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# Hairless Micropig Skin

## A Novel Model for Studies of Cutaneous Biology

**Robert M. Lavker, Gang Dong, Peishu Zheng, and George F. Murphy**

*From Dubring Laboratories, Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania*

***Reported here is the structural and immunohistochemical similarities between the Yucatan hairless micropig (HMP) skin and that of humans. Hairless micropig skin surface was composed of complex intersecting furrows that created geometric patterns remarkably similar to human skin surface glyphics. The dermal–epidermal interface consisted of undulant downgrowths that interdigitated with dermal papillae. Hairless micropig epidermis contained two morphologically distinct populations of basal keratinocytes (serrated and nonserrated). Similar heterogeneity has been seen only in human epidermis and primate palmar epidermis. Immunohistochemistry revealed that the HMP epidermis is reactive with monoclonal and polyclonal antisera to keratin proteins. Melanocytes reactive with antisera to S-100 protein, as in human skin, also were observed in HMP epidermis. Organization of dermal extracellular matrix, including collagen and elastic fibers, and the organization and reactivity of the microvasculature with antisera to factor VIII, were consistent with human skin. The costicosteroid-induced atrophy and subsequent rebound phenomenon after withdrawal of steroid observed in HMP skin was similar with that observed in humans. It is concluded that HMP skin approximates human skin significantly more precisely than most existing species and is an excellent model for studies of cutaneous physiology and pharmacology. (Am J Pathol 1991, 138:687–697)***

The integument of Homo sapiens is unique among higher primates. Skin surface markings,[1] an interdigitating, undulating dermal–epidermal relationship,[2] a dermis with distinct papillary and reticular zones,[3] and a microvasculature with superficial plexi[4,5] are but a few anatomic features that are distinctive in human skin. Furthermore there are innumerable biochemical and immunologic properties peculiar to human skin. Due to ethical limitations, human skin is difficult to use *in vivo* for experimental purposes. To circumvent this problem, cutaneous biologists have used a myriad of animal models[6] and/or *in vitro* systems[7] for skin experimentation. Glabrous skin of common laboratory animals such as mice, rats, guinea pigs, rabbits, dogs, as well as nonhuman primates demonstrates distinctive morphologic differences from human skin. Aside from palmar and plantar regions, the epidermis is markedly thinner, lacks basal cell heterogeneity and has a relatively flat dermal–epidermal interface devoid of rete ridges.[8] Dissimilarities are not only confined to the epidermis; these species also have a loosely organized dermal fabric,[9] a rudimentary vascular organization,[10] and dermal cells that often are phenotypically different from those of humans (ie, mast cells).[11] Consequently skin reactivity to a variety of substances (ie, phenol, calcium ionophores, retinoids) often is markedly altered when compared to humans.[12–14] A ubiquitous fur coat, acting in a protective manner, has been postulated as one of the reasons for the rudimentary nature of animal skin.[15] However hairless species (eg, mice, guinea pigs) also display a cutaneous visage that is markedly distinct from humans.[16–18]

*In vitro* systems for culture of human skin (eg, organ culture, cell culture, skin equivalent), while useful for studying certain aspects of skin biology, generally lack the ability to faithfully reproduce and/or maintain the *in vivo* situation.[7,19,20] Furthermore cells in culture often show disparate responses to exogenous compounds (growth factors, retinoids, corticosteroids) compared to *in vivo* situations.[21–23]

In the past several years, our laboratories have been involved in identifying animal models that better approximate the structural characteristics of human skin. In studies of simian models, we found many immunologic and kinetic analogies to human skin.[24–26] However only palm and sole skin was structurally analogous to that of

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Address reprint requests to Robert M. Lavker, PhD, Department of Dermatology, University of Pennsylvania, School of Medicine, Clinical Research Building, 422 Curie Blvd., Philadelphia, PA 19104.

humans.[25,26] In addition, size and inherent difficulties in handling limit routine use of nonhuman primates in many laboratory settings. Recently we investigated the Yucatan hairless micropig (HMP) as an animal model for use in cutaneous biology.[27] We report here that HMP skin shows important similarities in morphology, cellular composition, and immunoreactivity to human skin that are not present in rodents. Furthermore HMP skin appears to respond pharmacologically in a manner similar to human skin.

## Materials and Methods

### Animals

Female Yucatan HMPs (Charles River Laboratories, Boston, MA) aged 30 to 35 days and each weighing 5 to 8 kg were used throughout the study. After 4 or 5 months, these animals weighed 12 to 15 kg, were extremely docile, and easy to handle. The Yacatan HMP originally was developed at Colorado State University where workers using Yucatan hairless miniature pigs selectively bred the animals for smaller body size.

### Light and Transmission Electron Microscopy

Before biopsy animals were anesthetized with a combination of ketamine-HCl and innovar-vet. Three-millimeter punch biopsies were performed on the flanks of the HMPs. Each biopsy specimen was bisected. For light microscopy examination, tissues were minced and fixed in 10% buffered formalin, dehydrated, and embedded in either JB4 water-soluble embedding material (Polysciences, Fort Washington, PA) or paraffin. Two-micron–thick JB-4 plastic sections were cut with glass knives on a Reichert-Jung Supercut 2050 microtome (Cambridge Instruments Co., Buffalo, NY) and 5-μ–thick paraffin sections were cut on a conventional microtome.

One half of the biopsy was processed for transmission electron microscopy, as previously described.[28] Briefly, they were immediately fixed by immersion in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/l (molar) cacodylate-HCl, buffered (pH 7.4) for 2 hours at 24° C. Postfixation was for 1 hour in 1% osmium tetroxide. After dehydration through a graded series of alcohols, specimens were embedded in Epon 812 (Marivac, Halifax, Nova Scotia, Canada). Thin sections were cut with a diamond knife on a Porter-Blum MT-2B microtome (Sorvall Inc., Newtown, CT), stained with saturated uranyl acetate and lead citrate, and examined in a Hitachi HU-12A electron microscope (Tokyo, Japan).

One-micron–thick sections for light microscopy were prepared from the same Epon-embedded specimens. These were stained with toluidine blue and counterstained with basic fuchsin.

### Histochemical/Immunohistochemical Localization

Paraffin sections of HMP skin were stained with hematoxylin and eosin, Mowry's colloidal iron for glycosaminoglycans,[29] and Luna's stain for elastin.[30] In addition, paraffin sections were stained with polyclonal antibodies to epidermal keratin proteins, melanocytic and neural S-100 protein,[31] dermal endothelial factor VIII,[32] and mast cell chymotrypsin.[33] Frozen sections of HMP skin were stained for laminin, fibronectin, involucrin, filaggrin, and the Leu series (1, 2a, 3a, 4, 6)[34–37] (Table 1). JB-4 plastic sections were stained with toluidine blue and hematoxylin and esoin.

**Table 1.** *Antibodies Used in Immunohistochemistry Methodology and Rationale*

| Antibody | Source | Method | Rationale for use |
|---|---|---|---|
| Anti-keratin | Dako Corp. | PAP | Stains the intermediate filament of epidermal keratinocytes |
| Anti–S-100 protein | Dako Corp. | PAP | Stains melanocytes and neural cells |
| Anti-factor VIII-related antigen | Dako Corp. | PAP | Stains endothelial cells |
| Anti-chymotrypsin | Courtesy of Dr. Norman Schechter | PAP | Stains mast cells |
| Anti-laminin | Collaborative Research Inc. | Direct immunofluorescence | Stains basement membranes around dermal vasculature and dermal–epidermal junction |
| Anti-fibronectin | Dako Corp. | PAP | Same as above |
| Anti-involucrin | Biomedical Technology | PAP | Stains the protein envelope of the cells in the upper-third epidermis |
| Anti-filaggrin | Biomedical Technology | PAP | Stains the interfilamentous matrix protein of epidermal keratin |
| Anti-*leu* series | Becton Dickinson | PAP | Stains lymphocytes |

PAP, peroxidase-anti-peroxidase method.

### Steroid Atrophy-rebound Effect (SARE)

Six-week topical application of corticosteroids, under occlusion, has been shown to have profound effects on human skin.[28] By 3 weeks, the epidermis becomes thin and the normally cerebriform dermal–epidermal interface is flattened. Papillary dermal collagen and elastic fibers become compacted, which was associated with reduction of ground substance. These alterations became progressively more pronounced by 6 weeks.[28] Subsequent to discontinuation of steroid, the atrophy reverts. Epidermal hyperplasia typifies this recovery or 'rebound' phase. Epidermal and dermal restitution is rapid, with return of glycosaminoglycans within the dermal matrix and phenotypic features similar to normal, pretreatment skin, by 14 days after withdrawal.[38] Because corticosteroid treatment followed by removal evokes such varied cutaneous responses, this regimen was performed in HMP skin and morphologic assessments were made with the light microscope to determine how closely the responses of the HMP mimic the human.

Betamethasone dipropionate (Westwood Pharmaceuticals Inc., Buffalo, NY) cream (0.05%) was applied 3 times weekly (on Monday, Wednesday, and Friday) continuously for 6 weeks to 10 areas along the flanks of the HMPs. The cream (0.2 ml) was placed in 15-mm Duhring chambers, fastened to the skin with Elastikon porous tape (Johnson and Johnson, New Brunswick, NJ) to maintain an occlusive environment. Untreated skin and skin treated with the vehicle served as controls. Three-millimeter punch biopsies were taken at weekly intervals during treatment and at 0, 2, 4, 7, and 14 days after cessation of treatment. Sites treated with vehicle were biopsied after 6 weeks of occlusive treatment. Biopsies were processed for light microscopy and stained with hematoxylin and esoin as described above.

### Histometric Analysis

For estimation of epidermal thickness, care was taken to cut the sections perpendicularly to the surface. Histometric measurements of the viable epidermis were made on hematoxylin and eosin-stained sections, as previously described.[39] A Southern Micro Image Analysis system (Southern Micro Instruments, Inc., Atlanta, GA) was used for all histometric measurements. Histometric measurements also were made of ground substance on Mowry's colloidal iron-stained sections.

## Results

### Clinical Features

Hairless micropig skin is hairless, with the exception of sparse pilation around the back of the neck. Variation in degree of epidermal pigmentation ranged from pink to gray. Hairless micropig skin surface resembles xerosis vulgaris in humans with variably sized, focally ichthyosiform scales.[40] These scales often demonstrate a reticulate pattern, accentuated by skin surface markings (Figure 1a). These skin surface markings or glyphics are similar to those seen in humans[1] and consist of complex intersecting furrows that create a variety of geometric patterns. The most predominant pattern consisted of a latticework of squares and rectangles formed by the intersection of deep furrows.

### Morphology

#### *Light Microscopy*

Hairless micropig epidermis is organized in a series of alternating ridgelike downgrowths that interdigitate with dermal papillae, resulting in an undulating pattern (Figure 1b). Over the apices of dermal papillae, viable epidermis consists of 5 to 8 nucleated cell layers, whereas the epidermal down growths are considerably thicker (Figure 1b and c). Two morphologically distinct populations of basal keratinocytes are present, those located at the tips of the epidermal downgrowths displaying a smooth ('nonserrated') dermal–epidermal interface, and those located above the apices of dermal papillae (Figure 1c) showing a convoluted ('serrated') surface. This configuration of basal keratinocyte heterogeneity is identical to that seen in human epidermis from a variety of anatomic locations.[25,26]

A prominent 2- to 3-cell–thick granular layer was observed easily in plastic sections (Figure 1c). The other distinctive feature of HMP epidermis is a marked, hyperkeratotic stratum corneum, organized in the typical 'basket weave' pattern seen in humans[8] (Figure 1b). This thickened stratum corneum resembles ichthyosis vulgaris and reflects the clinically scaly surface (Figure 1a).

Hairless micropig papillary dermis consists of loosely arranged collagen fibers, organized in a thin feltwork, similar to the human papillary dermis. Reticular dermis is composed of thick collagen bundles that are aligned randomly (Figure 1b). In suitably stained preparations, elastic fibers are intertwined among the collagen fibers (data not shown). Ground substance appears as empty space between collagen bundles; however, when visualized with Mowry's colloidal iron, blue staining material (glycosaminoglycans) is prominent in the papillary dermis and around vessels. Fibroblasts are randomly distributed throughout the dermis (Figure 1b).

The most striking feature of HMP dermis is the well-defined, horizontally aligned superficial plexus of venules and arterioles that separate the papillary and reticular

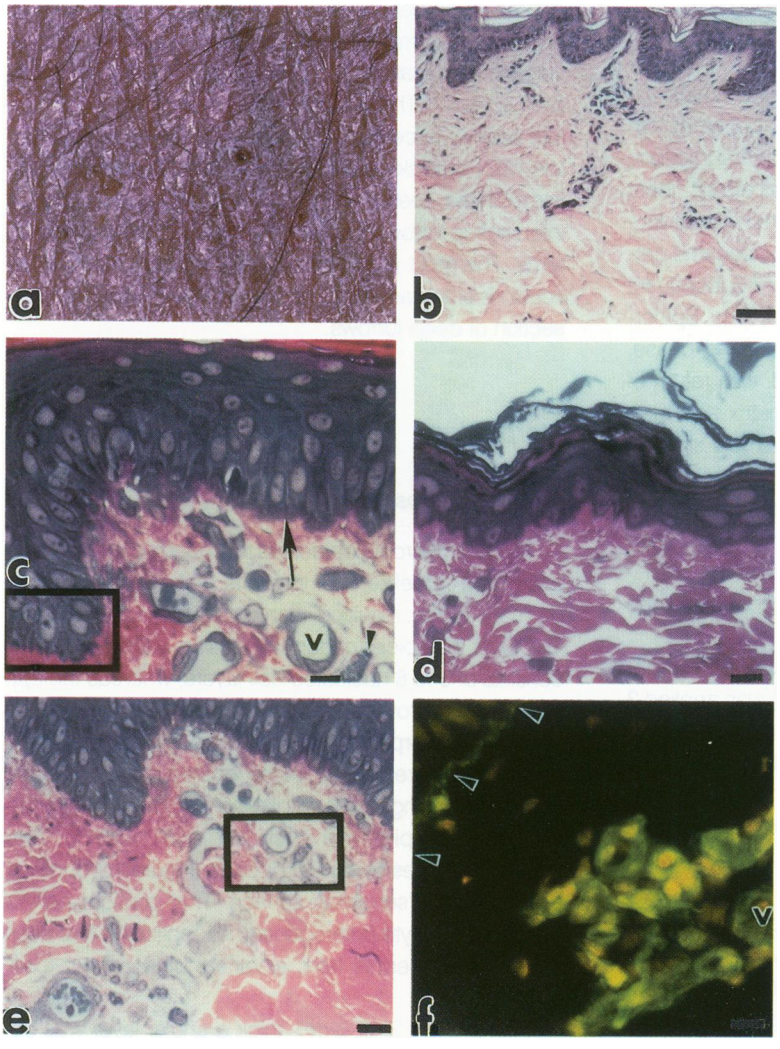


**Figure 1.** **a:** *Clinical photograph of pig skin showing skin surface markings similar to glyphics routinely seen in human skin.* **b:** *Light micrograph of hematoxylin and eosin-stained paraffin section of pig skin showing undulating dermal–epidermal junction consisting of epidermal downgrowths that interdigitate with dermal papillae (bar = 50 μm).* **c:** *Light micrograph of one-micron plastic section of pig skin showing two distinct populations of basal keratinocytes; those with a smooth (nonserrated) dermal–epidermal interface (located within the rectangle) and those with a convoluted (serrated-arrow) interface. Dermis contains numerous vessels (v). Arrowhead points to a mast cell (bar = 10 μm).* **d:** *Light micrograph of 1-μ plastic section of murine skin showing typical nonundulating thin epidermis overlying a compact dermis containing scant vascular profiles (bar = 10 μm).* **e:** *Light micrograph of 1-μ plastic section of pig skin showing network of vertically oriented capillary loops that emanate from the superficial vascular plexus similar to human skin. For contrast compare with* **d** *(murine skin) (bar = 10 μm).* **f:** *Light micrograph of frozen section of pig skin stained with FITC-laminin (green fluorescence) showing distribution around vasculature (v) and dermal–epidermal junction (arrowheads) (bar = 10 μm).*

dermis. A network of vertically oriented capillary loops that extend into each dermal papilla emanate from the superficial vascular plexus (Figure 1c and e). This vascular architecture is strikingly similar to that seen in humans[4,5] and is significantly different from that seen in murine animals (Figure 1d). Fully granulated mast cells are situated around the venules of this plexus and are observed easily in 1-μ–thick plastic sections stained with toluidine blue to highlight the metachromatic quality of mast cell granules (Figure 1c).

### *Transmission Electron Microscopy*

Fine structural features of individual basal, spinous, granular, and horny cells from HMP epidermis are similar to those from humans. Basal cells with serrated and nonserrated interfaces are distinguished easily (Figure 2a). Keratin filaments, membrane-coating granules, desmosomes, and keratohyalin granules all are present in normal amounts. Keratohyalin granules appear as angular, electron-dense bodies with keratin filaments embedded in the dense mass. This type of granule arrangement is characteristic for guinea pig and human epidermis, whereas rounded granules are typically seen in rodent and murine epidermis.[41] The stratum corneum consists of flattened anucleate cells composed of filaments embedded in an electron-dense matrix, enveloped by a thickened membrane.

Melanocytes were the next-most prevalent cell type found in HMP epidermis (Figure 2a). Situated primarily within the basal layer, these cells lacked desmosomal attachments and were recognized easily by their pale cytoplasm, containing fine filaments and melanosomes. Cells containing Birbeck granules, the distinctive ultrastructural marker for Langerhans cells,[42] were not observed in HMP epidermis. However cells devoid of desmosomes with pale cytoplasm sometimes were noted in the superficial layers of the epidermis. These cells lacked Birbeck granules and melanosomes and appeared to be similar to 'indeterminate cells' observed in humans.[43]

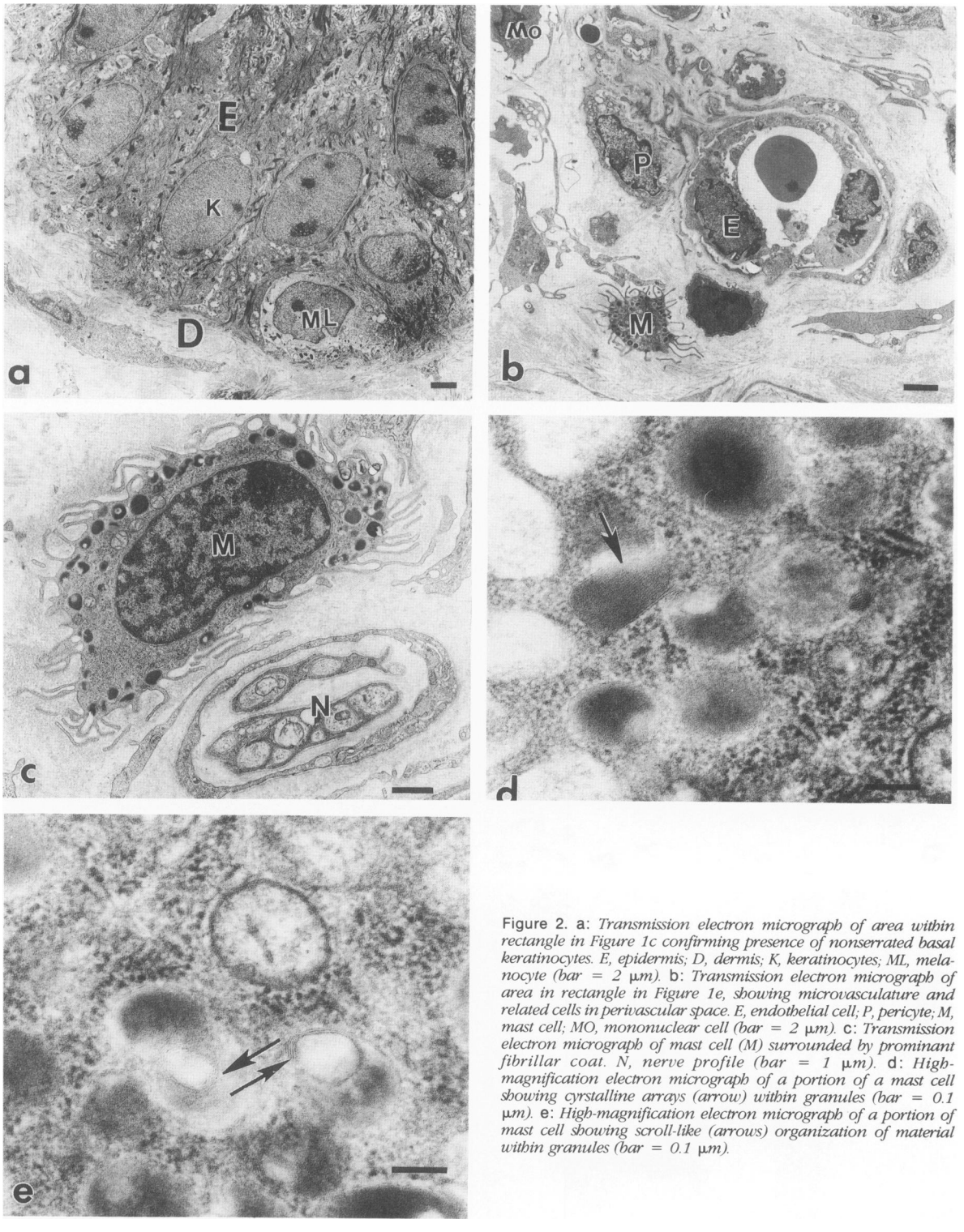


Figure 2. a: *Transmission electron micrograph of area within rectangle in Figure 1c confirming presence of nonserrated basal keratinocytes. E, epidermis; D, dermis; K, keratinocytes; ML, melanocyte (bar = 2 μm).* b: *Transmission electron micrograph of area in rectangle in Figure 1e, showing microvasculature and related cells in perivascular space. E, endothelial cell; P, pericyte; M, mast cell; MO, mononuclear cell (bar = 2 μm).* c: *Transmission electron micrograph of mast cell (M) surrounded by prominant fibrillar coat. N, nerve profile (bar = 1 μm).* d: *High-magnification electron micrograph of a portion of a mast cell showing cyrstalline arrays (arrow) within granules (bar = 0.1 μm).* e: *High-magnification electron micrograph of a portion of mast cell showing scroll-like (arrows) organization of material within granules (bar = 0.1 μm).*

Components of the dermis, including the extracellular matrix and the microenvironmental compartment of superficial microvasculature with associated mononuclear cells and mast cells, is remarkably similar to humans.[44] Microvasculature consists of well-developed endothelial cells, periocytes, and veil cells (Figure 2b). Endothelial cells exhibited Weibel–Palade bodies and micropinocytotic vesicles similar to those of human endothelial cells.[4,5] Mast cells most often were seen around the vasculature and nerve profiles (Figure 2b and c). Mast cells

appear to be surrounded by a distinctive network of fine flocculent material (Figure 2c). This extracellular coat appears identical in structure to material within the dermal matrix. Mast cell granules contain scroll-like and crystalline arrays, as well as homogeneous, electron-dense material (Figure 2d and e).

## *Histochemical/Immunohistochemical Characterization*

Immunohistochemical evaluation demonstrated patterns of immunoreactivity for polyclonal antibodies to epidermal keratin protein (Figure 3a), melanocytic and neural S-100 protein (Figure 3b), dermal endothelial factor VIII (Figure 3c), and mast cell chymotrypsin that were identical to those previously described in human skin[31,32] (Figure 3d). Hairless micropig skin stained with fluorescein isothiocynate laminin showed a continuous linear distribution around the vasculature and at the dermal–epidermal junction (Figure 1f). In addition, reaction patterns similar to those observed in humans were noted for HMP skin stained with involucrin,[35] filaggrin,[36] Luna's stain[30] for elastic tissue, and periodic acid-Schiff (data not shown).

Hairless micropig skin did not cross-react with monoclonal antibodies for the following human leukocytes: Leu-1 (pan T cells), Leu-2a (T cytotoxic/suppressor cells), Leu-3a (T helper/inducer cells), Leu 4 (pan T cells), and Leu-6 (prothymocytes and Langerhans cells).

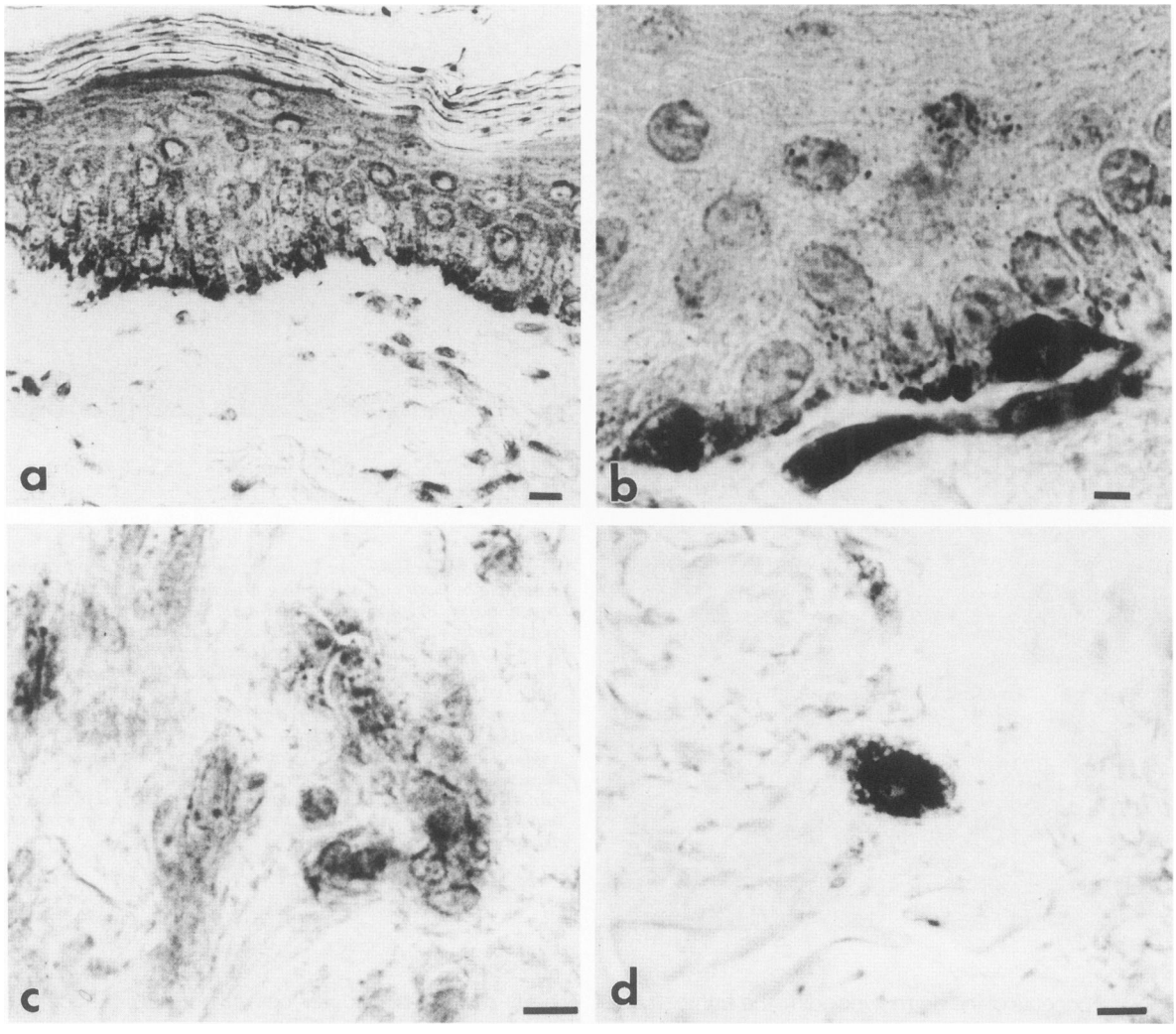


**Figure 3.** *Light micrographs of paraffin sections of pig skin incubated with screening antibodies to human skin antigens. Cross-reactivity for the following molecules is demonstrated.* **a**: *Keratin proteins distributed throughout the entire epidermal layer (PAP method; DAB substrate) (bar = 10 μm).* **b**: *S-100 protein within the cytoplasm of basal melanocytes and in small unmyelinated dermal nerve twig (bar = 5 μm).* **c**: *Factor VIII within the cytoplasm of endothelial cells of the microvascular plexus (bar = 10 μm).* **d**: *Alpha-1 chymotrypsin in a granular pattern within a dermal cell with characteristics of a mast cell (bar = 10 μm).*

## Steroid Atrophy Rebound Effect

Animals suffered no weight changes or systemic signs during or after corticosteroid treatment and thus did not appear to develop acute or chronic stigmata of systemic steroid absorption. Clinically steroid-treated skin was less pigmented and had a thin, finely wrinkled appearance. Some effacement of skin surface markings was noted; however telangiectasia was not apparent.

Image analysis of histometric data disclosed a steady decrease in viable epidermal thickness (VET) as treatment with betamethasone dipropionate progressed (Figure 4a) with a maximal reduction of 50% after 6 weeks of treatment. At this juncture, the epidermis was devoid of mitotic figures and had a flattened dermal–epidermal interface identical to that observed previously in similarly treated human skin.[28] Observed reduction in VET results not only from a flattening of existing cells but also from an overall decrease in cell layers from 10 to 6 (Figure 4b). Remaining keratinocytes were unaffected as regards their fine structure. Amount and appearance of keratin filaments, membrane-coating granules, and keratohyalin granules appeared normal. The stratum corneum was unaffected by corticosteroid application, unlike that of similarly treated human skin, which displayed a markedly attenuated stratum corneum at this point[28] (Figure 4b). Although melanocytes were not decreased in number, their cytoplasm appeared attenuated with few if any melanosomes visible (data not shown).

Dermal architecture also was noticeably altered after 6 weeks of treatment. In hematoxylin and eosin-stained sections, the dermis showed pallor, apparent homogenization, compaction of collagen bundles, and an attenuation of the vasculature (Figure 4b). Vessels were less prominent due to fewer and smaller profiles. Laminin staining of the basement membrane zones of dermal vessels was much thinner after steroid treatment, which resulted in an overall less-intense staining pattern compared with controls (data not shown). With regard to ground substance, an 88.3% diminution in blue staining material, representative of acid mucopolysaccharide, was observed after treatment.

By 2 to 4 days after termination of treatment, mitotic figures were observed within the epidermis. Mitoses rarely were encountered in untreated HMP epidermis and their appearance was correlated with the subsequent increase in VET. Within 7 days after cessation of treatment, VET was 25% thicker than that of controls and returned to normal values by 14 days (Figure 4a and c). Reaccumulation of ground substance in the papillary dermis as well as increased numbers and thickness of laminin reactivity around vessels were seen 14 days after treatment was stopped (data not shown). The blue stain-

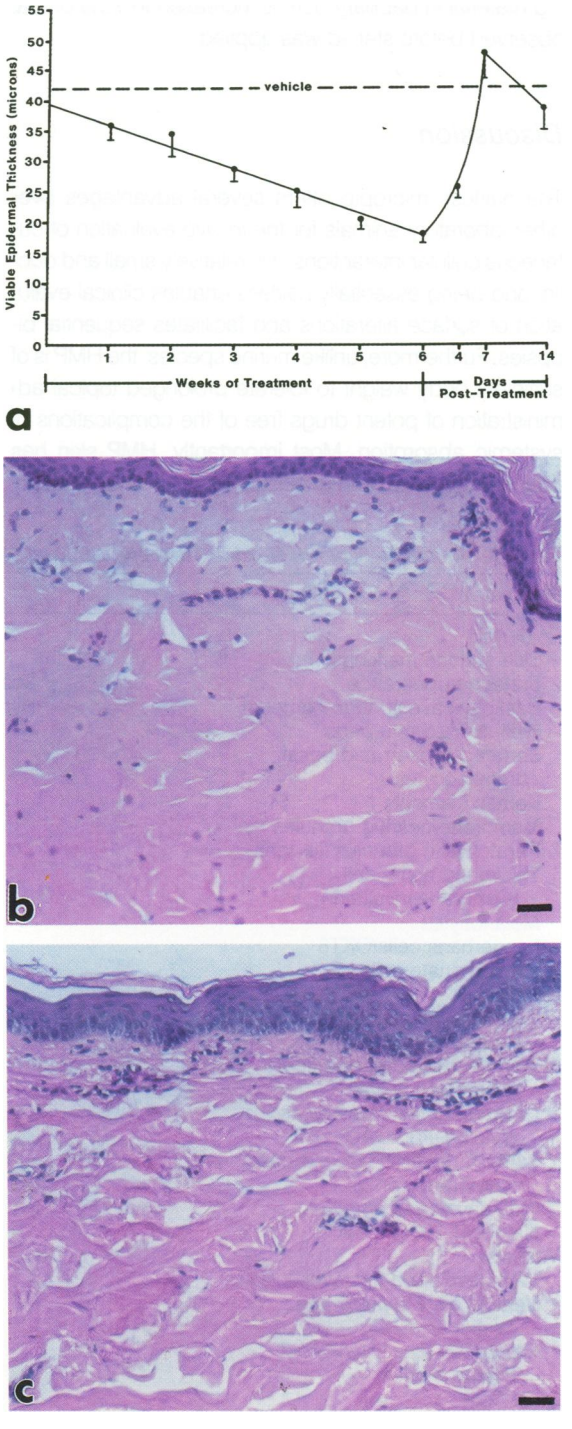


**Figure 4.** **a**: *Histometric analysis of viable epidermal thickness (VET) after occlusion with 0.05% betamethasone dipropionate cream and vehicle Purpose, showing the corticosteroid-induced atrophy and the rebound after discontinuation of treatment.* **b**: *Light micrograph of hematoxylin and eosin-stained section of pig skin treated continuously for 6 weeks with betamethasone dipropionate under occlusion. Epidermis shows loss of rete ridge pattern and thinning (atrophy). The dermis shows pallor, apparent homogenization, and compaction of collagen bundles (bar = 50 μm).* **c**: *Light micrograph of hematoxylin&eosin-stained section of pig skin 14 days after cessation of treatment. Epidermis thickness is returning toward normal. Papillary dermis shows features similar to untreated skin (bar = 50 μm).*

ing material in papillary dermis increased to 75% of that observed before steroid was applied.

## Discussion

The hairless micropig offers several advantages over other laboratory animals for the *in vivo* evaluation of cutaneous cellular interactions. It is relatively small and docile, and being essentially hairless enables clinical evaluation of surface alterations and facilitates sequential biopsies. Furthermore, unlike murine species, the HMP is of sufficient body weight to tolerate prolonged topical administration of potent drugs free of the complications of systemic absorption. Most importantly, HMP skin has clinical, structural, immunohistochemical, and reactivity features that are remarkably similar to human skin. These features are summarized in Tables 2 and 3.

Organization of skin surface into geometric patterns, basal cell heterogeneity, an interdigitating dermal–epidermal architecture, a prominent network of vertically oriented capillary loops emanating from the superficial vascular plexus, and mast cells with granules displaying whorls and scrolls and lattice formations are structural features of HMP skin that are found routinely in human skin but rarely, if ever, are manifested in other animal species.[1–5,11] Similarly distribution patterns of a wide range of polyclonal antibodies are identical to those of humans.[34,36]

Functionally the response of HMP skin to the suppressive stimulus of corticosteroids manifested by 1) thinning of the viable epidermis, 2) alteration of the papillary and

**Table 2.** *Similarity Between Hairless Micropig Skin and Human Skin*

| Parameter | HMP | Human | Reference |
|---|---|---|---|
| Epidermis | | | |
| Skin-surface markings | + | + | 1 |
| Surface appearance | Scaly | Smooth | 40 |
| Basket-weave stratum corneum | Hyperkeratotic | Normal | 2 |
| Well-defined rete pegs | + | + | 2 |
| Serrated/nonserrated basal keratinocytes | + | + | 25,26 |
| Keratin filaments | + | + | 57 |
| Membrane-coating granules | + | + | 58 |
| Keratohyalin granules/filaggrin | + | + | 38,36 |
| Thickened horny cell membrane/involucrin | + | + | 59,60,35 |
| Melanocytes | + | + | 2 |
| Langerhans cells/OKT6 | − | + | 61 |
| Indeterminate cells | + | + | 43 |
| Dermis | | | |
| Papillary–reticular collagen organization | + | + | 3 |
| Well-developed elastic fiber network/Luna stain | + | + | 30 |
| Ground substance/Mowry's colloidal iron-GAGs | + | + | 28 |
| Laminin reactivity around basement membrane zones | + | + | 34 |
| Well-developed fibroblast | + | + | 3 |
| Nerves/S-100 protein | + | + | 31 |
| Vessels | | | |
| Well-developed microvasculature | + | + | 4 |
| Vertical capillary/rete peg relationships | + | + | 4 |
| Factor VIII reactivity/Weibel Palade bodies | + | + | 4 |
| Mast Cells | | | |
| Mast cells/chymotrypsin | + | + | 46,62 |
| 'Whorl and Scroll' mast cell granule structure | + | + | 11 |
| Flocculent encasement around mast cells | + | − | 27 |
| Mast cell depletion *via* corticosteroids | − | + | 46 |
| Miscellaneous | | | |
| Macrophages | + | + | 3 |
| *Leu* series reactivity | − | + | 37 |
| Corticosteroid suppression epidermis/dermis | + | + | 28 |
| Corticosteroid rebound | + | + | 38 |

**Table 3.** *Structural Characteristics of the Skin of Human and HMP* versus *Murine*

| Characteristics | Human and HMP | Murine |
|---|---|---|
| Complex surface | + | − |
| Thick epidermis with distinct strata | + | − |
| Basal cell heterogeneity | + | − |
| Undulating dermal–epidermal junction | + | − |
| Well-defined superficial microvasculature | + | − |
| Defined internal structure in mast cell granule | + | − |
| Well-defined papillary and reticular dermal zones | + | − |
| Dermal keratinizing cyst | − | + |
| Response to SARE | + | −* |

* It is difficult to perform experiment of SARE on murine not only because of the thinness of epidermis but also because the smallness of body weight makes the systemic effect of corticosteroid unacceptable, as are some other topical drugs.

reticular dermis, and 3) diminution of the vasculature was analogous to that seen in humans.[28] Furthermore the pronounced epidermal hyperplasia, along with dermal and vascular restitution following removal of corticosteroids, are additional cutaneous reactions that mirror those of humans.[39]

While similarities between HMP and human skin are numerous, there are some differences with respect to structure, immunohistochemistry, and function. A scaly, ichthyosiform surface whose morphologic counterpoint is a thickened, hyperkeratotic stratum corneum is the primary morphologic difference between HMP and human skin. This thickened stratum corneum responded differently to topical application of potent corticosteroids than human stratum corneum. In human skin, 6 weeks of steroid treatment resulted in a stratum corneum that was dramatically thinned, appearing as a wispy layer of horny cells,[28] whereas HMP stratum corneum remained unchanged after a similar exposure to steroid. However human xerotic or ichthyotic stratum corneum is relatively steroid unresponsive (unpublished data), and thus the failure of corticosteroids to affect HMP stratum corneum is consistent with the similar situation in humans.

Hairless micropig mast cells are unique in that they are encased in a fibrous mantle whose biologic significance is unknown. Unlike human dermal mast cells, which can be depleted by long-term topical corticosteroids,[45] HMP dermal mast cells do not appear to be diminished by topical corticosteroids. A possible explanation for this difference in response to corticosteroids is that the fibrous mantle may provide some protective or insulating function for HMP mast cells.

Tennis racquet-shaped Birbeck granules, the characteristic ultrastructural marker for human and rodent Langerhans cells,[42] were not observed in electron lucent, dendritic cells located in the superficial HMP epidermis. In canine epidermis, cells devoid of desmosomal attachments, having an electron-lucent cytoplasm without melanin granules and keratin filaments, were identified as Langerhans cells.[46] However Birbeck granules were not observed in any of 589 cells examined. Similarly Birbeck granules rarely were observed in epithelial cells from goat and cattle skin.[47] Thus the observed lack of Birbeck granules in 'indeterminant-type' cells within HMP epidermis is consistent with findings in other domestic animals and these cells probably represent Langerhans cells.

The major immunohistochemical difference was lack of epitopes defined by monoclonal antibodies to human T-cell subsets and Langerhans cells

## *Implications for Future Studies*

Hairless micropig epidermis represents an informative model for investigations on factors governing proliferation and homeostasis. Based on anatomic location, morphologic features, and kinetic properties, nonserrated basal keratinocytes have been postulated to represent epidermal stem cells that give rise to suprabasally located transient amplifying cells.[25,26,48] Serrated keratinocytes are believed to be in the transient amplifying compartment. Basal keratinocyte heterogeneity (existence of serrated and nonserrated cells) of this nature previously were observed only in palmar and plantar skin from humans and primates and in glabrous skin of humans.[25,26] Palmar and plantar epidermis, which is normally hyperproliferative,[26] is refractory to most external perturbations and responds only to drastic stimuli (unpublished data). Existence of discrete populations of serrated and nonserrated cells, in conjunction with a pharmacologically responsive skin (ie, SARE), thus provides cutaneous biologists an excellent opportunity to investigate effects of various proliferative conditions on stem and transient amplifying cells.

One of the earliest sites of injury in graft-*versus*-host disease are those cells located at the tips of the rete pegs (the 'nonserrated' or presumptive stem cells).[49] It has been hypothesized that natural killer cells lyse rapidly proliferating cells,[50] thus the putative stem cell and transient amplifying cells present at tips of rete may be selected targets in graft-*versus*-host disease. Hairless micropig skin configured with an interdigitated dermal–epidermal junction with nonserrated cells at the tips of epidermal downgrowths may be an important model for cytotoxicity studies that address this issue.

Hairless micropig skin is ideal for investigations involving perturbation of the microenvironment because it has a dermal microvasculature, surrounded by mononuclear and mast cells, in close proximity to the epidermis. Preliminary studies revealed that initial steroid administration resulted in apparent downregulation of both epidermal interleukin-1$\alpha$ and incorporation of 3H-TdR.[51] This appar-

ent ability of corticosteroids to act as a suppressive stimulus on the HMP microanatomic compartment should provide a means to study cytokine-mediated interrelationships between skin cells. Another result of corticosteroid treatment of skin is a depletion of Langerhans cells,[28,52] which may simulate an immunocompromised state.[52,53] Thus angioproliferation after removal of corticosteroids may reflect different influences than vascular hyperproliferation previously observed after wound healing.

Mast cell granules in human skin contain characteristic scroll-like and crystalline arrays, features not present in mast cells of rodent skin.[11] Hairless micropig skin mast cells, in addition to displaying granule fine structure consistent with human mast cells, appear to be surrounded by a distinctive network of fine flocculent material. This extracellular coat appears identical in structure to material within dilated cisternae of rough endoplasmic reticulum of fibroblasts, and within the dermal matrix. Fibroblast–mast cell interactions led to speculation concerning a possible homeostatic link between these two cell types.[54–56] Furthermore studies on the changes in the fibrous mantle after pharmacologic-induced degranulation may yield some new insights into interrelationships between mast cell granules and mast cell membrane during exocytosis.

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