Merkel Cells of the Terminal Hair Follicle of the Adult Human Scalp

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Human scalp skins were treated with 20 mM ethylendiaminetetraacetic acid and terminal hair follicles were extracted with the epidermis. Some terminal hair follicles were morphologically preserved well and provided opportunity to examine three-dimensional distribution of CAM5.2 (K8, 52.5 kD) reactive Merkel cells. In anagen terminal hair of the scalp numerous immunoreactive Merkel cells were distributed in the presumptive bulge area. Distinct swelling as in the bulge of human vellus hair was usually absent; however, in rare instances anagen terminal hair demonstrated unilateral prominent swelling with dense aggregation of Merkel cells. In telogen hair the bulge becomes indistinguishable from the regressed end of the club hair follicle but Merkel cells continued to be abundant. We found morphologic variation of the bulge such as formation of knoblike swellings and villous projections. Interestingly, Merkel cells were also located in these structures. Palisading stockade-like nerve endings were observed surrounding the follicular epithelium at the sebaceous gland level. Merkel cells were sparse in this follicular segment. Variable number of Merkel cells were also scattered in the infundibulum of terminal hair in no association with peripheral nerves. *Key words: knob/villi. J Invest Dermatol 102:506–510, 1994*

Human Merkel cells have been considered as tactile receptors of the epidermis and hair follicles. For example, an abundance of Merkel cells has been reported in human facial hairs [1,2] and they were assigned a function to subserve the tactile sensation of the facial skin. However, the presence or absence of Merkel cells in terminal hairs of human scalp has not been studied. In this study, we confirmed the presence of Merkel cells in the terminal scalp hairs in anagen and telogen phases and further analyzed their three-dimensional distribution using extracted terminal hair follicles. It was found that the follicular Merkel cells are most concentrated in the bulge areas where slow cycling stem cells for anagen hair matrix were identified [3].

MATERIALS AND METHODS

Tissue Samples of terminal hair follicle obtained from the scalp of five patients during routine surgical procedures contained vellus and terminal hairs. In the present study, we used the following definition [4] of vellus and terminal hairs. Vellus hair is soft and short, usually not longer than 2 cm, often colorless. Terminal hair is larger and coarse, endowed with medulla and pigment, and can vary in length. Tissue specimens were cut into two samples. One was immediately embedded in O.C.T. compound (Lab-Tek Products, Naperville, IL), snap-frozen in liquid nitrogen, and stored in −70°C until use. Another was embedded in paraffin and 8-μm serial transverse sections (200 sections) prepared.

Preparation of Terminal Hair Follicles The skin was placed in phosphate-buffered saline, pH 7.3, at room temperature and then incubated in 20 mM ethylendiaminetetraacetic acid in phosphate-buffered saline for 3–12 h at 37°C. The epidermal sheets with attached hair follicles were gently removed with forceps and rinsed in phosphate-buffered saline. The morphologic preservation of vellus hair follicles were much better than terminal hair follicles [5]. Terminal hair follicles (25 samples) were observed three-dimensionally with light microscope in the wet whole mount.

Electron Microscopy The scalp skin of a 55-year-old white woman was fixed with 5% glutaraldehyde in cacodylate buffer, pH 7.2. The fixed tissue was embedded in Araldite and thin sections were double stained with lead citrate and uranyl acetate. Thin sections were observed in a Hitachi H-300 electron microscope.

Immunohistochemistry Frozen sections of human scalp skin were immunostained for monoclonal antibody against Merkel cells using the avidin-biotin-peroxidase complex (Vector Kit, Vector Laboratories, Burlingame, CA) and diaminobenzidine coloration technique. Frozen tissue specimens were serially cut into 10-μm sections (150 sections) in a cryostat and fixed in cold acetone for 10 min. Monoclonal murine antibody CAM5.2 (Becton-Dickinson, San Jose, CA) [6] specific for the simple epithelial type cytokeratin (52.5 kD) was used as a marker for Merkel cells [7]. Immunoelectron microscopy confirmed that CAM5.2-positive cells contain Merkel cell granules [7]. Frozen sections were incubated for 30 min with primary antibody at room temperature, and stained immunohistochemically with biotinylated anti-mouse IgG horse serum as secondary antibody and avidin-biotin-peroxidase complex as third reagent. The peroxidase color reaction was developed under microscopic observation in the presence of diaminobenzidine, washed with water, and counterstained with hematoxylin.

The separated epidermal sheets with terminal hair follicles were similarly immunostained by the avidin-biotin-peroxidase complex method, washing with phosphate-buffered saline three times for 10 min and fixing in a cold acetone for 30 min. Incubation with CAM5.2 took overnight and the reaction time with biotinylated anti-mouse Ig horse serum as secondary antibody and avidin-biotin-peroxidase complex as third reagent took 60 min each at room temperature. The peroxidase color reaction was monitored under microscopic observation in the presence of diaminobenzidine.

Double-Immunoenzyme Labeling Serial transverse sections were deparaffinized prior to immunostain. A double immunoenzyme staining method with peroxidase (for cow S-100 protein) and alkaline phosphatase (for keratin) as labels was performed to study the topographical relationship between immunoreactive Merkel cells in the hair follicle and myelinated nerve surrounding the hair follicle. A double immunoenzyme technique was previously described [8]. In brief, for myelinated nerve demonstration rabbit anti-cow S-100 protein antiserum (DAKO) was applied, and visualized with

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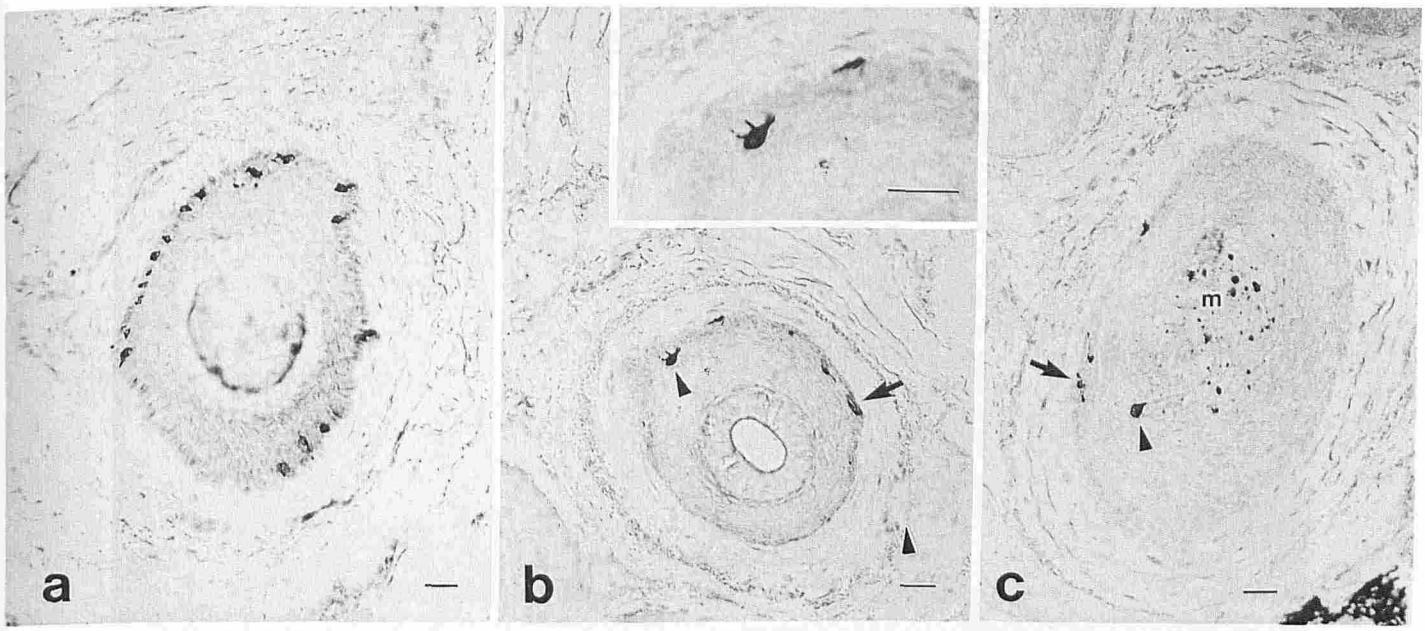

Figure 1. Transverse sections of terminal hair follicles of human scalp skin at the level of the bulge. The distribution patterns of Merkel cells are shown. *a)* Merkel cells are situated along the most circumference of a terminal hair in the outermost cell layer. *b)* Merkel cells are located in the outermost layer *(arrow)* as well as a relatively inner layer *(arrowhead)* of the outer root sheath of a terminal hair. *Inset,* high magnification of the area pointed to by the *arrowhead.* Merkel cell shows dendritic processes. *c)* Merkel cells are situated in the outermost *(arrow)* as well as inner *(arrowhead)* layers of the outer root sheath. m, melanin granules. Frozen sections stained with CAM5.2. *Bars,* 25 μm.

diaminobenzidine. S-100 protein is absent in neurofilament or unmyelinated small fibers and nerve endings. CAM5.2 for Merkel cell was then applied and alkaline-phosphatase activity was developed with Fast red. The double-stained sections were counterstained with hematoxylin. Counterstaining was sometimes omitted to improve the color resolution between diaminobenzidine and Fast red.

RESULTS

Transverse sections of human scalp skin showed the irregularly shaped knoblike or villous projections at the sebaceous gland level. Knoblike structures indicated dome-shaped epithelial swellings and villous structures represented slender protrusions, respectively. Transverse sections of human scalp skin demonstrated that Merkel cells were situated in the outermost (Fig 1*a*) as well as the inner cell layers of the outer root sheath (Fig 1*b,c*). The distribution of Merkel cells tended to be heavier on one side of the follicle (Fig 1*a–c*), particularly on knoblike projections, where the bulge was better developed to anchor arrector pili muscle. The outer root sheaths of the lower portions were variably stained with CAM5.2.

Nerve–Merkel Cell Relationship Double immunoenzyme technique using CAM5.2 and S-100 protein antibodies on the serial transverse sections enables us to roughly evaluate the topographical relationship between Merkel cells and myelinated peripheral nerves around human terminal hair follicles. In the infundibulum of terminal hair follicles several Merkel cells were present in the outermost cell layer in no association with myelinated nerves. At the sebaceous gland level perifollicular nerve endings labeled with S-100 protein antibody were demonstrated in a palisading arrangement surrounding the outer root sheath of terminal hair follicles, but Merkel cells were absent in this follicular segment, excepting occasional Merkel cells free from myelinated nerve fibers. At the lower level of the sebaceous gland, Merkel cells were observed in the outermost cell layer, but not associated with S-100 reactive myelinated nerve fibers or nerve endings (Fig 2*a*). Some Merkel cells closely faced circular S-100 reactive fibers, but were not attached to them (Fig 2*b*). Interestingly, Merkel cells were more concentrated in knoblike or villous structures (Fig 3*a*) of the terminal hair follicles; in some instances these structures were closely encircled with circular myelinated nerve fibers (Fig 3*b*).

Electron Microscopy Nerve endings were not found in apposition to Merkel cells in the bulge (Fig 4). Five Merkel cells, three in the outer layer along the lamina densa and two in the inner layer, did not show any associated nerve fibers, either myelinated or unmyelinated. These Merkel cells were followed by serial sections and still not found to be associated with neural elements.

Three-Dimensional Views In wet mount specimens some terminal hair follicles had knoblike or villous variations below the sebaceous gland, which corresponded to the bulge areas as described above in transverse sections (cf Fig 3*b*). The number of knoblike or villous structures was variable. The presence or absence and the shape of these structures were not influenced by the hair cycle. The anagen terminal hair follicles usually did not show swellings below the sebaceous glands. However, in rare instances prominent swelling was observed in the location corresponding to the bulge. In the telogen terminal hair follicles the bulge areas became indistinguishable from the clubbed end. The irregular shaped knoblike swellings were also found in the outer root sheath covering the club hair, thus identifying this area with the bulge that was obscured by the clubbing. Knoblike or villous structures observed in the whole mount of extracted terminal hair follicles were structurally similar to epithelial projections shown in the transverse sections of human scalp skin (cf Fig 3).

Merkel Cells in Extracted Hair Five hundred seventy-five Merkel cells were counted in 25 extracted hairs. However, it was hard to count accurately the number of Merkel cells because whole mount of terminal hair follicle was thick. Many Merkel cells were observed below the sebaceous glands, especially concentrated in the presumptive bulge areas in the anagen follicles (Fig 5*a*) and also in the clubbed end areas in the telogen follicles (Fig 5*b*). Merkel cell population was especially dense in the knobs and villi, although a cluster of Merkel cells were also observed in smooth portion of the bulge (Fig 5*a*). The structures such as knobs and villi and general density of Merkel cells were not influenced by the hair cycle. In some terminal hair follicles variable numbers of Merkel cells were scattered in the infundibulums.

In smaller vellus hairs of human facial skin, a skirtlike structure or hood [5] surrounding the entire circumference of hair follicle was

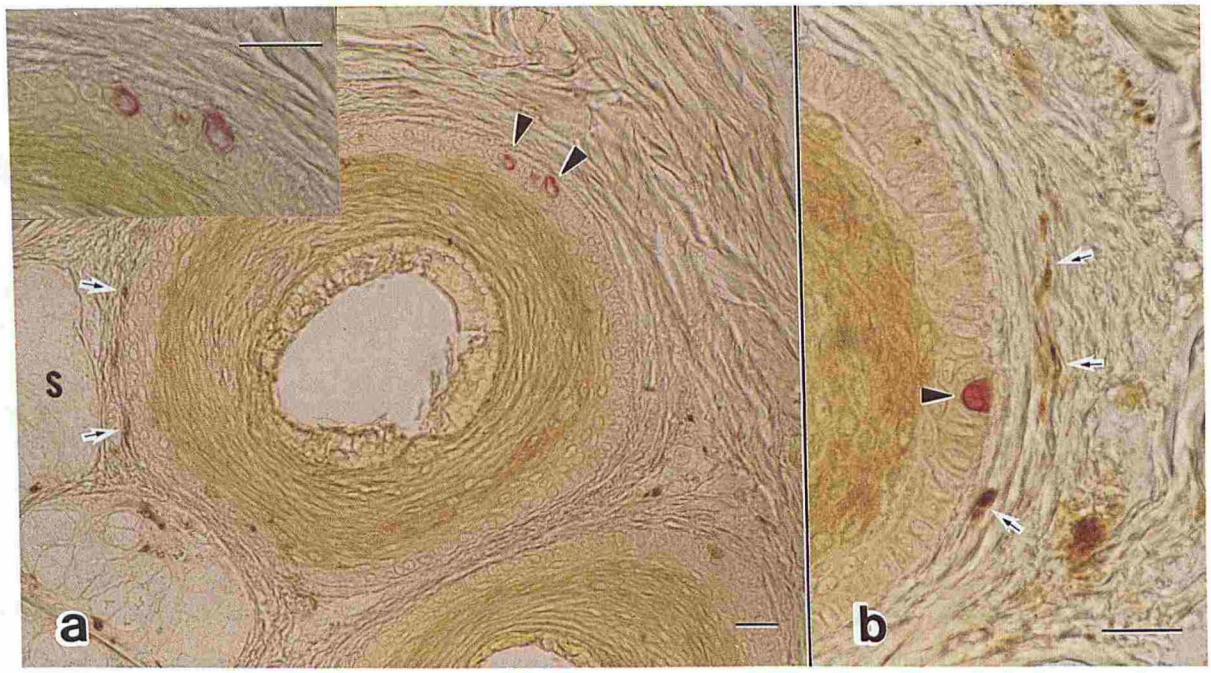

Figure 2. Transverse sections of terminal hair follicle of human scalp skin. The topographic relationship between CAM5.2–reactive Merkel cell and S-100 protein–reactive nerve fibers are shown. *a* and *b)* Merkel cells *(arrowheads)* are stained red and situated in the outermost cell layer of the terminal hair follicle but separated from S-100–reactive myelinated nerves *(arrows)* at the lower level of the sebaceous gland(s). *Inset,* high magnification of Merkel cells pointed to by *arrowheads* in the main picture of *a.* S-100 protein/peroxidase/diaminobenzidine for nerve. CAM5.2/alkaline phosphatase/Fast red for Merkel cell. *Bars,* 25 μm.

always present and some Merkel cells were found there [5]. However, this structure was absent in terminal hairs of human scalp skin.

DISCUSSION

Although the presence of Merkel cells in the adult human hair follicle and perifollicular sheath has been reported sporadically by electron microscopic survey [1,9–11], there has been no systemic studies by immunostain, which can scan large areas [12]. In the present study, we have studied Merkel cell distribution of human terminal hair follicle three-dimensionally and described the concentrated localization of CAM5.2 reactive Merkel cells in the bulge. S-100 protein-reactive nerve fibers are myelinated and therefore large; such fibers were absent in contact with follicular Merkel cells. Electron microscopy failed to detect unmyelinated fine fibers such as those found in association with the epidermal Merkel cells. The bulge and knobs of vellus hair were rich in Merkel cells but the skirt was only sparsely populated with Merkel cells [5]. We found the

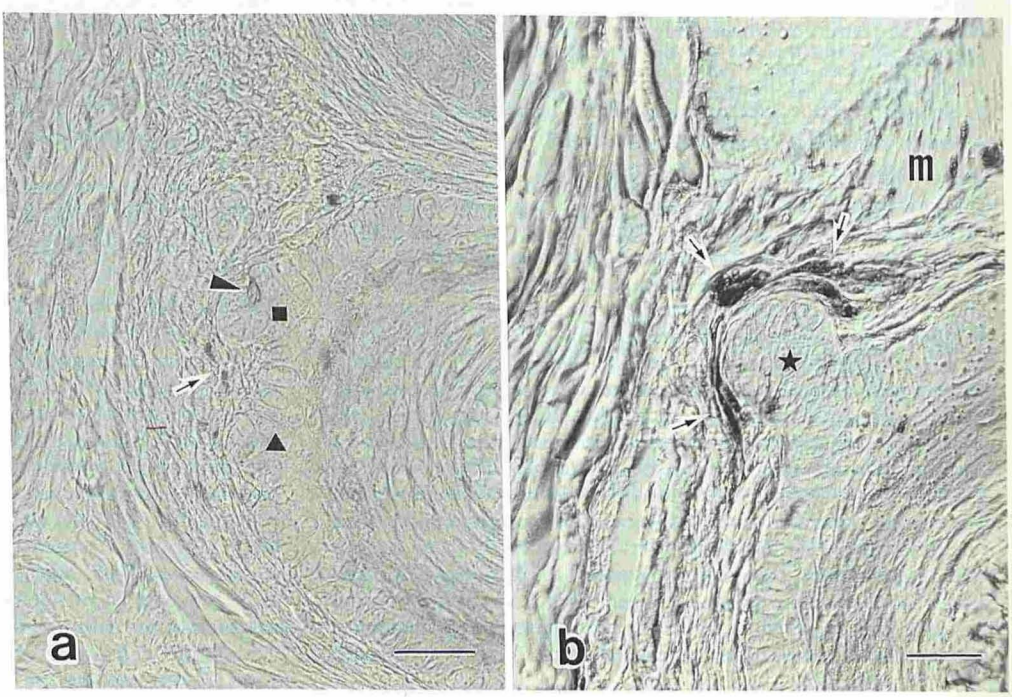

Figure 3. Transverse sections *(a,b)* of terminal hair follicles of human scalp skin at the bulge. The villous (■,▲) and knoblike (★) projections and arrector muscle (m) are observed. Merkel cells *(arrowhead)* are situated in the villous-like (■) projection. In this specimen villouslike (▲) and knoblike (★) projections are surrounded by circular nerve fibers *(arrows).* S-100 protein/peroxidase/diaminobenzidine for nerve. CAM5.2/alkaline phosphatase/Fast red for Merkel cell. *Bars,* 25 μm.

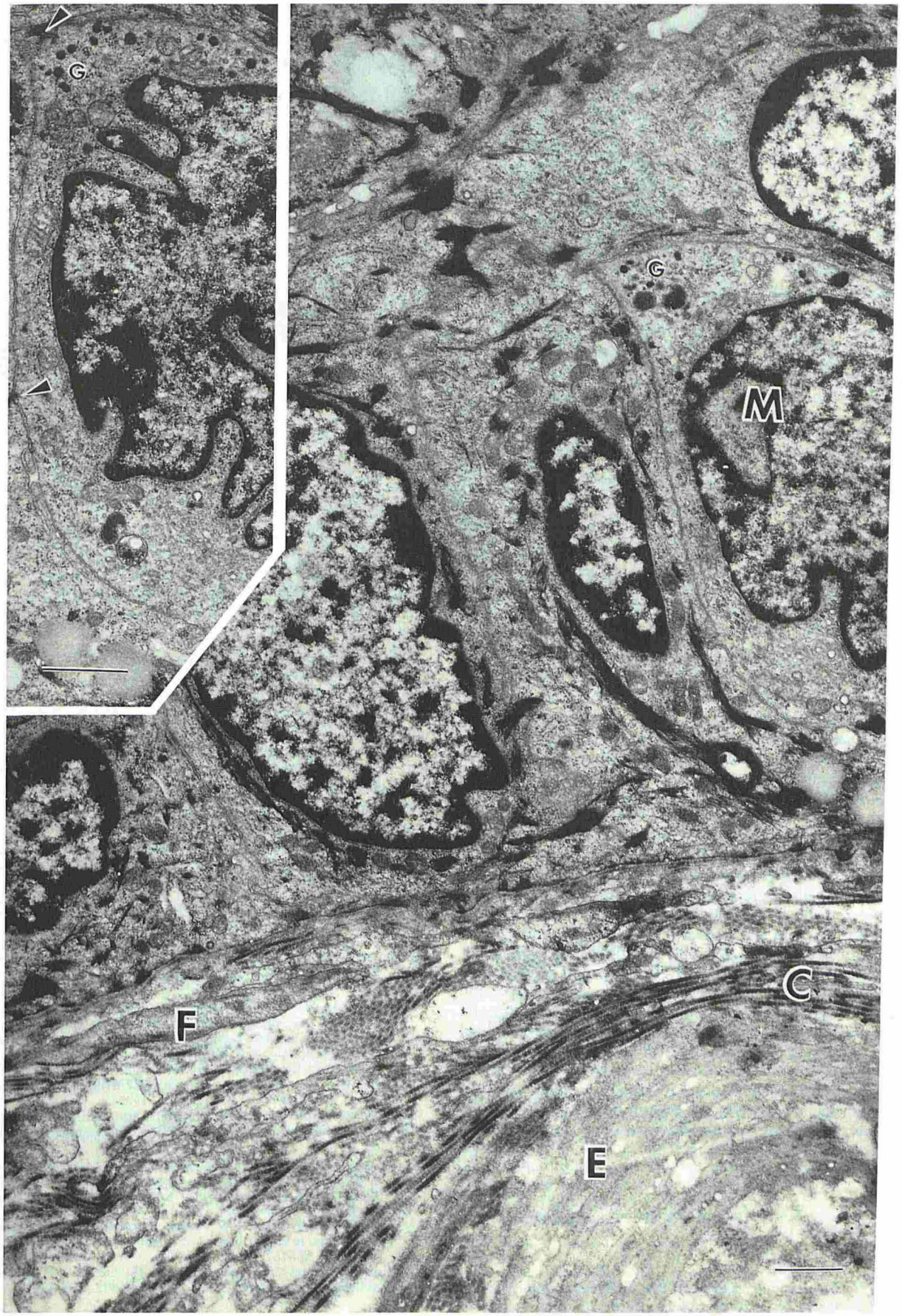

Figure 4. Electron microscopy of a Merkel cell (M) in the bulge of a terminal hair follicle of human scalp skin. A serial section of this cell shown in the *inset* exhibits desmosomal connections *(arrowheads)* to surrounding basal cells and typical Merkel cell granules (G). Perifollicular connective tissue sheath contains collagen fibers (C), an elastic fiber (E), and processes of fibroblasts (F) but no nerve endings. No neural elements could be detected in sections serial to this one. *Bars*, 1 μm.

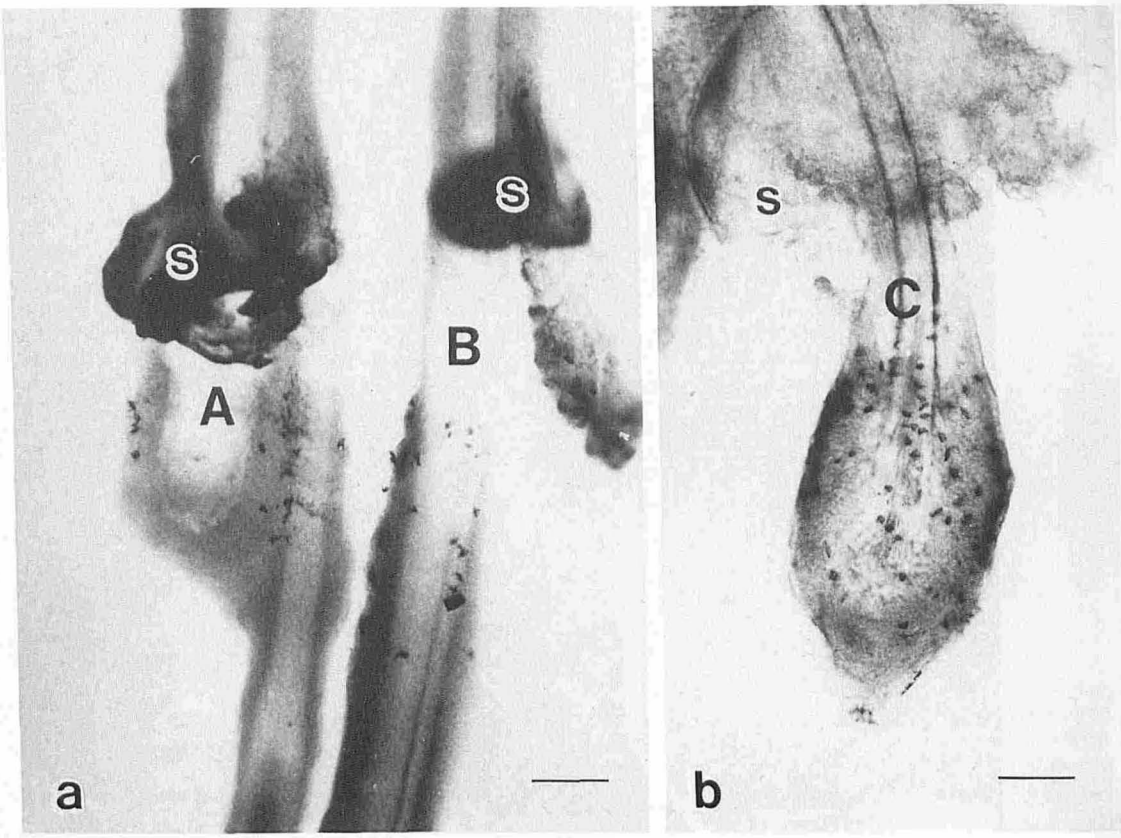

Figure 5. Three-dimensional views *(a,b)* stained with CAM5.2. A large number of Merkel cells are scattered in the bulge areas of early anagen (A), anagen (B), and telogen (C) terminal hair follicles below the sebaceous gland(s). *Bars,* 100 μm.

same features in the terminal hairs of the human scalp except for the absence of skirt. Knoblike or villous structures have not been described in detail except for a collar-like protrusion [10,11]. The functional significance of newly discovered knobs and villi is not clear; we presumed that they may serve as anchoring points for arrector muscle fibers.* We further speculated that the concentrated Merkel cells in these structures may have something to do with the attachment and orientation of arrector pili muscles [13].

Previous observations [14,15] showed CAM5.2 immunoreactivity in the deep outer root sheath in hair follicles. In the present study CAM5.2 also stained the lower portion of the outer root sheath of terminal hair follicles, but not the bulge area except for CAM5.2 reactive dendritic cells as observed in the epidermis presumably indicating Merkel cells. Preferential localization of Merkel cells in the bulge areas where human terminal and vellus hair follicles begin a new anagen growth may imply some functional roles related to hair growth. Telogen hair follicle devoid of hair shaft certainly has no function in generating tactile sensation; nevertheless, an abundance of Merkel cells persist in the bulge. More recently, Cotsarelis *et al* [3] proposed a hypothesis that the "bulge" houses slow cycling stem cells that provide the matrix cells of early anagen hair. It is possible that Merkel cells secrete some growth factors or cytokines that initiate a new hair cycle. Although this idea is highly speculative, we would not be surprised if new cytokines of Merkel cells responsible for the initiation of new hair cycle are identified in the future.

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