intracellular localization is tightly regulated, thereby ensuring proper function of this tissue (Resing and Dale, 1991; Rawlings et al., 1994; Kalinin et al., 2002). Many features of the epidermis are conserved in evolutionary distant mammalian species, suggesting that the basic structure of the epidermis and its molecular regulation were established in early forms of mammals (Sokolov, 1982; Alibardi and Maderson, 2003).

Hairs are epidermal appendages characteristic of mammalian skin that develop by a specific process of epidermal invagination (Hardy, 1992; Millar, 2002; Botcharev and Paus, 2003). Hairs are made of concentric layers of cor-

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*Correspondence to: Lorenzo Alibardi, Dipartimento di Biologia, University of Bologna, Via Selmi 3, 40126, Bologna, Italy. Fax: 39-051-2094286. E-mail: alibardi@biblio.cib.unibo.it

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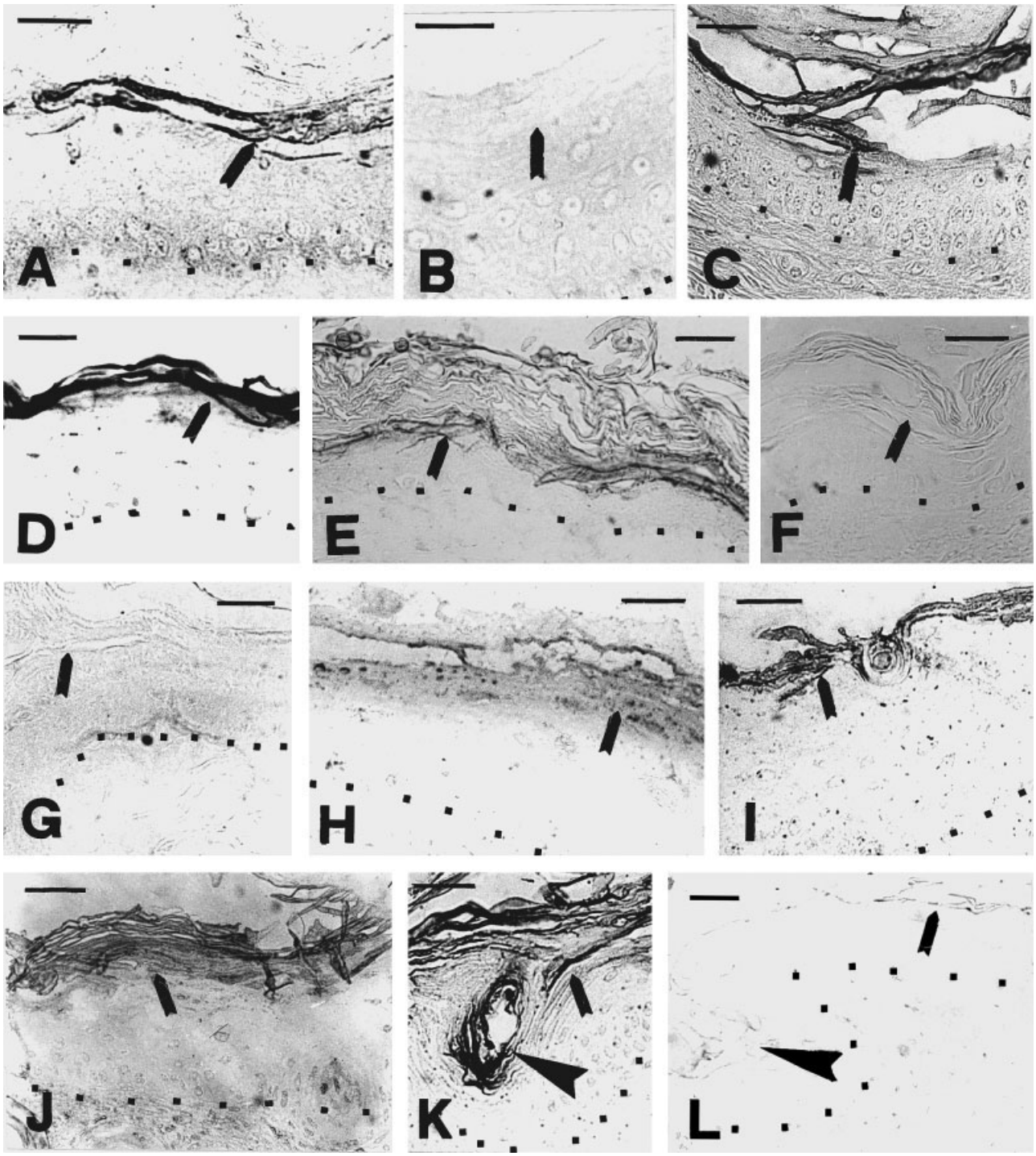

Fig. 1. Immunocytochemical light microscopy of caspase-14 distribution in the epidermis. Dots indicate the basal membrane. Scale bars = 20 μm. **A:** Immunoreactive transitional and lowermost stratum corneum (arrow) of echidna epidermis. Antimurine caspase-14 antibody (ab). **B:** Negative control of echidna epidermis (arrow on the corneous layer). **C:** Reactive lowermost part of the stratum corneum (arrow) of platypus epidermis. Antimurine caspase-14 ab. **D:** Reactive stratum granulosum and corneum (arrow) of possum epidermis. Antimurine caspase-14 ab. **E:** Reactive transitional and corneous layer (arrow) of kangaroo epidermis. Antihuman caspase-14 ab. **F:** Negative control of kangaroo epidermis (arrow on corneous layer). **G:** Preabsorbing control of kangaroo epidermis (arrow indicates the corneous layer). **H:** Weak reactivity of stratum granulosum (arrow) and corneum of cat epidermis. Murine caspase-14 ab. **I:** Reactive corneous layer (arrow) of hamster epidermis. Antimurine caspase-14 ab. **J:** Reactive corneous layer (arrow) of rat epidermis. Antihuman caspase-14 p17 ab. **K:** Detail of intense reactivity in the corneous layer (arrow), including that of the apical part of the hair canal (arrowhead), of rat epidermis. Antimurine caspase-14 ab. **L:** Preabsorbing control (with caspase-14 p17) of rat epidermis (arrow on corneous layer, arrowhead on upper part of the hair canal).

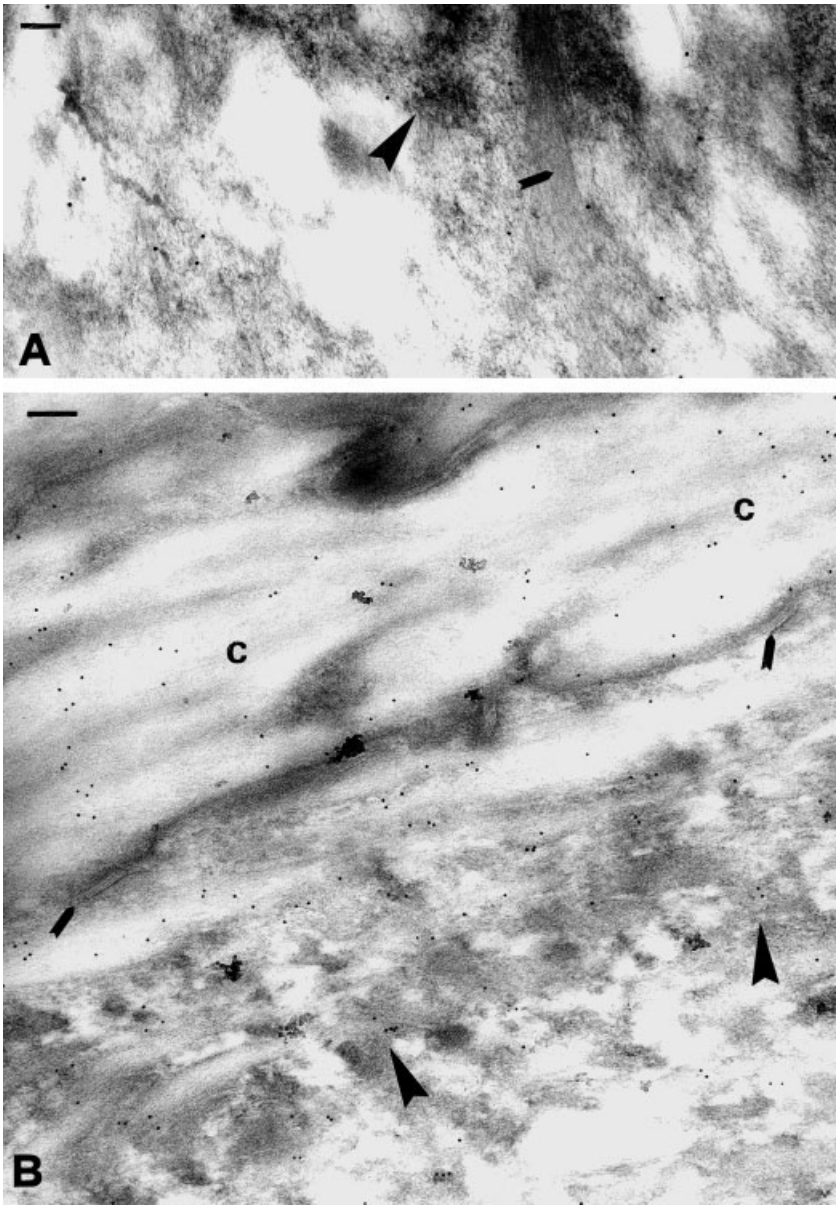

Fig. 2. Ultrastructural distribution of caspase-14 in the epidermis of echidna as detected with immunogold-labeled antimurine caspase-14 antibody. **A:** Granular layer showing diffuse labeling. The arrowhead points to denser keratohyalin. The arrow points to keratin bundle. Scale bar = 100 nm. **B:** Labeling over keratohyalin of transitional layer (arrowheads) and in the corneous layer (c). Arrows over desmosomes. Scale bar = 200 nm.

neous materials with different composition and properties: the central hair fiber is made from inside out by a medulla (often absent), cortex, and cuticle, and the fiber is ensheathed within the follicle by an inner and outer root sheath (Orwin, 1981; Rogers, 2004). Like interfollicular epidermis, hair follicles have a similar organization in all mammals (Sokolov, 1980; Alibardi, 2004a, 2004b, 2004c).

A series of enzymes are activated specifically during cornification. Transglutaminases and sulfhydryl oxidases play an essential role in forming the cornified cytoplasm and cell envelope (Polakowska and Goldsmith, 1991; Hashimoto et al., 2000). Proteases such as matriptase and stratum corneum chymotryptic enzyme are required for maturation of filaggrin and for shedding of superficial corneocytes, respectively (List et al., 2003; Caubet et al., 2004). Another protease implicated in the homeostasis of the stratum corneum is caspase-14, a member of the caspase family of cysteine proteases (Eckhart et al., 2000a, 2000b; Lippens et al., 2000). Caspase-14 is converted from a catalytically inactive proenzyme into the active enzyme, which is present in high amount in human stratum corneum (Fischer et al., 2004). Endopeptidase activity of caspase-14 has been demonstrated in vitro, but the endogenous substrate of this enzyme is elusive (Mikolajczyk et al., 2004). Whereas most caspases such as caspase-3 are detectable in the skin and in a wide variety of other tissues, caspase-14 has a highly tissue-restricted expression pattern that suggests a specific role in the epidermis (Eckart et al., 2000a).

The caspase-14 gene is expressed in the upper spinous and, most strongly, in the granular layer of human epidermis (Eckhart et al., 2000b). The protein is detectable in these layers as well as in the stratum corneum (Eckhart et al., 2000a; Lippens et al., 2000, 2003; Rendl et al., 2002; Alibardi et al., 2004). At the ultrastructural level, human caspase-14 localizes to the amorphous material of kerato-

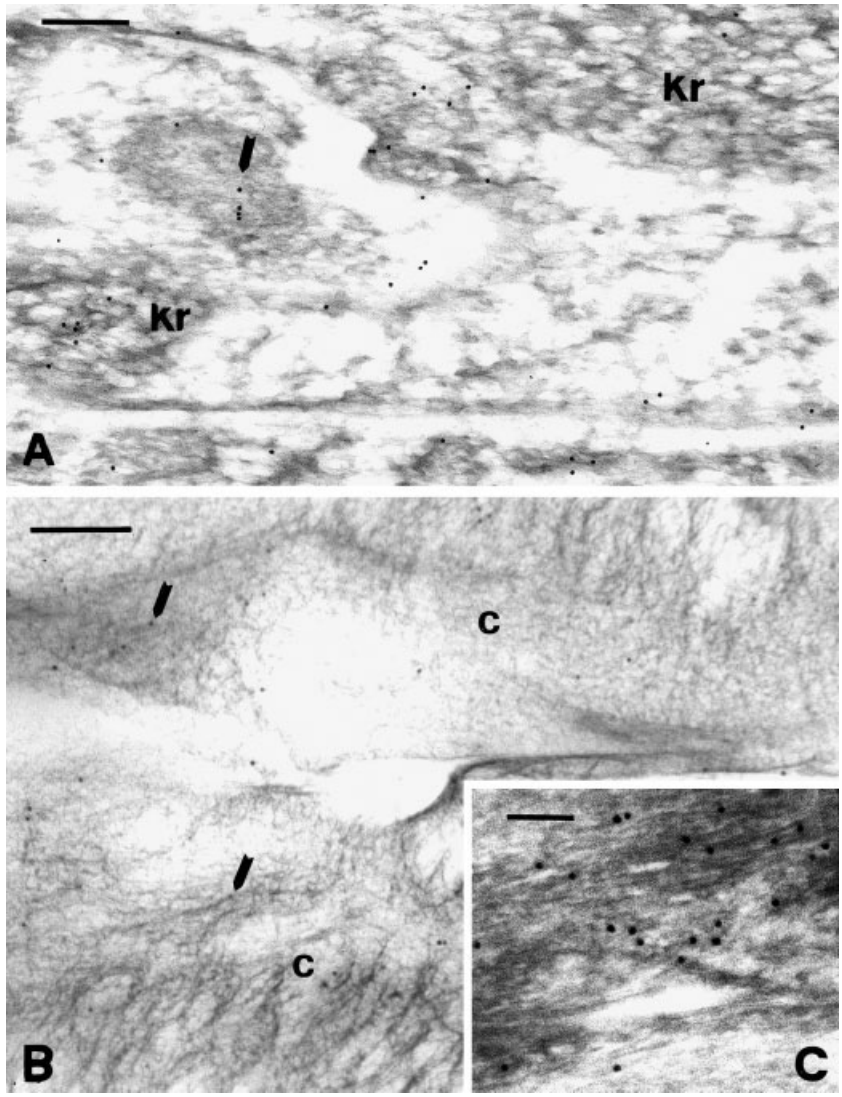

Fig. 3. Ultrastructural distribution of caspase-14 in the epidermis of echidna as detected with immunogold-labeled antihuman caspase-14 p17 antibody. **A:** Labeling over alveolate (Kr) and amorphous (arrow) keratohyalin of the granular layer. Scale bar = 250 nm. **B:** Diffuse labeling (arrows) of corneocytes (c). Scale bar = 250 nm. **C:** Detailed view of labeling among keratin filaments of corneocyte. Scale bar = 100 nm.

hyalin granules and to the nucleus of granular layer cells (Alibardi et al., 2004). In stratum corneum, it is associated with corneodesmosomes, the cornified envelope and keratin filaments embedded in the corneous material of mature corneocytes. The above studies have suggested that the activity of caspase-14 may be involved in the establishment or maturation of these subcellular structures.

In situ hybridization and immunostaining of human skin have revealed that caspase-14 is also expressed in hair follicles (Eckart et al., 2000b; Lippens et al., 2003). In the latter, caspase-14 is present in the inner root sheath (IRS) as well as the innermost part, but not in the basal layer of the outer root sheath (Eckhart et al., 2000b; Lippens et al., 2003). In addition, keratinocytes differentiating into cells of the sebaceous glands express caspase-14, whereas keratinocytes of the sweat glands are devoid of caspase-14 (Lippens et al., 2000).

The present study investigates the fine distribution of caspase-14 in the skin of selected representative species of monotreme, marsupials, and placental mammals in order to evaluate whether caspase-14 is ubiquitously associated with terminal differentiation of mammalian epidermis and hairs. This study also provides for the first time data on the ultrastructural localization of caspase-14 in hair follicles and reveals an association of caspse-14 with keratinocyte-specific cellular organelles.

MATERIALS AND METHODS

Skin samples (2–4 × 2–4 mm) were collected from the following species: platypus (*Ornithorhynchus anatinus*, foot skin, n = 3), echidna (*Tachyglossus aculeatus*, belly skin, n = 2), possum (*Trichosurus vulpecula*, ear skin, n = 2), red kangaroo (*Macropus rufus*, trunk skin, n = 1), mole (*Talpa europaea*, belly and tail skin, n = 1), cat (*Felis catus*, belly skin, n = 2), hamster (*Mesocricetus auratus*, tail and belly skin, n = 3), rat (*Rattus norvegicus*, belly and tail skin, n = 3), and mouse (*Mus musculus*, tail skin, n = 3). The monotreme and marsupial specimens were collected as previously reported (Alibardi, 2004a, 2004b).

Tissues were immediately fixed at 0–4°C for 4–6 hr with a modified Carnoy's fluid (nine parts of 90% ethanol and one part of acetic acid), and other tissues were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4, dehydrated at 0–4°C, and embedded in bioacryl resin

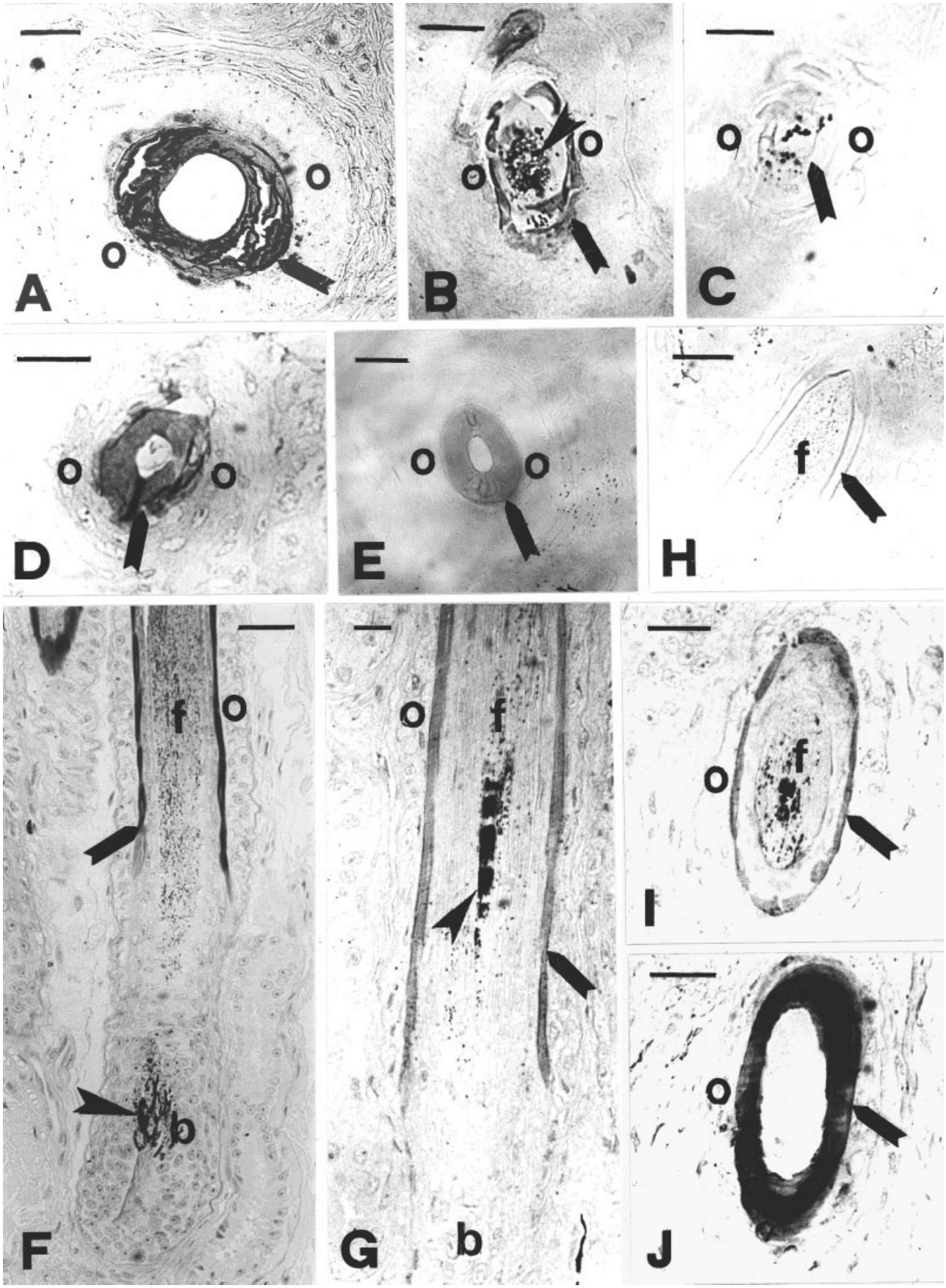

Figure 4.

under UV polymerization at 0–4°C (Scala et al., 1992). Using an ultramicrotome, tissues were sectioned at 1–2 μm thickness for light microscopic observations. Parallel sections were collected over chromoallume-coated slides for the following immunocytochemical reaction. From tissues fixed in 4% paraformaldehyde, thin sections at 40–80 nm thickness were collected on nickel grids for ultrastructural immunogold cytochemistry.

Three types of antibodies against caspase-14 were used: rabbit antimurine caspase-14 antiserum raised against recombinant mouse caspase-14 was kindly provided by Dr. Wim Declercq (Flanders Interuniversity Institute for Biotechnology and Ghent University, Ghent, Belgium) (Lippens et al., 2000). Antihuman caspase-14 and antihuman caspase-14 p17 were raised against the recombinant proenzyme and the p17 subunit of human caspase-14, respectively (Eckhart et al., 2000a, 2000b). The specificity of the antibodies and the cross-reactivity with human caspase-14 of the antimurine caspase-14 antibody have been demonstrated previously (Alibardi et al., 2004). Recombinant human caspase-14 and caspase-14 p17 proteins were used for preabsorption controls.

Antibodies were diluted 1:500 in 0.05 M Tris-HCl at pH 7.6 with 2% BSA for light immunocytochemistry, or 1% cold water fish gelatin for ultrastructural immunocytochemistry. In controls, the primary antibody was omitted from the incubating solution and, in some cases, the primary antibody was preabsorbed with recombinant antigen as described previously (Alibardi et al., 2004). Light immunocytochemistry for caspase-14 was performed using a secondary HRP-conjugated antirabbit IgG antibody (Sigma, St. Louis, MO) at dilution of 1:50, which was revealed by a DAB reaction. For TEM immunocytochemistry, the secondary (antirabbit IgG) antibody was a 10 nm gold conjugate (dilution 1:40; Sigma). Thin sections were lightly stained in 4% uranyl acetate. The ultrastructural immunocytochemical observation was done using a Philips CM-100 electron microscope operating at 80 kV.

RESULTS

Epidermis: Light Microscopy Immunocytochemistry

All the employed antibodies produced a similar labeling pattern on sections of epidermis of all three groups of mammals. The detection by peroxidase in plastic sections produced a weak to moderate brown reaction, which was completely abolished in negative or preabsorbed controls.

In general, the anticaspase-14 antibodies stained the granular, transitional, and corneous layers of mammalian epidermis, while the spinosus and basal layers were immuno-negative (Fig. 1). In the hairy epidermis of echidna and platypus, the immunoreactivity was uneven over the transitional layer and the lowermost stratum coneum (Fig. 1A–C). In the glabrous web epidermis of platypus, where the granular layer is absent, no reactivity was observed. Controls (omission of the primary antibody and antigen preabsorption) were negative (Fig. 1B).

The epidermis of the opossum showed regions with a reactive stratum granulosum and corneum (Fig. 1D), whereas other regions were stained very weakly. The kangaroo epidermis showed a constant immunoreactivity in the precorneous and corneous layer, which was absent in controls (Fig. 1E–G). Variable immunoreactivity was seen in the stratum granulosum and corneum of the cat epidermis (Fig. 1H), and more constant in that of hamster (Fig. 1I). In mouse, human, and rat (the positive controls), immunolabeling revealed a gradient of increasing intensity from the upper spinous layer to the stratum corneum (Fig. 1J and K). No immunoreactivity was seen in controls (Fig. 1L).

Under the electron microscope, no labeling was seen in the basal and spinous layers, but gold particles became more common in the granular and especially in the corneous layers. In the epidermis of echidna, the labeling was diffuse among keratin bundles mixed with keratohyalin in granular and transitional cells (Fig. 2). Also, the heterochromatin and condensing nuclei of some transitional cells appeared labeled (data not shown). The labeling was more intense among aggregated keratin filaments of mature cells of the corneous layer.

In the epidermis of the rat, the caspase-14 labeling was also present in keratin bundles mixed with keratohyalin in the granular cells (Fig. 3A). Keratohyalin was made by a meshwork of 10–12 nm filaments forming F-bodies merged with keratin-like material. Finally, in cells of the stratum corneum, the labeling was diffuse among the dense keratin filaments (Fig. 3B and C). In the control epidermis of the various species used for the ultrastructural analysis (echidna, kangaroo, and rat), no labeling was present, both in negative (omitting the primary antibody) or preabsorbing with the caspase-14 p17 antigen (data not shown).

Hair: Light and Electron Microscopy Immunocytochemistry

In general, using the antihuman and murine caspase-14 antibodies, the only labeled structures of hair of all mammalian species were the cornifying IRS and the cornified outer root sheath at the top of the hair canal, which surrounded the hair fiber (Figs. 1K, 4, and 5). The early differentiating IRS was unlabeled, as were the remaining hair layers.

Specifically, in large hair of echidna ventral skin, the reaction was seen in the thick cornified IRS and surrounding layer (indicated as companion layer; Fig. 4A). In kangaroo hair, the reaction was seen mainly in the cornified Henle layer and upper cornified part of the external hair canal (Fig. 4B and C). The same pattern was also observed in large hairs of opossum skin (the reaction was uncertain in smaller hairs; Fig. 4D), in hair of the mole (Fig. 4E), cat (Fig. 4F–J), rat (Fig. 5A–C), and mouse (Fig. 5D–F).

The first layer of the IRS to cornify, the external Henle layers, was also the first to react with the caspase-14

Fig. 4. Light immunocytochemistry of caspase-14 distribution in hairs. o, outer root sheath; f, hair fiber; b, hair bulb. Scale bars = 20 μm in all panels except G, which = 10 μm. **A:** Cross-section of echidna hair with immunoreactive inner root sheath (arrow). **B:** Kangaroo hair in cross-section with positive inner root sheath (arrow) and in some part of the cortex (arrowhead). **C:** Preabsorption control of the parallel section of previous figure with negative inner root sheath. **D:** Cross-sectioned opossum hair with labeled inner root sheath (arrow). **E:** Cross-sectioned mole hair with positive inner root sheath (arrow). **F:** Longitudinal section of cat hair with positive cornifying Henle layer (arrow). The arrowhead indicated melanocytes. **G:** Higher magnification of cat hair with labeled cornifying Henle layer (arrow). The arrowhead indicates dark (pigments or immunopositive) medulla cells. **H:** Immunonegative cat hair (arrows points to the IRS) in preabsorbing control. **I:** Detail of obliquely sectioned cat hair with labeled Henle layer (arrow). **J:** At higher level, all the IRS of this cat hair is immunoreactive (arrow).

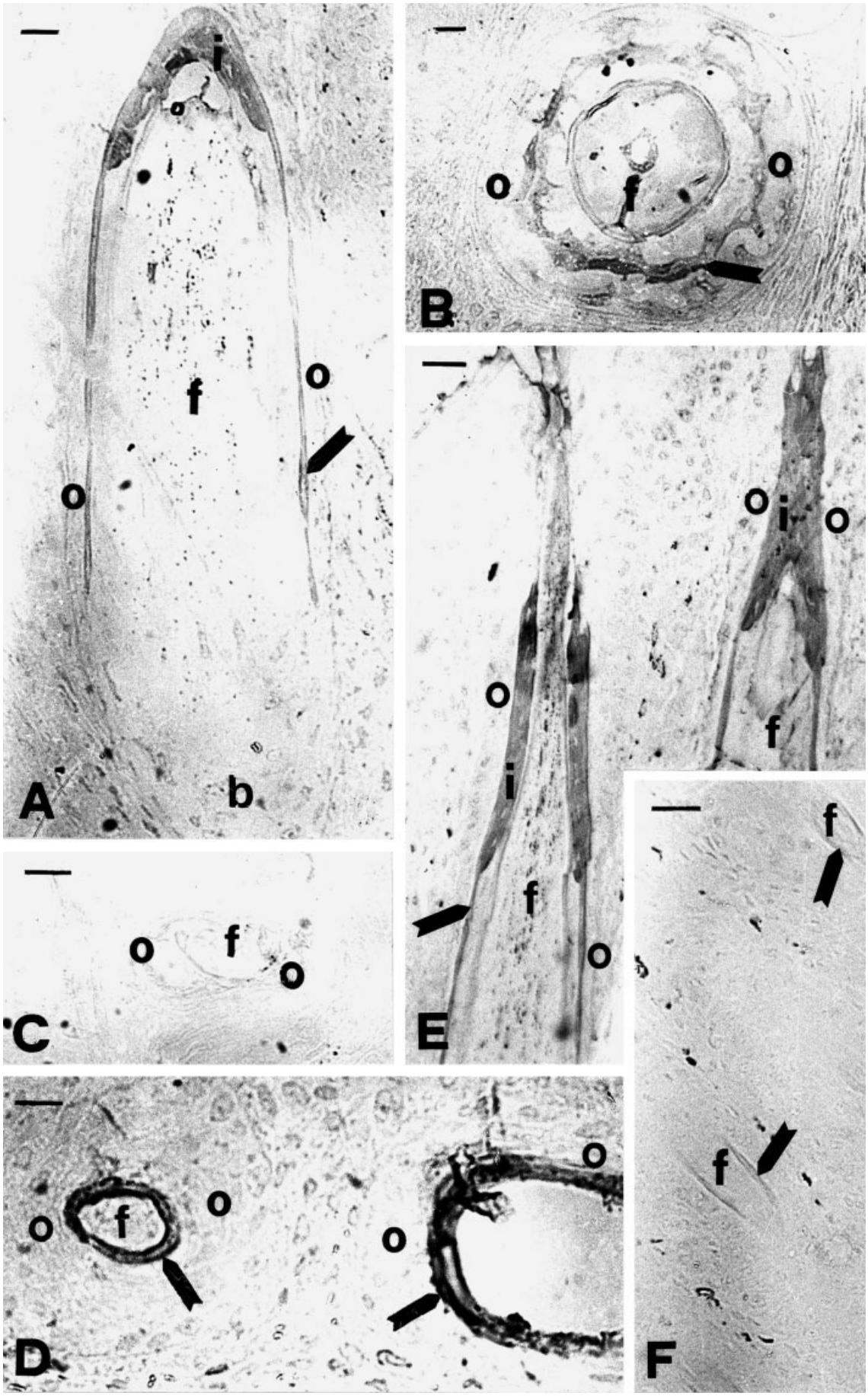

Figure 5.

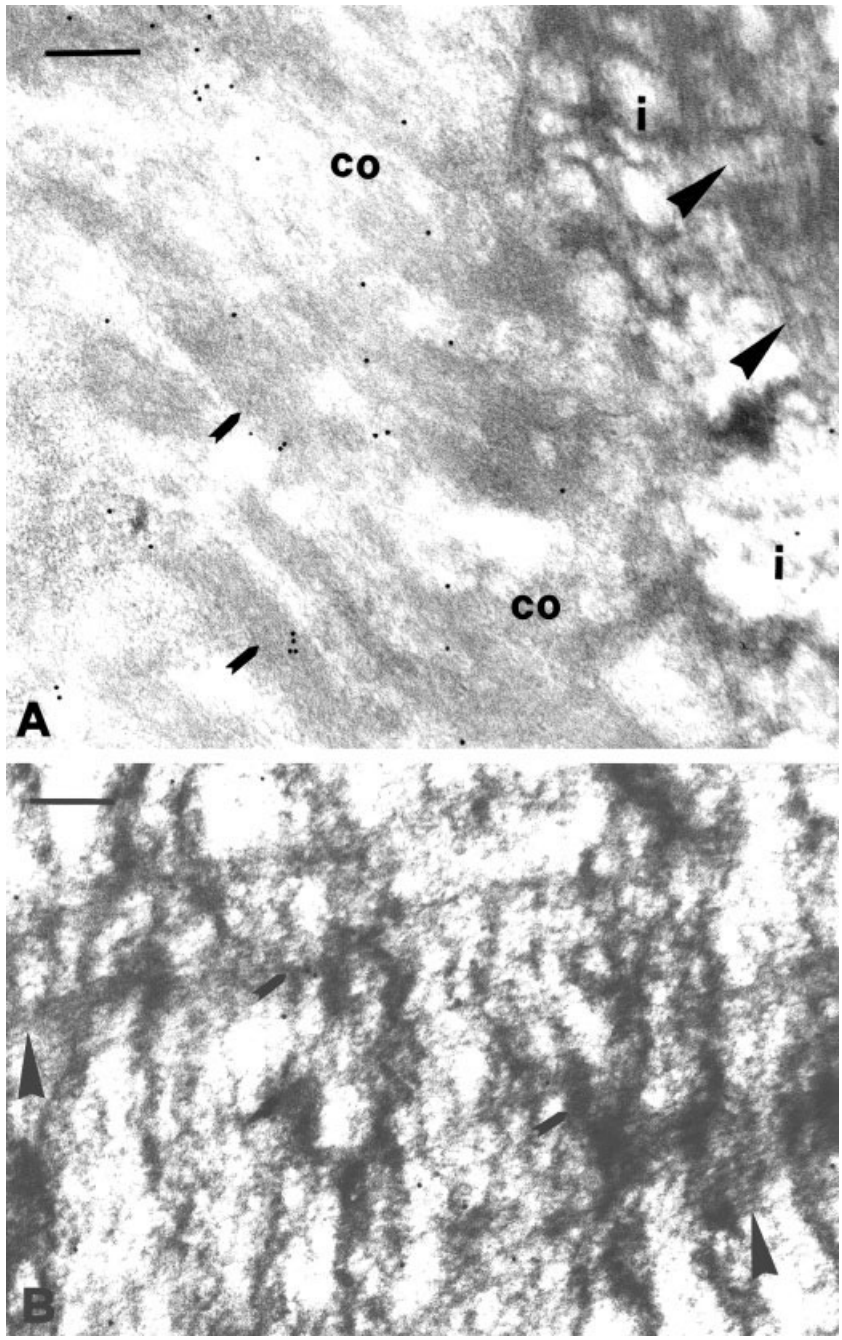

Fig. 6. Immunogold localization of caspase-14 in echidna hair. **A:** Detail of companion layer (co) and inner root sheath (i, arrowheads on fibrous matrix). The labeling is diffuse among keratin filaments (arrows). Scale bar = 200 nm. **B:** Detail of diffuse labeling among amorphous keratin-trichohyalin material (arrows). The arrowhead indicates parallel fibers of cornifying inner root sheath. Scale bar = 250 nm.

Fig. 5. Light immunocytochemical distribution of caspase-14 in hairs. o, outer root sheath; i, inner root sheath; f, hair fiber; b, hair bulb. Scale bars = 10 μm in A, B, D, and E; 20 μm in C and F. **A:** Oblique section of rat hair with positive cornifying Henle layer (arrow) and complete inner root sheath at higher levels. **B:** Cross-sectioned rat hair with mainly positive Henle layer (arrow). **C:** Negative control of rat hair. **D:** Cross-sectioned mouse hairs with positive inner root sheath (arrows). **E:** Longitudinally sectioned mouse hairs with mainly positive Henle layer (arrow) and inner root sheath. **F:** Immunonegative hairs of preabsorbed control of mouse hairs (arrows).

antibodies (Figs. 4F, G, and I and 5A, B, and E). In the cornifying region of the hair (particularly in kangaroo, cat, and rat hairs), where the Huxley and IRS cuticle were cornifying, caspase-14 labeling became visible. The staining observed in cells of the cortex and medulla of hair fibers was sometimes confusing due to the dark pigmentation deposited in its cells (Fig. 4B, C, F, G, and I). Some weak staining was seen in some levels of the hair surrounding the IRS, possibly indicating labeling in the companion layer. A stronger labeling was seen in cornified cells of the outer root sheath lining the upper part of the hair canal and continuing with the corneous layer of the epidermis.

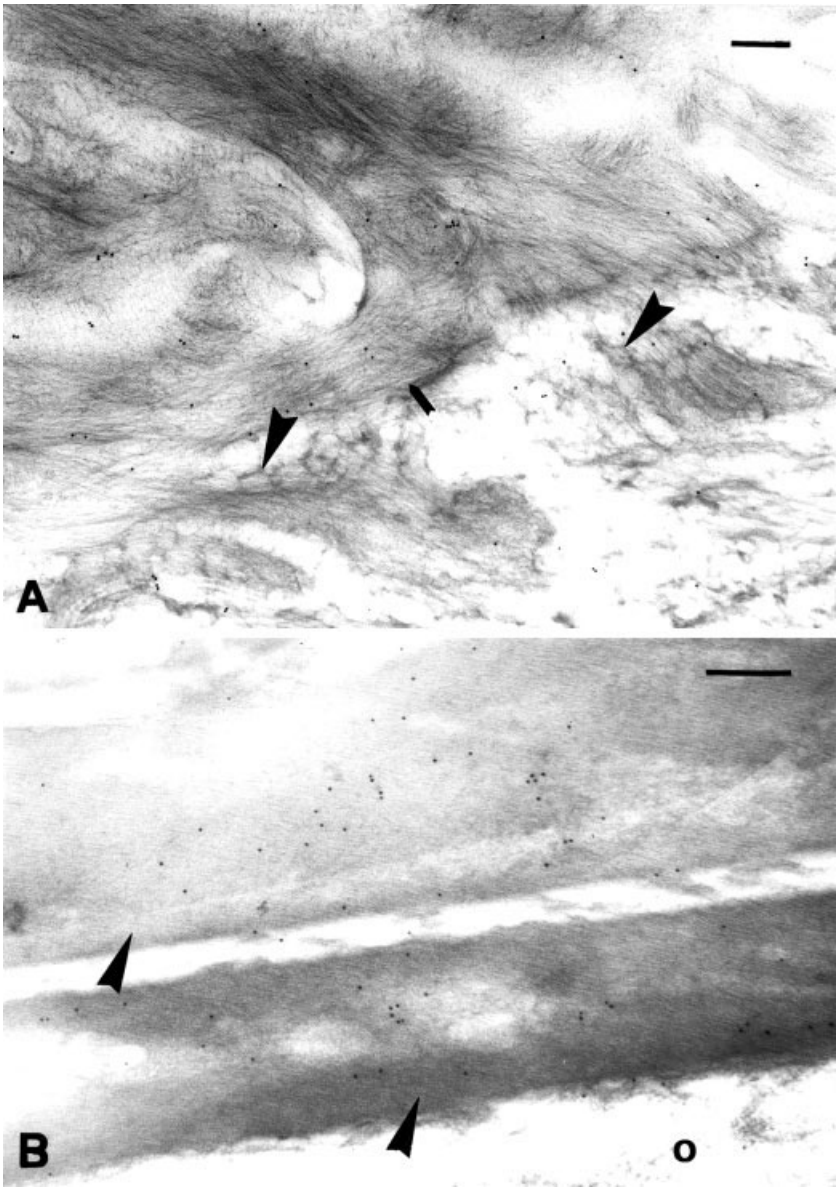

Fig. 7. Caspase-14 immunogold labeling of echidna and kangaroo hairs. **A:** Labeling in cornifying cell of the upper hair canal of echidna hair (the arrow points to keratin filaments). Arrowheads indicate coarser filaments among keratin. Scale bar = 250 nm. **B:** Labeling of IRS cells (arrowheads) of kangaroo hair. Scale bar = 250 nm

The ultrastructural study was done on echidna (ventral), kangaroo (trunk), and rat (tail) hair only. In general, the labeling with both the antihuman caspase-14 p17 and antimurine caspase-14 antibodies was diffuse (no clumps of gold particles) in living cells of the outer and inner root sheath and in the companion layer. Gold particles were more concentrated in cornifying cells of the outer or inner root sheath. In some cytoplasmic areas of the cuticle and cortex, gold particles were occasionally seen, but never over bundles of keratin or keratin-like filaments (data not shown). Both negative (omitting the first antibody) and preabsorbing (with recombinant caspase-14 p17 protein) controls showed an almost complete lack of gold particles.

The study on echidna hair showed a diffuse labeling among keratin filaments of the companion layer, connected with the cornified IRS (Fig. 6A). In the latter, sparse gold particles were seen among the fibrous remnants of the trichohyalin granules, which were mixed with irregularly or linearly oriented keratin filaments (Fig. 6B). An intense labeling was seen among keratin filaments in cornifying cells of the outer root sheath located in the upper part of the hair canal that surrounded the hair fiber (Fig. 7A). A more diffuse labeling was sometimes observed in cornifying cells of the cortex.

Also in kangaroo hair, most of labeling (which was eliminated in controls) was seen in cornifying cells of the IRS (Fig. 7B) and outer root sheath. A diffuse labeling was also seen in the cytoplasm of immature cortical cells. Only a diffuse labeling made of scattered gold particles was seen in living cells of the outer root sheath.

Finally, also in rat hair, the labeling was present mainly in cornifying IRS and was more diffuse in outer root sheath. Trichohyalin granules were generally unlabeled while a diffuse labeling was present among the surrounding keratin-trichohyalin complex filamentous network (Fig. 8). Some nuclear labeling was also seen in condensing nuclei of IRS cells, especially of the Henle layer, where the labeling was the most intense (data not shown). An

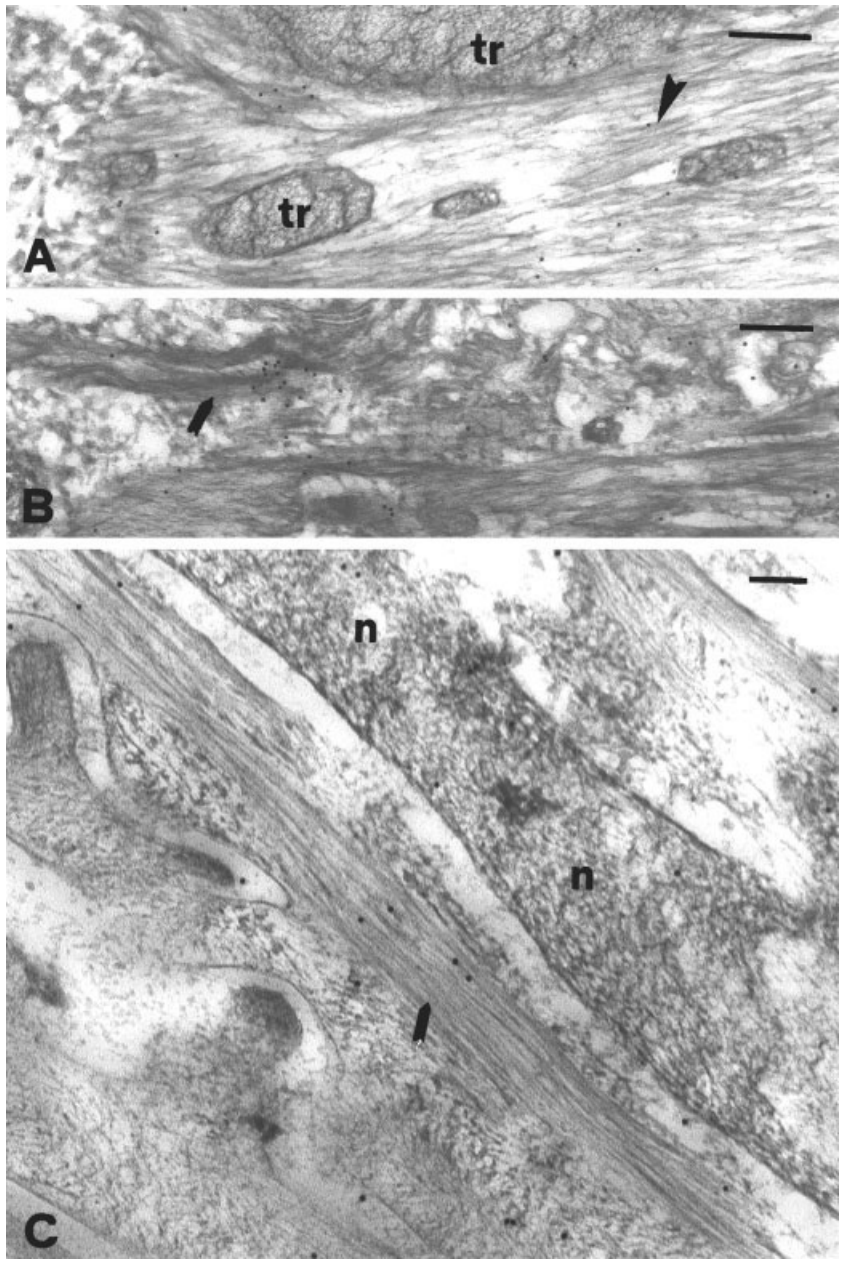

Fig. 8. Caspase-14 immunogold labeling of rat hair. **A:** Diffuse labeling among parallel keratin-trichohyalin fibrils (arrowhead) of Huxley cell. Scale bar = 250 nm. **B:** Detail of labeling associated with keratin-trichohyalin material (arrow) in rat Huxley cell. Scale bar = 200 nm. **C:** Other diffuse labeling among oriented fibrils (arrow) of inner root cell. n, nucleus. Scale bar = 100 nm.

intense labeling was present in cornified cells of the upper part of hair canal representing cornified cells of the outer root sheath (data not shown). A diffuse labeling was also seen in immature cortical cells. The negative and preabsorbing controls showed nearly complete depletion of the gold particles (data not shown).

DISCUSSION

Epidermis

In the present comparative study, expression of caspase-14 is shown to be upregulated during differentiation of epidermal keratinocytes of all mammalian species investigated. In agreement with previous studies on human and mouse skin, the highest abundance of caspase-14 was consistently found at sites of keratinocyte cornification, suggesting that caspase-14 has a general role in maturation of mammalian epidermis.

At the ultrastructural level, caspase-14 of monotreme, marsupial, and placental mammalian epidermis localized mainly among keratin filaments mixed with keratohyalin in upper spinosus, granular, and corneous layers. This pattern is very similar to that previously observed in the ultrastructural investigation of caspase-14 in human epidermis, which showed that keratohyalin associated to keratin (the amorphous component of keratohyalin) was the main site of immunolabeling for caspase-14 (Alibardi et al., 2004). Therefore, both cornification proteins (loricrin, involucrin, etc.) and associated keratins (K1, K10, K18) are possible targets of caspase-14 during formation of the epidermal barrier in mammals. Specific protein sub-

strates of caspase-14 in the epidermis remain unknown and further biochemical analysis is needed. It is unknown whether caspase-14 may also be involved in the process of sloughing of corneocytes on the surface of the stratum corneum, a process known to be driven by proteolytic enzymes (Menon and Norlen, 2002; Milstone, 2004).

Interestingly, caspase-14 immunolocalization is similar to that recently observed for transglutaminase (Alibardi and Toni, 2004). Transglutaminases crosslink proteins during apoptosis in a wide variety of cell types (Lorand and Graham, 2003). In the epidermis, transglutaminases-1 and -3 crosslink proteins such as involucrin, loricrin, and sciellin, thereby forming the cornified cell envelope that functions as the basic building block of the stratum corneum barrier (Kalinin et al., 2002). The cornified envelope represents the terminal stage of keratinocyte differentiation, where both nucleus and most cell organelles are degraded, with their proteins contributing to the formation of the corneous mass and cell envelope (Holbrook, 1989; Ishida-Yamamoto et al., 2000; Kazerounian and Aho, 2003). On this ground, it is conceivable that caspase-14 has evolved from the caspase family of general cell death-mediating proteases to become a regulator of the formation of this specific cell death product.

Hair

This study is the first to deal with the fine localization of caspase-14 in hair follicles. Previous light microscopic studies indicated that caspase-14 mRNA and the enzyme are present in IRS and keratogenous zone of the hair of human and mouse (Eckhart et al., 2000b; Lippens et al., 2003). The present study in part confirms the above initial studies but indicates that, immunologically, caspase-14 is confined to the cornifying layers of the hairs (keratogenous zone), and especially in the IRS and corneous cells of the outer root sheath above the sloughing zone of the IRS. The present study also indicates that the role of caspase-14 in hairs is widespread among all mammals.

The IRS region surrounds the moving hair fiber and is constructed in such a manner as to allow fiber movement until it is sloughed near the neck of the hair follicle at catagen. The IRS together with the companion layer constitute the slippage plane along which the hair fiber moves during growth at anagen (Stenn and Paus, 2000). It is conceivable that specific types of keratins (K6 and others) (Rothnagel and Roop, 1995; Mahony et al., 1999; Langbein et al., 2001) and/or interkeratin or matrix proteins such as trichohyalin may be degraded specifically to facilitate this dynamic process. A role for caspase-14 in this process remains, however, to be shown. Caspase-14 may intervene in the proteolysis of keratin/trichohyalin during the cornification of IRS. This notion is supported by the finding that, like in interfollicular epidermis, caspase-14 localization overlaps with that of transglutaminase (Polakowska and Goldsmith, 1991; Rogers et al., 1999; Alibardi, 2004a, 2004b), possibly indicating that both the enzymes target related protein substrates during the cornification of IRS. Biochemical studies are required to test these possible protein substrates of caspase-14.

The distribution of caspase-14 in the hair follicle was highly similar in representatives of monotreme (echidna), marsupial (red kangaroo), and placental (rat) mammals, suggesting that there is a general pattern of caspase-14 localization in mammalian hairs. Furthermore, the presence of caspase-14 both in the cornifying sheaths of the hair follicle and in the cornifying cells of the epidermis supports the hypothesis that the IRS is an evolutionary specialization of the stratum corneum of the epidermis that has become internalized in the hair follicle (Alibardi, 2004c).

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LITERATURE CITED

Alibardi L, Maderson PFA. 2003. Distribution of keratin and associated proteins in the epidermis of monotreme, marsupial, and placental mammals. J Morphol 258:49–66.

Alibardi L. 2004a. Fine structure and immunocytochemistry of monotreme hairs with emphasis on the inner root sheath and trichohyalin-based cornification during hair evolution. J Morphol 261: 345–363.

Alibardi L. 2004b. Fine structure of marsupial hairs with emphasis on trichohyalin and the structure of the inner root sheath. J Morphol 261:390–402.

Alibardi L. 2004c. Comparative aspects of the inner root sheath in adult and developing hairs of mammals in relation to the evolution of hairs. J Anat 205:179–200.

Alibardi L, Toni M. 2004. Immuno-cross reactivity of transglutaminase and cornification marker proteins in the epidermis of vertebrates suggest common processess of soft cornification across species. J Exp Zool 302B:526–549.

Alibardi L, Dockal M, Reinisch C, Tschachler E, Eckhart L. 2004. Ultrastructural localization of caspase-14 in human epidermis. J Histochem Cytochem 52:1561–1574.

Botcharev VA, Paus R. 2003. Molecular biology of hair morphogenesis: development and cycling. J Exp Zool 298B:164–180.

Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, Egelrud T, Simon M, Serre G. 2004. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J Invest Dermatol 122: 1235–1244.

Eckhart L, Declercq W, Ban J, Rendl M, Lengauer B, Mayer C, Lippens S, Vandenabeele P, Tschachler E. 2000a. Terminal differentiation of human keratinocyets and stratum corneum formation is associated with caspase-14 activation. J Invest Dermatol 115: 1148–1151.

Eckhart L, Ban J, Fischer H, Tschachler E. 2000b. Caspase-14: analysis of gene structure and mRNA expression during keratinocyte differentiation. Biochem Biophys Res Commun 277:655–659.

Fischer H, Stichenwirth M, Dockal M, Ghannadan M, Buchberger M, Bach J, Kapetanopoulos A, Declercq W, Tschachler E, Eckhart L. 2004. Stratum corneum-derived caspase-14 is catalytically active. FEBS Lett 577:446–450.

Fuchs E. 1990. Epidermal differentiation: the bare essentials. J Cell Biol 111:2807–2814.

Hardy MH. 1992. The secret life of the hair follicle. Trends Gen 8:55–61.

Hashimoto Y, Suga Y, Matsuba S, Mizoguchi M, Takamori K, Seitz J, Ogawa H. 2000. Immunohistochemical localization of sulfhydryl oxidase correlates with disulfide crosslinking in the upper epidermis of rat skin. Arch Dermatol Res 292:570–572.

Holbrook KA. 1989. Biologic structure and function: perspectives on morphological approaches to the study of the granular layer keratinocyets. J Invest Dermatol 92(Suppl 4):84S–104S.

Ishida-Yamamoto A, Takahashi H, Iizuka H. 2000. Immunoelectron microscopy links molecules and morphology in the studies of keratinization. Eur J Dermatol 10:429–435.

Kalinin AE, Kajava AV, Steinert PM. 2002. Epithelial barrier function: assembly and structural features of the cornified cell envelope. BioEssays 24:789–800.

Kazerounian S, Aho S. 2003. Characterization of periphilin, a widespread, highly insoluble nuclear protein and potential constituent

of the keratinocyte cornified envelope. J Biol Chem 278:36707–36717.

Langbein L, Rogers MA, Praetzel S, Aoki N, Winter H, Schweizer J. 2002. A novel epithelial keratin, hK6irs1, is expressed differentially in all layers of the inner root sheath, including specialized huxley cells (Flugelzellen) of the human hair follicle. J Invest Dermatol 118:789–799.

Lippens S, Kockx M, Knaapen M, Mortier L, Polakowska R, Verheyen A, Garmyn M, Zwijsen A, Formstecher P, Huylebroeck D, Vandenabeele P, Declercq W. 2000. Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing. Cell Death Differ 7:1218–1224.

Lippens S, VandenBroecke C, Van Damme E, Tschachler E, Vandenabeele P, Declercq W. 2003. Caspase-14 is expressed in the epidermis, the choroid plexus, the retinal pigment epithelium and thymic Hassall's bodies. Cell Death Differ 10:257–259.

List K, Szabo R, Wertz PW, Segre J, Haudenschild CC, Kim SY, Bugge TH. 2003. Loss of proteolytically processed filaggrin caused by epidermal deletion of Matriptase/MT-SP1. J Cell Biol 163:901–910.

Lorand L, Graham RM. 2003. Transglutaminases: crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol 4:140–156.

Mahony D, Karunaratne S, Rothnagel JA. 1999. The companion layer and outer root sheath of the anagen follicle. Exp Dermatol 8:329–331.

Menon GK, Norlen L. 2002. Stratum corneum ceramides and their role in skin barrier function. In: Leyden JJ, Rawlings AV, editors. Skin moisturization. New York: Marcel Dekker. p 31–60.

Mikolajczyk J, Scott FL, Krajewski S, Sutherlin DP, Salvesen GS. 2004. Activation and substrate specificity of caspase-14. Biochemistry 43:10560–10569.

Millar SE. 2002. Molecular mechanisms regulating hair follicle development. J Invest Dermatol 118:216–225.

Milstone LM. 2004. Epidermal desquamation. J Dermatol Sci 36:131–140.

Orwin DFG. 1979. The cytology and cytochemistry of the wool follicle. Int Rev Cytol 60:331–374.

Polakowska RR, Goldsmith LA. 1991. The cell envelope and transglutaminases. In: Goldsmith LA, editor. Physiology, biochemistry and molecular biology of the skin, vol. 1. Oxford: Oxford University Press. p 168–201.

Rawlings AV, Scott IR, Harding CR, Bowser PA. 1994. Stratum corneum moisturization at the molecular level. J Invest Dermatol 103:731–740.

Rendl M, Ban J, Mrass P, Mayer C, Lengauer B, Eckhart L, Declerq W, Tschachler E. 2002. Caspase-14 expression by epidermal keratinocytes is regulated by retinoids in a differentiation-associated manner. J Invest Dermatol 119:1150–1155.

Resing KA, Dale BA. 1991. Proteins of keratohyalin. In: Goldsmith LA, editor. Physiology, biochemistry and molecular biology of the skin, vol. 1. Oxford: Oxford University Press. p 148–167.

Rogers G, Dunn S, Powell B. 1999. Late events and the regulation of keratinocyte differentiation in hair and feather follicles. In: Chuong CM, editor. Molecular basis of epithelial appendages and morphogenesis. Austin, TX: R.G. Landes. p 315–338.

Rogers G. 2004. Hair follicle differentiation and regulation. Int J Dev Biol 48:163–170.

Rothnagel JA, Roop DR. 1995. Hair follicle companion layer: reacquanting an old friend. J Inv Dermatol 104:42S–43S.

Scala C, Cenacchi G, Ferrari C, Pasquinelli G, Preda P, Manara GC. 1992. A new acrylic resin formulation: a useful tool for histological, ultrastructural, and immunocytochemical investigation. J Histochem Cytochem 40:1799–1804.

Sokolov VE. 1982. Mammalian skin. Berkeley, CA: University of Califorrnia Press.

Stenn KS, Paus R. 2001. Control of hair follicle cycling. Physiol Rev 81:449–494.