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Epidermal Stem Cells

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Stem cells are by definition present in all self-renewing tissues and are believed to play a central role in cell growth and differentiation. Existing evidence suggests that a subpopulation of epidermal basal keratinocytes represents stem cells; however, these cells have never been positively identified. In this paper we review evidence that in monkey palm epidermis there exist two morphologically distinct subpopulations of basal keratinocytes that are spatially segregated. One population, located in the shallow rete ridges, is characterized by a cytoplasm filled with tonofilaments and a highly convoluted ("serrated") dermal-epidermal junction; these cells may play a role in anchoring the epidermis to the dermis. In contrast, the other population, located at the tips of deep rete ridges, is characterized by a "primitive" cytoplasm containing abundant melanosomes and a relatively flattened ("nonserrated") dermal-epidermal junction. Tritiated thymidine labeling experiments suggest that the nonserrated basal keratinocytes are slow-cycling; however, a highly proliferative population of keratinocytes can be identified immediately above these basal cells. These findings are consistent with the concept that the nonserrated basal keratinocytes may represent stem cells that give rise to suprabasally located, transient amplifying cells before undergoing terminal differentiation. Monkey palm epidermis provides a model system for further studies of primate epidermal stem cells.

The epidermis is a classic example of a renewing tissue [1]. To fulfill its role in protection, the epidermis elaborates a tough, cohesive, highly resistant outer layer (stratum corneum) comprised of flattened, anucleated horny cells. These horny cells are constantly shed or lost into the environment, only to be continuously replaced by new cells. Thus a perpetual cell replacement is of paramount importance for proper epidermal maintenance. Since skin is one of the largest organs of the body, the epidermis is also one of the major cell-renewal systems.

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Abbreviations:
EPU: epidermal proliferative unit
[^{3}H]-TdR: tritiated thymidine
TA cells: transient amplifying cells

Morphologically, the epidermis has been considered a four-compartment system wherein relatively undifferentiated basal cells sequentially mature into spinous, granular, and horny cells [2]. However, in kinetic terms, the epidermis has been regarded as a two-compartment system [3]. The proliferative compartment encompasses basal and some suprabasal cells, functioning to maintain the germinative cell population and to supply cells to the differentiative compartment. The differentiative compartment consists of upper spinous, granular, and horny cells; within this compartment, cell maturation and eventual cell loss occur without any new cell production. Thus the proliferative compartment, which is ultimately governed by stem cells, is solely responsible for the maintenance of the "steady-state" conditions of the epidermis.

STEM CELLS

Stem cells are by definition present in all renewing tissues [4,5]. These cells are long-lived, have great potential for cell division (i.e., are clonogenic), and are ultimately responsible for cell replacement. Although the epidermis is one of the major cell-renewal systems, relatively little is known about the identity and properties of epidermal stem cells. Most of our knowledge of stem cells comes from studies on blood cells and several other cell-renewing tissues (e.g., intestinal epithelia, seminiferous epithelia). These investigations suggest that stem cells possess the following properties: (1) their cytoplasm appears "primitive" and contains few, if any, differentiation products; (2) they have very low mitotic activity and thus are slow-cycling; (3) they give rise to transient amplifying (TA) cells that amplify the number of cells derived from each stem-cell mitosis by undergoing a few rounds of cell division; and (4) they can be induced to proliferate by tissue demand or specific stimuli [4–7]. The properties of stem, transient amplifying, and postmitotic cells are summarized in Table I.

Since the epidermis is prototypical of a tissue governed by stem cells and is so readily accessible to experimentation, and since the stages of differentiation (keratinization) are so well documented, it is somewhat paradoxical that epidermal stem cells have not yet been identified. This can be partially explained by the customary view regarding basal keratinocytes as a morphologically homogeneous population.

MORPHOLOGIC HETEROGENEITY OF BASAL KERATINOCYTES

The basal layer of the human epidermis was classically described as consisting of a homogeneous population of cylin-

TABLE I. *General properties of stem, transient amplifying, and postmitotic cells*

Cell type	Location	Differentiated state	[^{3}H]-TdR incorporation	Cell-division potential
Stem cell	Well protected	Primitive	Low	High
Transient amplifying cell	Usually adjacent to stem cell	↓	High	Low (finite)
Postmitotic cell	Close to environment	Well differentiated	0	0

drical cells oriented perpendicularly to the surface of the skin [8]. Early pioneering work by Odland [10], Selby [9], Brody [11], and others in the late 1950s and early 1960s elucidated the structure of the desmosome and the hemidesmosome and described the dermal-epidermal junction in relation to the histologic concept of the basement membrane. In defining this latter region, almost all investigators observed some types of basal-cell processes extending into the dermis. Odland [10] was the first to describe in detail the irregular finger-like processes of the basal epidermal cells that projected downward toward the dermis. He stated that these processes varied in width and depth of penetration into the papillary layer of the dermis, although no mention of basal-cell heterogeneity was made. Hibbs and Clark [12] were the first to note morphologic differences among basal cells at different regions of the epidermis. In their investigations of the human abdomen skin, they noted that in rete ridges the basal layer was composed of cuboidal cells and these cells became taller as the region overlying the apices of dermal papillae were approached. Furthermore, it was observed that basal cells at the apex of rete ridges had less pronounced finger-like projections into the dermis than basal cells at the apex of the dermal papillae. These interesting observations were overlooked, however, by subsequent investigators and consequently dropped into obscurity. In the mid-1960s to the early 1970s, investigators concentrated on elucidating the sequential morphologic and biochemical changes occurring during keratinization [13–15]. Thus attention largely shifted from the basal cells to spinous, granular, and horny cells, and in the schema of keratinization that evolved, basal-cell homogeneity was frequently implied and accepted. In fact, the most frequent mention of basal-cell heterogeneity was with regard to the presence of various cell types, such as melanocytes, Langerhans cells, Merkle cells, and keratinocytes, leading to the classification of cells in the basal layer into the two broad categories of keratinocytes and nonkeratinocytes [16].

KINETIC HETEROGENEITY OF BASAL KERATINOCYTES

While most of the morphologists were assuming that basal keratinocytes represented a homogeneous cell population, kinetic data strongly suggested basal-keratinocyte heterogeneity. Early observations of Mackenzie [17], Christophers [18], and Menton and Eisen [19] showed that in some regions of laboratory animals as well as the human body, the epidermis was arranged in vertical columns extending downward from the stratum corneum through the granular and spinous cell layers. A cluster of 10 to 12 basal keratinocytes was positioned under each column. These basal keratinocytes were organized such that a peripheral ring of six to seven cells and a central core of approximately three cells were positioned under each column. Potten [20] regarded the epidermal-cell column as a unit of cells called the *epidermal proliferative unit* (EPU). Kinetic data suggested that the peripheral ring of basal cells was the preferential site of colchicine-arrested mitotic cells [21,22], as well as the region of selected tritiated thymidine ([^{3}H]-TdR) incorporation [20]. Potten [20] also demonstrated that the central basal cells could be induced to proliferate following wounding.

Potten and Hendry further reported that fewer than 10 percent of basal cells were capable of regeneration after severe radiation damage [23]. These observations suggested that the cells in the basal layer did not have a uniform proliferative potential, as was originally proposed by Weinstein and Frost [24], and led to the hypothesis of a two-compartment proliferative model for skin [20]. In this model, the proliferative compartment is postulated to contain both slow-cycling stem cells and transient amplifying cells that can undergo a finite number of divisions. This model, when combined with earlier findings that a significant proportion of basal keratinocytes are postmitotic maturing cells that remain in the basal layer for a period of time prior to migrating to the suprabasal layers [25–27], results in the concept of a mixed population of basal keratinocytes functioning as follows:

Stem Cells → transient amplifying cells → postmitotic cells

The major problem with this hypothesis has been the inability to correlate the kinetic data with the morphologic information. Recent studies using monkey palm epidermis as a model system, however, have provided evidence for the existence of these three cell types in the epidermis of the primates [28].

MONKEY PALM EPIDERMIS AS A MODEL SYSTEM

During a study of the tissue distribution of keratin antigens in various monkey tissues [29], we noted that monkey palm epidermis was unique in that there were two morphologically distinct, spatially segregated populations of basal keratinocytes. One population had features typical of stem cells, and the other appeared as typical basal keratinocytes presumably involved in anchoring the epidermis to the dermis [28].

Morphology

Cynomolgus monkey palm epidermis consists of alternating deep and shallow downgrowths (rete ridges). The shallow ridges interface with troughs formed between the apices of bifurcated dermal papillae (Fig. 1). The surface horny layer has alternating ridges and sulci, which correspond to the deep and shallow rete ridges, respectively. This alternating pattern of ridges and sulci is visible as dermatoglyphics (Fig. 1). Basal keratinocytes at the tips of the deep rete ridges are more heavily pigmented than those in the shallow ridges (Figs. 1,2), resulting in a dark and

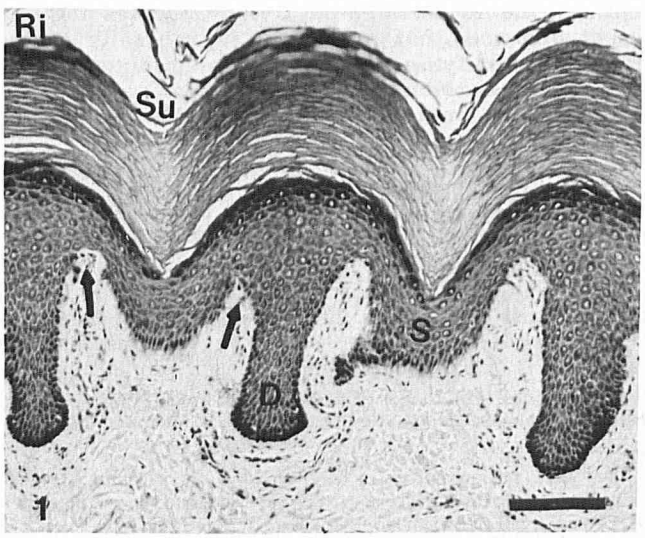

FIG 1. Paraffin section of cynomolgus monkey palm epidermis. Deep, pigmented rete ridges (*D*) alternate with shallow, less pigmented ones (*S*). Arrows indicate apices of the bifurcated dermal papillae. The horny layer has alternating ridges (*Ri*) and sulci (*Su*) (scale bar = 100 μm; ×125).

light pattern which is visible as dark ridges and light sulci. Aside from the marked differences in pigmentation, paraffin sections cut at conventional 5- to 7-μm thickenss fail to resolve the striking contrast in the organization of the dermal-epidermal junction that exists between deep and shallow ridges. However, 1-μm plastic sections clearly reveal the well-developed cytoplasmic projections extending into the dermis that characterize the basal keratinocytes in the shallow ridges (Fig. 2*B*). In contrast, basal keratinocytes at the tips of the deep ridges have a much smoother epidermal-dermal interface (Fig. 2*A*). Accordingly, we have termed the two cell types *serrated* and *nonserrated* basal keratinocytes [28].

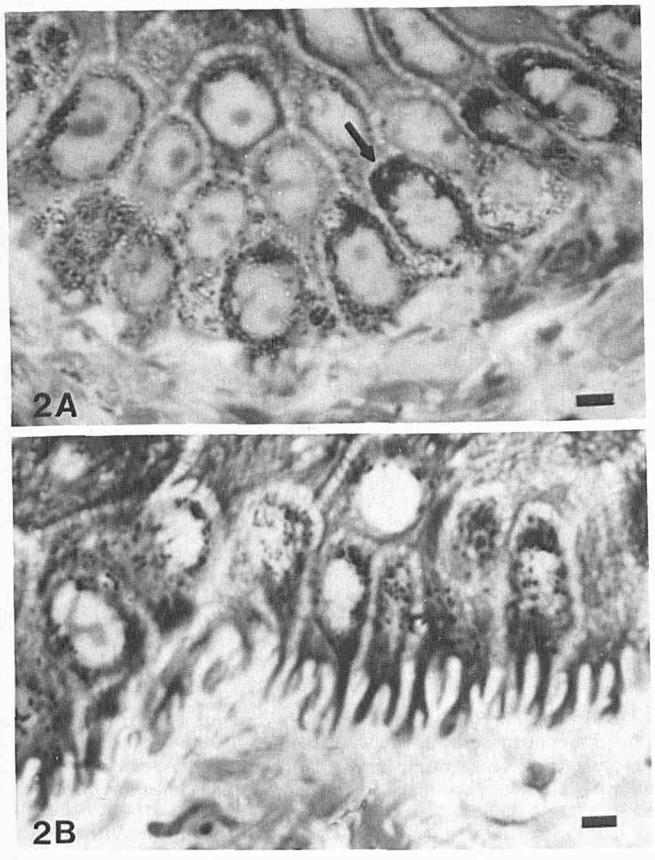

FIG 2. One-micron plastic sections showing (*A*) nonserrated basal keratinocytes of the deep rete ridges and (*B*) serrated keratinocytes of the shallow rete ridges. *A*, Nonserrated basal keratinocytes are cuboidal in shape with prominent melanosomes concentrated around the apical portion of the nucleus (*arrows*). *B*, Serrated basal keratinocytes are columnar in shape with distinctly less melanosomes (scale bar = 10 μm; ×450).

The architecture of the basal surface of these two cell types is dramatically different, as depicted in the scanning electron microscope. The nonserrated basal keratinocytes of the deep rete ridges possessed round to polygonal outlines (Fig. 3*A*); the undersurface of these cells (facing the dermis) consists of small projections of cytoplasm, resulting in a ruffled appearance. In contrast, the corresponding cell surface of the serrated cells showed a stalagmite-like arrangement of cytoplasmic protrusions extending deeply into the dermis (Fig. 3*B*). These projections are of such magnitude and are so numerous that the outline of individual cells is frequently obscured.

Aside from differences in undersurface configurations, nonserrated and serrated cells have other distinguishing morphologic features. Nonserrated cells are small, cuboidal, and have a large nuclear:cytoplasmic volume ratio (0.5–0.6) (Fig. 2*A*). Free ribosomes and melanosomes are the most conspicuous organelles in the nonserrated-cell cytoplasm (Fig. 4*A*). The melanosomes are frequently concentrated around the apical portion of the nucleus. Another hallmark of the nonserrated cell is the paucity of keratin filaments.

In contrast, serrated cells are relatively large, columnar in shape, and have a relatively small (0.3–0.4) nuclear:cytoplasmic ratio (Fig. 2*B*). These cells contain copious bundles of keratin filaments that extend all the way to the tips of the cytoplasmic projections (Figs. 4*B*,5) and have less melanin compared with the nonserrated cells.

To determine whether nonserrated and serrated cells were unique to monkey palm epidermis, we examined 1-μm plastic sections and thin sections (400 Å) of human epidermis from the palm, face, upper arm, forearm, back, abdomen, and lower leg. All the samples contained both nonserrated and serrated basal cells (Fig. 6). Serrated cells were seen along the thinner, more flattened portions of the epidermis, while nonserrated cells, many of them heavily pigmented, were confined to the tips of the rete ridges. Thus, although the morphologic heterogeneity among basal keratinocytes was more obvious in monkey and human palmar epidermis owing to spatial segregation, it can also be demonstrated in human epidermis from other anatomic locations.

These observations firmly establish the presence of two morphologically distinct types of basal keratinocytes: the nonserrated cell, having features characteristic of more "primitive" cells, and the serrated cells, which appears more differentiated and resembles the "classical" keratinocyte.

Cell Kinetics

[^{3}H]-TdR was injected subcutaneously into monkey palm and biopsies were taken from injection sites 30 minutes later. Autoradiographic data showed that the incorporation of the tracer in palm epidermis was nonrandom. Over 80 percent of the labeled nuclei were located in deep rete ridges; of these, 75 percent were present in the suprabasal cells. The suprabasal

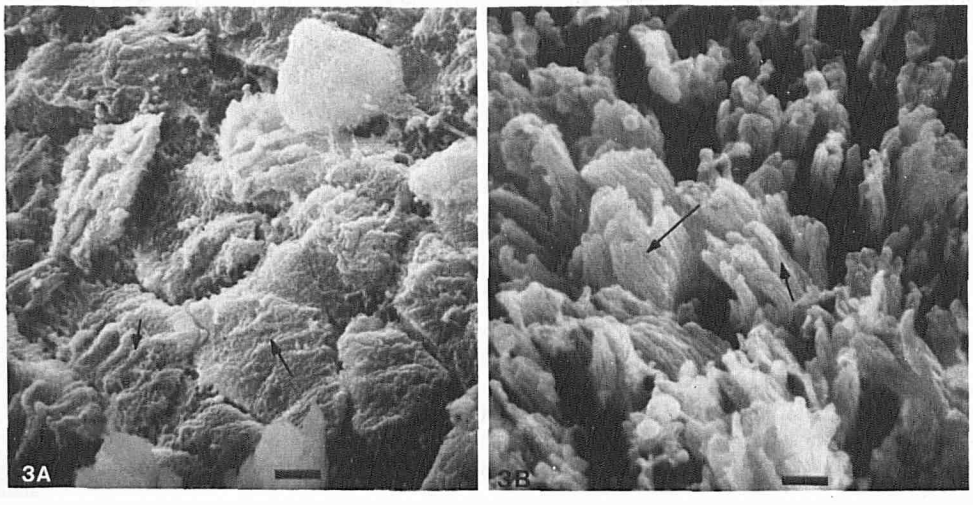

FIG 3. Scanning electron micrographs showing the undersurface of (*A*) the nonserrated basal keratinocytes of the deep rete ridges and (*B*) the serrated basal keratinocytes of the shallow rete ridges. *A*, Undersurface of round to polygonal nonserrated keratinocytes has a ruffled appearance (*arrows*). *B*, Undersurface of serrated cells consists of stalagmite-like arrangement of cytoplasmic protrusions (*arrows*) (scale bar = 2 μm; ×3000).

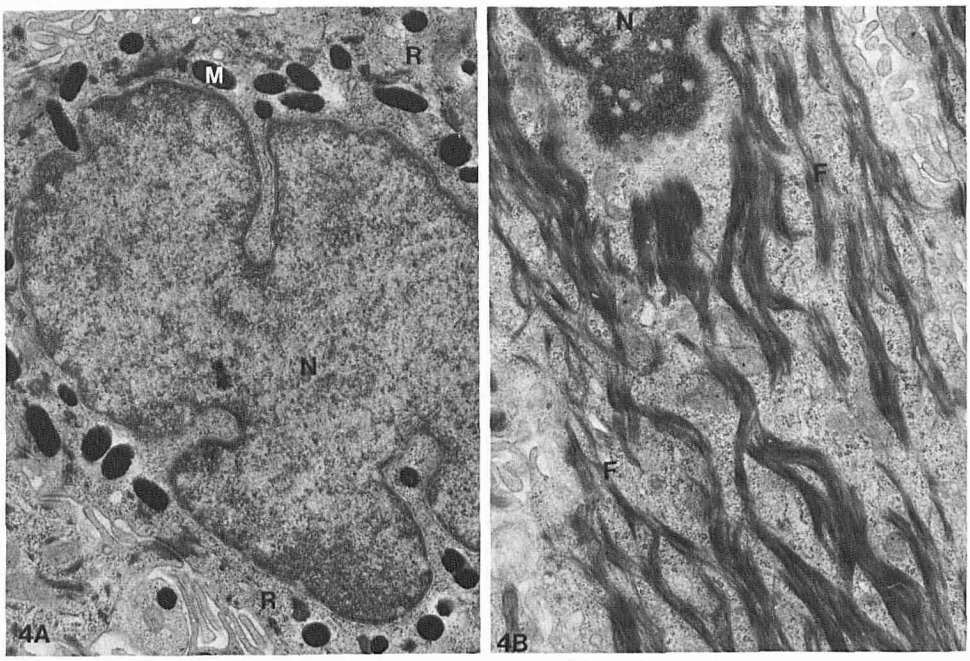

FIG 4. Transmission electron micrographs showing (*A*) nonserrated basal keratinocytes and (*B*) serrated basal keratinocytes. *A*, nonserrated cell cytoplasm is characterized by abundant amounts of ribosomes (*R*) and melanosomes. Note relative absence of keratin filaments. N = nucleus. *B*, Serrated cell cytoplasm is characterized by copious amounts of keratin filaments (*F*). N = nucleus (×13,500).

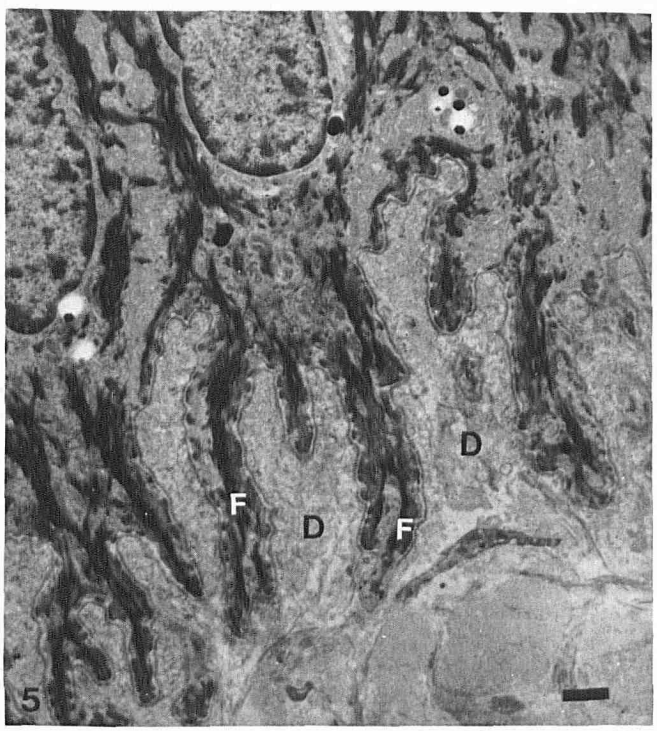

FIG 5. Transmission electron micrograph showing the serrated basal keratinocyte with typical cytoplasmic projections extending deeply into the papillary dermis (*D*). Bundles of keratin filaments (*F*) extend all the way to the tips of the cytoplasmic projections (scale bar = 1 μm; × 6,000).

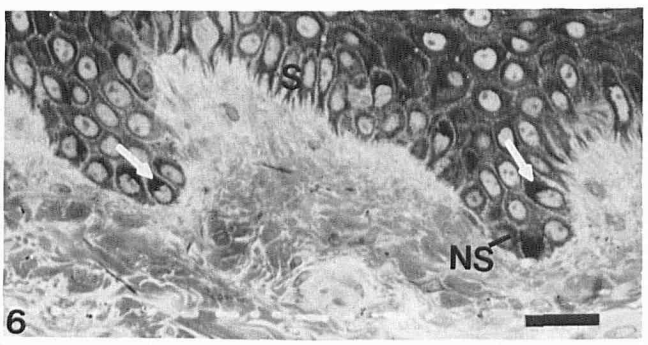

FIG 6. One-micron plastic section of human epidermis from the upper inner arm showing nonserrated (*NS*) and serrated (*S*) basal keratinocytes. Nonserrated cells at tips of epidermal downgrowths contain prominent perinuclear capping of melanosomes (*arrows*) (scale bar = 50 μm; ×200).

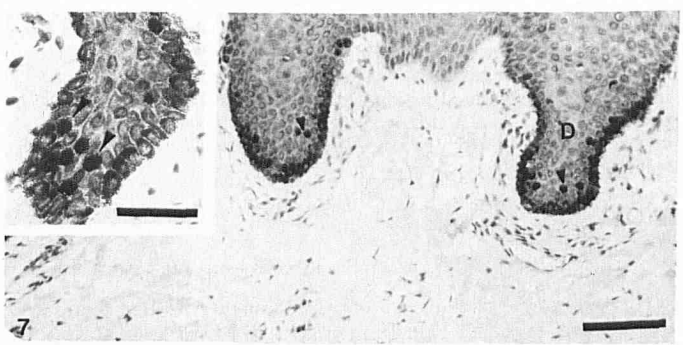

FIG 7. Autoradiograph of monkey palm skin 30 minutes after injection of [^{3}H]thymidine showing nonrandom incorporation of the tracer. Most labeled nuclei (*arrowheads*) were found in the suprabasal cells of the deep rete ridges (*D*) (scale bar = 50 μm). *Inset*, Higher magnification of a portion of a deep rete ridge showing suprabasal location of labeled nuclei (*arrowheads*) (scale bar = 25 μm).

location of these labeled cells was established by three-dimensional reconstructions from 6 to 10 serial sections, each 7 μm thick. Basal cells at the very tips of the deep rete ridges (nonserrated cells) were not labeled; the few labeled basal cells in deep rete ridges were present along the shoulders of the ridge (Fig. 7).

In the shallow ridges only a small percentage of the basal cells were labeled. These data are summarized in Table II. A similar preferential uptake of [^{3}H]-TdR by cells in the deeper rete ridges, along with a heavy suprabasal labeling pattern, was seen in in vitro [^{3}H]-TdR experiments on human palms.

Significance of Suprabasal Labeling

The occurrence of suprabasal labeling that we observed in the deep rete ridges of monkey palm epidermis (Table II) deserves some discussion, since it may seem contradictory to the early concepts that cell division was restricted to cells

TABLE II. *Differential labeling of monkey palm epidermis*

Overall	Type of rete ridge[a]		
	Shallow	Deep	
		Basal[b]	Suprabasal
9.6 ± 0.5[c]	2.1 ± 0.3	3.4 ± 0.7	9.7 ± 1.0

[a] Thirty-three percent of the basal cells were located in the shallow ridges and 66 percent were in the deep ridges.

[b] The labeled basal cells were predominantly located along ascending shoulders of rete ridges; the percentage of labeled nuclei of nonserrated basal cells at the tips of ridges was less than 0.5 percent.

[c] Each value (mean ± standard deviation) represents labeled nuclei per 100 basal cells expressed as a percentage.

resting on the basal layer. This idea originated from studies of [^{3}H]-TdR incorporation in rodents, which have a relatively thin epidermis. In these cases, only basal-cell nuclei are labeled 1 hour after injection of the tracers [30–32]. In one of the earliest investigations on human epidermal kinetics using autoradiographic techniques, Weinstein and Van Scott [33] reported that labeled epidermal cells were located only in the basal layer of the epidermis 1 hour after local injection of [^{3}H]-TdR. Epstein and Maibach [34] were the first to note a small but significant number of labeled cells in the layer immediately above the basal layer in human epidermis. Further evidence of suprabasal labeling was provided by Ashihara et al. [35] in their studies on cell proliferation in human breast skin epidermis. These investigators observed more labeled cells in the epibasal (suprabasal) layer than in the deepest basal layer and further commented that heavily pigmented basal cells rarely incorporated [^{3}H]-TdR. In the first attempt to quantify the degree of suprabasal labeling in human epidermis, Pennys et al. [36] reported that about 30 percent of labeled cells were in the suprabasal layers. In a more recent work, Briggamen [37] grafted normal human upper thigh skin to the backs of nude mice. Beginning 6 weeks later, [^{3}H]-TdR was injected every 6 hours for 16 days. Autoradiographs revealed preferential incorporation of [^{3}H]-TdR in the deeper rete ridges, with a high degree of suprabasal labeling.

While it is now generally accepted that in human epidermis there exists a cohort of suprabasal cells with proliferative capabilities, the finding of 75 percent suprabasal labeling in palmar epidermis may seem extraordinarily high. A careful survey of the literature showed, however, that the degree of suprabasal labeling in various human stratified squamous epithelia is directly proportional to their labeling index (Fig. 8). Thus the high percentage of suprabasal labeling that we observed in monkey palm epidermis reflects the fact that it is a highly proliferative tissue.

Epidermal Stem Cells and Transient Amplifying Cells: A Unifying Concept

A theoretical basis to explain all these data can be provided by the earlier proposed model:

Stem cells → transient amplifying cells → postmitotic cells

As previously discussed, this concept was established mainly on the basis of data obtained from studies of blood cells. While the existence of these three cell types in the basal layer of the epidermis was suggested by Potten and other workers [20,23], there has been, until now, no supporting data in primate epidermis. In our present study, we found that in deep rete ridges, the nonserrated basal cells rarely incorporated [^{3}H]-TdR, while adjacent suprabasal cells demonstrated a high degree of [^{3}H]-TdR uptake. These findings suggest that the nonserrated cells are slowly cycling stem cells, whereas the [^{3}H]-TdR-incorporating suprabasal cells adjacent to the nonserrated cells most likely represent the transient amplifying cells. The more superficially located nonlabeled cells are considered postmitotic cells. Such a scheme is illustrated in Fig. 9. It should be emphasized, however, that to substantiate these conclusions, one needs to

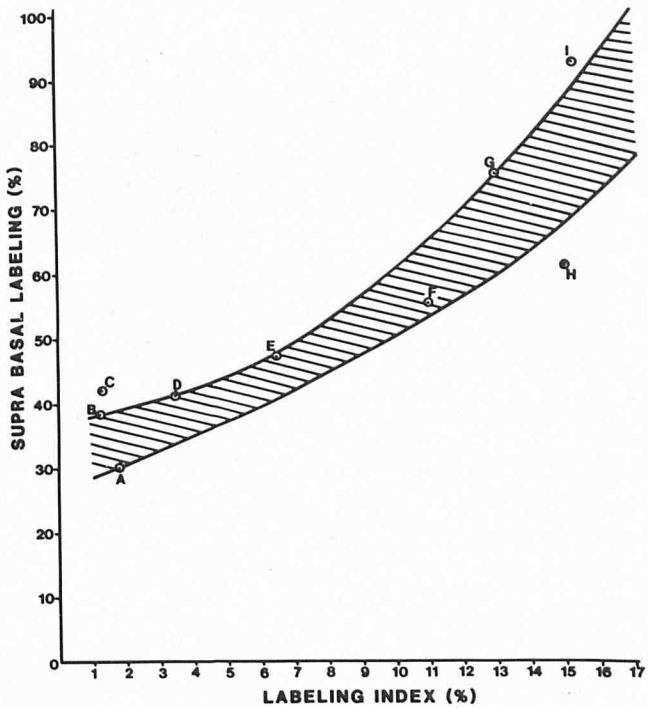

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FIG 8. Correlation between suprabasal labeling and the mitotic activity of the epidermis. *A* = normal human epidermis [33,36,45], *B* = X-linked ichthyosis [36], *C* = ichthyosis vulgaris [36], *D* = lamellar ichthyosis [36], *E* = epidermolytic hyperkeratosis [36], *F* = psoriasis [36,46–48], *G* = monkey palm epidermis [28], *H* = human palm epidermis [49], *I* = vaginal epithelium [50]. Where several values were available, an average was calculated.

know more about the mitotic properties of these cells. This is so because recent work has demonstrated that [^{3}H]-TdR incorporation data should be treated with caution owing to the potential problems of thymidine pool and various thymidine metabolic pathways [38,39]. Nevertheless, it is interesting to note that prior to the advent of autoradiographic techniques using tritiated thymidine as a marker for DNA synthesis, early investigators often observed numerous mitotic figures in the suprabasal layers [40–43]. In fact, Thuringer [40,41] reported that over 90 percent of the mitotic figures in normal, non-drug-treated human scalp and prepuce epidermis were located suprabasally.

Further support for the concept that the nonserrated cells are slowly cycling and thus relatively permanent can be obtained from an analysis of the distribution of melanin and melanocytes. We found that the distribution of melanocytes was uniform throughout the entire monkey palm epidermis (data not shown). However, the distribution of melanin among basal keratinocytes was clearly nonrandom (heavily melanized nonserrated cells versus lightly melanized serrated cells). This would suggest that the nonserrated cells became heavily melanized as a result of long contact with melanocytes, whereas the serrated cells contained less melanin owing to fewer infusions from melanocytes because, presumably, they moved off the basal layer (i.e., turn over) quicker.

The correlation between labeling index and suprabasal labeling (Fig. 8) can also be explained using the model shown in Fig. 9. If we assume transient amplifying cells can undergo 2 to 4 divisions, as proposed by Potten [44], then under normal conditions, each stem-cell division would result in 4 to 16 terminally differentiated cells. In "normal" epidermis, which has a relatively low labeling index, the basal region of the epidermis would be relatively "uncrowded," and thus most of the [^{3}H]-TdR-uptaking transient amplifying cells would find room in the

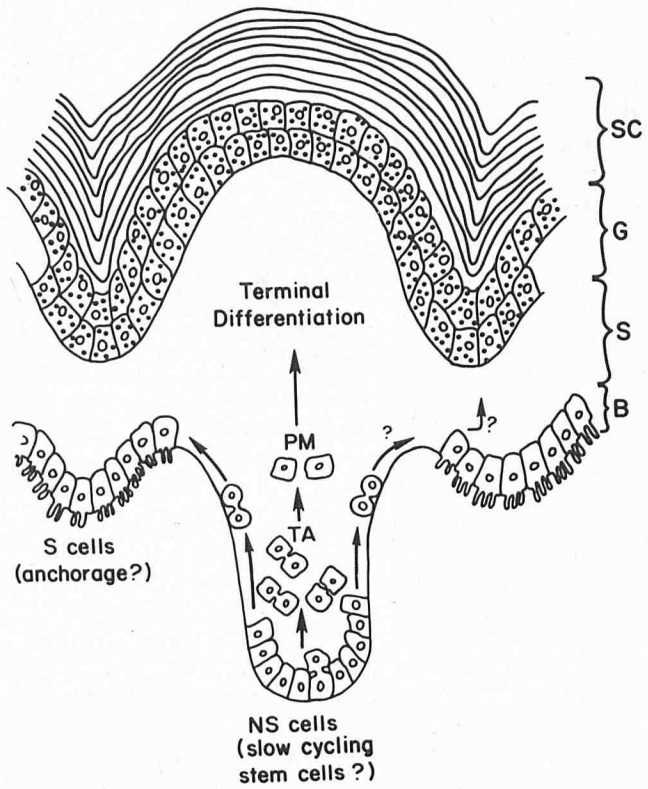

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FIG 9. Schematic representation of heterogeneity in basal keratinocytes. In this scheme, nonserrated (*NS*) cells at the tips of the deep rete ridges are believed to be slowly cycling stem cells. These cells give rise to suprabasally located transient amplifying (*TA*) cells which actively incorporate [^{3}H]thymidine. The TA cells give rise to the more superficially located, nonlabeled postmitotic (*PM*) cells. The serrated (*S*) cells located in the shallow rete ridges are believed to have an anchoring function. *B* = basal; *S* = spinous; *G* = granular; *SC* = stratum corneum.

basal layer, with relatively few (up to 30 percent) located in a suprabasal position. If, however, there were numerous cell divisions, as in the case of hyperproliferative conditions, then the basal layer would become "crowded," and more transient amplifying cells would be pushed up into a suprabasal position, thus resulting in a higher percentage of suprabasal labeling (Fig. 8).

CONCLUSIONS

In this paper we have reviewed evidence that the nonserrated basal keratinocytes may represent epidermal stem cells. These cells have the following properties: they have a high nuclear:cytoplasmic ratio; they are characterized by a relatively undifferentiated cytoplasm; they appear to be slow-cycling, but they give rise to a highly proliferative population of (transient amplifying) cells; they are located at the tips of the deepest rete ridges and are heavily melanized; and thus they are maximally protected from environmental insults, including ultraviolet radiation.

We have also presented evidence supporting the possibility of stem cell → transient amplifying cell → postmitotic terminally differentiated cells. Such a scheme provides a unifying concept that helps to explain several well-established, but so far poorly understood observations, including the structural and functional heterogeneity of basal keratinocytes and the occurrence of suprabasally located replicating cells.

It is generally believed that stem cells play a crucial role in tumorigenesis. However, although a great majority of human neoplasms are epithelial-derived, relatively little is known about epithelial stem cells. Monkey palm epidermis provides a unique model system for further studying epithelial stem cells, since (1) the epidermis is readily accessible to experimental manipulation; (2) in histologic sections, all sequential stages of cell maturation are well preserved; (3) aside from monkey palm, no other experimental animal has demonstrated in their epidermis the existence of two morphologically distinct, spatially segregated populations of basal keratinocytes; and (4) the cynomolgus monkey palm has a pigmentation pattern that corresponds precisely to the location of the nonserrated (presumptive) stem cells, thus providing a convenient morphologic marker for areas rich in this cell type.

Further characterization of various cellular compartments of the monkey palm epidermis should lead to a better understanding of the role of stem cells in epithelial growth control, differentiation, and carcinogenesis.

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Selective Enrichment of Human Epidermal Cell Subpopulations Using Monoclonal Antibodies

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In studying the mechanisms that regulate the growth and differentiation of the human epidermis, it would be helpful to obtain relatively pure populations of the different epidermal cell types. We have used a solid-phase immunoabsorption method termed "*panning*" to positively select two types of epidermal cells: Langerhans cells and the keratinocytes found in the basal cell layer (basal cells). To attach basal cells to a goat anti-mouse IgG-coated plastic surface, we used murine monoclonal antibodies (VM-1 or VM-2), which were recently produced in our laboratory and bind specifically to antigens on human basal cells. Using antibodies VM-1 or VM-2, we panned for basal cells and obtained a yield of about 40 percent (an enrichment of about 2.5-fold). The cells enriched for basal cells demonstrated much better growth and DNA synthesis than did the cell fraction depleted of basal cells.

For positive selection of Langerhans cells, we used OKT6, a murine monoclonal antibody that binds specifically to Langerhans cells in the epidermis. We determined that of those cells preincubated with OKT6 and adherent to an antibody-coated petri dish surface, about 70 percent demonstrated OKT6 binding by fluorescence microscopy. This represents a 15- to 20-fold enrichment for Langerhans cells. The nonadherent cell fraction contained less than 1 percent OKT6-positive cells. Ultrastructural studies showed that the cells thus separated were Langerhans cells. The OKT6-positive but not the OKT6-negative cells were capable of stimulating allogeneic lymphocytes in the skin-cell-lymphocyte reaction. Thus the panning technique is an effective method for obtaining greatly enriched subpopulations of viable epidermal cells.

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Abbreviations:
EDTA: ethylenediaminetetraacetic acid
FACS: fluorescence-activated cell sorter
FCS: fetal calf serum
R/M-FITC: fluorescein-isothiocyanate conjugated rabbit anti-mouse Ig
HLA-Dr antigen: human equivalent of murine Ia or immune-response-associated antigen
LC: Langerhans cells
MLR: mixed leukocyte reaction
PBML: peripheral blood mononuclear leukocytes
PBS: phosphate-buffered saline
SLR: skin cell lymphocyte reaction
^{3}H-T: tritiated thymidine
T cells: thymus-derived lymphocytes

The human epidermis consists of a heterogeneous population of cells, including keratinocytes in various stages of differentiation, Langerhans cells (LC), melanocytes, and Merkel cells. Langerhans cells, which comprise only 2 to 6 percent of the