what we believe to be the first example of loss of a transmitter-specific cell population in a major disorder of higher cortical function and, as such, represents a significant step in the understanding of the pathophysiology of this neurological disorder.

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Heterogeneity in Epidermal Basal Keratinocytes: Morphological and Functional Correlations

Abstract. *Two structurally distinct populations of basal keratinocytes, nonserrated and serrated, were observed in cynomolgus monkey and human palm epidermis. Anatomical location, fine structural features, and kinetic properties suggest that nonserrated cells represent a stem cell population and that serrated cells help anchor the epidermis to the dermis.*

Although relatively little is known about epidermal stem cells, studies of other renewing cell populations, such as blood cells and seminiferous and intestinal epithelia, suggest that stem cells possess the following properties: (i) ultrastructurally, their cytoplasm is primitive and contains few, if any, differentiation products; (ii) they have very low mitotic activity; (iii) they give rise to transient amplifying cells that undergo several rounds of division before terminal differentiation; and (iv) they can be induced to proliferate by tissue demand or specific stimulation (*1*).

While studying epidermal differentiation in cynomolgus monkeys and humans, we noted that palmar epidermis has two morphologically distinct, spatially segregated populations of basal keratinocytes. We report here that one population has features typical of stem cells and that the other may anchor the epidermis to the dermis. The two populations are present in epidermis from different regions of the body and can easily be identified with the light microscope.

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 hyperkeratotic layer reflects the alternating pattern of ridges and sulci. This is visible as dermatoglyphics. Basal cells in the tips of the deep rete ridges are more heavily pigmented than those in the shallow ridges, resulting in a dark and light pattern in the epidermis. These regional differences have been noted in the palms and soles of other primates (*2*); however, it has not been appreciated that the architecture of the dermal-epidermal junction is very different in the deep and shallow ridges. In 1-μm plastic sections (*3*), basal keratinocytes at the tips of deep ridges are flattened and slightly convoluted at the dermal-epidermal junction (Fig. 2A). In contrast, keratinocytes in shallow ridges display well-developed cellular projections extending deep into the papillary dermis, resulting in a highly convoluted dermal-epidermal junction (Fig. 2B). Accordingly, we call the two cell types nonserrated and serrated basal keratinocytes.

Nonserrated cells are small and cuboidal and have a large ratio of nuclear to cytoplasmic matter (Fig. 2A). The cell periphery has numerous microvilli, which occasionally are punctuated by desmosomes, and the cytoplasm is filled with free ribosomes, mitochondria, and melanosomes (Fig. 3A). Frequently the melanosomes are concentrated around the apical end of the nucleus. In addition, the cells show a paucity of keratin filaments and a diffuse distribution of nuclear chromatin. These characteristics suggest a relatively primitive cell (*4*). In contrast, serrated basal cells contain copious bundles of keratin filaments extending all the way to the tips of the

0036-8075/82/0305-1239$01.00/0

cytoplasmic projections and have a relatively straight periphery. These features are characteristic of basal keratinocytes (Fig. 3B) (*5*).

To determine whether these morphologically distinct cells proliferate at different rates, we injected monkey palm epidermis with [^{3}H]thymidine in vivo and 30 minutes later made autoradiographs of samples removed by biopsy. As shown in Fig. 4, incorporation of the tracer was nonrandom. (The labeling indices of the various cell compartments are presented in Table 1.) Over 80 percent of the labeled nuclei were in deep rete ridges; of these, 75 percent were present in the suprabasal cells (*6*). Most labeled basal cells in deep rete ridges were present along the ridge shoulders. Basal cells at the tips were not labeled. In shallow rete ridges, few of the serrated basal cells were labeled. This labeling pattern indicates that there are three distinct cell populations in deep rete ridges: (i) a layer of nonserrated basal cells with low mitotic activity, (ii) a highly proliferative suprabasal compartment (*7*), and (iii) a postmitotic, differentiating population of more superficially located cells.

Table 1. Differential labeling of monkey palm epidermis. Tritiated thymidine (10 μCi; specific activity, 80 Ci/mmole) in 0.1 ml of sterile saline was administered intradermally into each of four monkey palms. Samples were taken 30 minutes later and processed for paraffin section autoradiography (*9*). Each value (mean ± standard deviation) represents labeled nuclei per 100 basal cells, expressed as a percentage.

Overall	Type of rete ridge*		
	Shallow	Deep	
		Basal†	Suprabasal
9.6 ± 0.5	2.1 ± 0.3	3.4 ± 0.7	9.7 ± 1.0

*Thirty-three percent of the basal cells were located in the shallow ridges and 66 percent were in the deep ridges. †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 < 0.5 percent.

The nonserrated basal cells resemble stem cells in several ways. Their ultrastructural characteristics are primitive in nature, they have slow cycling kinetics but can be induced to undergo more rapid division during healing after linear incision (*8*), and normally they are close to the rapidly proliferating suprabasal cells. Finally, they are located at the tips of the deepest rete ridges and are heavily melanized, providing maximum protection from ultraviolet radiation.

The ultrastructural morphology and proliferative capacity of serrated cells closely resemble those of "classical" basal keratinocytes (*5*). The serrations of such basal cells increase the surface area of the dermal-epidermal junction. These cells, usually found along the thinnest portion of the epidermis, also enhance resistance to shearing forces and hence aid in anchoring the epidermis to the dermis. Serrated cells have been noted in 1-μm plastic sections of human epidermis and by electron microscopy (*5*); however, to our knowledge their anatomical significance has never been recognized.

Human palmar epidermis is very similar to that of the monkey palm. As in monkeys, human palmar epidermis shows a preferential uptake of [^{3}H]thymidine by cells in the deeper rete ridges, with suprabasal cells being labeled most heavily (*9*). To determine whether nonserrated and serrated cells are unique to the palms in humans, we examined 1-μm plastic sections of human epidermis from the face, upper arm, forearm, back, abdomen, and lower leg. All the samples contained both nonserrated and serrated basal cells (Fig. 5). 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 rete ridges. Thus, the morphological heterogeneity of basal keratinocytes, although more obvious in palmar epidermis due to spatial segregation, can also be demonstrated elsewhere in the epidermis (*10*). In addition, there is no apparent relation

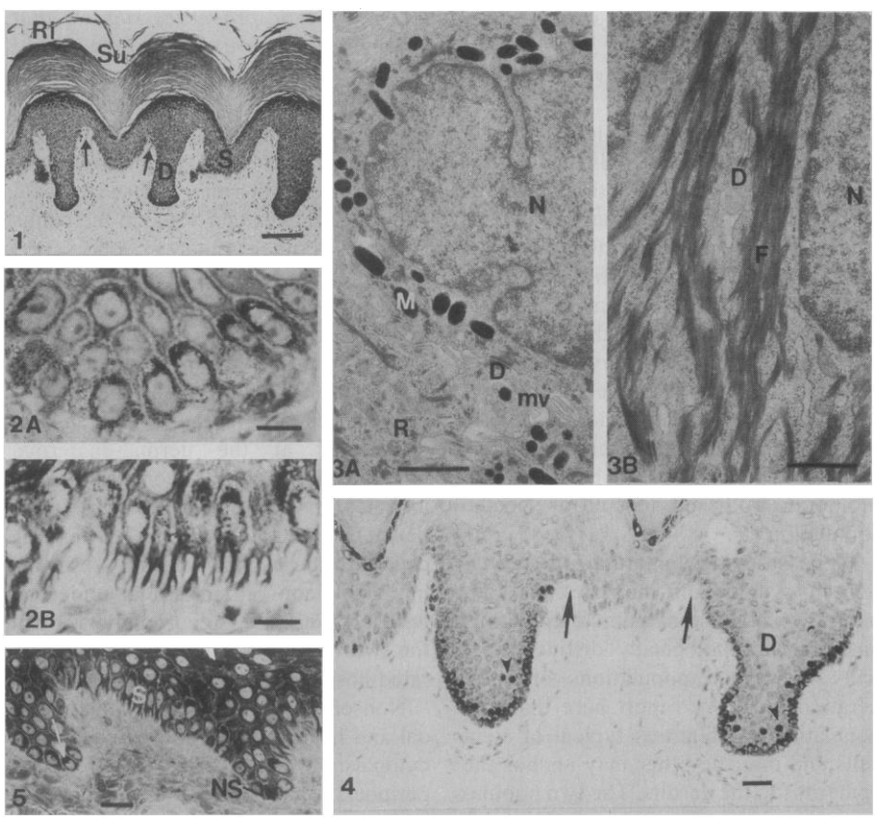

Fig. 1. Paraffin section of cynomolgus monkey palm epidermis. Deep, pigmented rete ridges (*D*) alternate with shallow, less pigmented ones (*S*). The arrows indicate apices of the bifurcated dermal papillae. The horny layer has alternating ridges (*Ri*) and sulci (*Su*). Scale bar, 100 μm. Fig. 2. Plastic sections showing (A) nonserrated basal keratinocytes of the deep rete ridges and (B) serrated keratinocytes of the shallow rete ridges. Scale bar, 10 μm. Fig. 3. Transmission electron micrographs showing (A) nonserrated basal cells and (B) serrated basal cells. The nonserrated cells contain numerous melanosomes (*M*), ribosomes (*R*), and microvilli (*mv*) on the cell periphery, whereas the serrated cells contain abundant amounts of keratin filaments (*F*) and a relatively straight cell periphery (*D*, desmosomes; *N*, nucleus). Scale bar, 1 μm. Fig. 4. Autoradiograph of monkey palm skin exposed for 30 minutes to [^{3}H]thymidine, labeled nuclei (arrowheads) in the suprabasal cells of the deep rete ridges (*D*). Arrows point to apices of the bifurcated dermal papillae. Scale bar, 20 μm. Fig. 5. Plastic section of human epidermis from the upper inner arm, showing nonserrated (*NS*) and serrated (*S*) basal cells. Note the perinuclear capping of melanosomes (arrow) in nonserrated cells at the tips of epidermal downgrowths. Scale bar, 20 μm.

between the two cell types and the intensity with which their cytoplasm is stained (*11*).

In some regions of the epidermis, cells of the stratum corneum are organized into discrete columns (*12*). These columns extend downward through the granular and spinous cell layers. A cluster of 10 to 12 basal keratinocytes is positioned beneath each column. This arrangement has been termed an epidermal proliferative unit (*13*). It has been proposed that the basal cells in the center of each column are stem cells. However, radiobiological and other data (*14*) indicate that stem cells are more likely to reside in the tips of deep rete ridges or follicles than in the epidermal proliferative unit. Our findings strongly support this prediction.

The structural and kinetic observations presented here demonstrate the existence in primate epidermis of a discrete population of basal cells whose properties satisfy the general criteria established for stem cells. These presumptive stem cells have a specific distribution in the epidermis and are easily identified morphologically. The epidermis, in which sequential stages of cell maturation are well preserved in histological sections, is readily accessible to experimental manipulation, and thus provides an excellent model for studies on the biological properties of stem cells.

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Ultrastructure of 40-Million-Year-Old Insect Tissue

Abstract. *Examination of the ultrastructure of preserved tissue in the abdomen of a fossil fly (Mycetophilidae: Diptera) entombed in Baltic amber revealed recognizable cell organelles. Structures that corresponded to muscle fibers, nuclei, ribosomes, lipid droplets, endoplasmic reticulum, and mitochondria were identified with the transmission electron microscope. Preservation was attributed to inert dehydration as well as the presence of compounds in the original sap which functioned as natural fixatives. This evidence of cell organelles in fossilized soft tissues represent an extreme form of mummification since Baltic amber is considered to have formed about 40 million years ago.*

Well-preserved fossilized soft tissues of animals are rare and have been examined with the light (*1*, *2*) or scanning electron microscope (*3*, *4*). Cell ultrastructure has apparently not been studied in soft tissues more than 1 million years old.

During routine examination of insect fossils in Baltic amber, a well-preserved female fly (Mycetophilidae: Diptera), which appeared to have internal soft tissues, was observed. Baltic amber is considered to have originated in the late Oligocene to early Eocene, or about 40 million years ago (*5*).

The piece of yellow transparent Baltic amber was broken through the abdomen of the fly. Some of the body cavity was filled with the original plant sap, which had since become amber. The rest of the body cavity was filled with Araldite 6005 and polymerized for 8 hours at 60°C. The blocks were trimmed and sectioned with glass knives (Porter-Blum MT2 ultramicrotome). Sections were treated with saturated aqueous uranyl acetate for 20 minutes and then with lead citrate for 5 minutes. The sections, mounted on slot grids, were observed in a Philips EM 300 electron microscope.

The amber specimen containing the fly was confirmed as being Baltic amber by infrared spectroscopy (*6*). Beck *et al.* (*7*) have shown that Baltic amber is distinguishable from related ambers by an absorption band of medium intensity at 1160 to 1150 cm^{-1} (8.6 to 8.7 μm) which is preceded by a more or less flat shoulder nearly 0.5 μm wide. From a 1.5-mg sample of the amber containing the fly specimen, a spectrum was obtained which matched that of well-preserved Baltic amber (*6*).

Observations of the ultrastructure showed a strip of tissue adjacent to the cuticle and approximately 12 μm wide in the abdomen of the fly; it showed a high degree of preservation. From the location, we concluded that this tissue represented epidermis and associated muscle. The degree of preservation varied considerably and a few areas contained cellular remains with morphologically identifiable organelles. Nuclei were identified on the basis of their size, location within the cell, structure, and clearly defined boundaries (Fig. 1A). The nuclei were generally flattened and elongate, with a slightly undulate border as is normally observed in insect epidermal

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