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# Thy-1 Antigen-Bearing Dendritic Cells Populate Murine Epidermis

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**Two distinct cell populations, melanocytes and Langerhans cells (LC), have been recognized previously to possess dendritic configuration in normal mammalian epidermis. Employing immunofluorescence microscopy with monoclonal antibodies against Thy-1.2 antigen to identify cells in whole mounts of murine epidermis, we have identified a third dendritic cell population which differs from both LC and melanocytes. Thy-1 antigen-bearing (Thy-1$^+$) epidermal cells are primarily dendritic, although round and angular forms may be found. They are distributed relatively evenly across skin surfaces, although densities vary greatly from site to site and from strain to strain. Densities were highest in ear epidermis from the pigmented strain B10.A (580 cells/mm$^2$), a value approaching that of epidermal LC, and were lowest in ear epidermis from the albino strain BALB/c (5 cells/mm$^2$). Thy-1$^+$ epidermal cells possess neither Ia antigens nor substantial amounts of melanin, and their surface distributions are disparate from those of both LC and mature melanocytes. We propose that at least some of these cells are T lymphocytes whose malignant counterparts account for cutaneous T-cell lymphomas.**

Melanocytes and Langerhans cells (LC) have been recognized previously to possess dendritic configuration in normal mammalian epidermis [1]. In the experiments reported here we have identified in normal murine skin a previously unrecognized Thy-1 antigen-bearing population of epidermal cells, a population which is distinct from dendritic LC and from mature melanocytes.

## MATERIALS AND METHODS

Skin specimens from 9 selected inbred murine strains were examined: B10 (Thy-1.2), B10.A (H-2 Ia$^k$, Thy-1.2), B10.BR (H-2 Ia$^k$, Thy-1.2), C57BL/6 (Thy-1.2), C3H (Thy-1.2), A/J (Thy-1.2), BALB/c (Thy-1.2), AKR (Thy-1.1), and PL/J (Thy-1.1). Cells were identified by immunofluorescence microscopy in whole mounts of epidermis from ear, tail, and abdominal wall skin as described previously [2,3].

Immunoreagents were purchased commercially and employed at the listed concentrations (Table I). After acetone fixation, each epidermal specimen was washed in 0.05 M phosphate-buffered saline (PBS) and then incubated overnight at 4°C in the appropriate monoclonal antibody. Following a second wash in PBS each specimen was incubated for 90 min in the appropriate secondary reagent. Washed specimens were placed as whole mounts on glass slides in 90% glycerol:10% PBS.

For electron microscopy, abdominal wall epidermal cells were disaggregated into a single cell suspension with trypsin, as described by Stingl et al [4]. These cells were then incubated in unconjugated antimouse Thy-1.2 followed by rabbit antimouse IgG and then peroxidase-antiperoxidase. After fixation in glutaraldehyde, an electron-dense precipitate was produced by the peroxidation of *p*-phenylenediamine in the presence of pyrocatechol [5]. Electron microscopy of stained cells was performed with standard techniques.

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Abbreviations:
LC: Langerhans cell(s)
PBS: phosphate-buffered saline

## RESULTS

Employing either directly fluoresceinated monoclonal antimouse Thy-1.2 or monoclonal antimouse Thy-1.2 followed by fluoresceinated goat antimouse IgG, regularly spaced and brightly stained dendritic cells were observed in the epidermis from abdominal wall, ear, and tail skin specimens in all murine strains that express the Thy-1.2 allele (Fig 1). The majority of epidermal Thy-1$^+$ cells appeared dendritic (Fig 1*a*), although round, oval, and angular forms were also observed (Fig 1*b*). To determine whether there might be site or strain variations in surface densities of Thy-1$^+$ cells, epidermal whole mounts from 3 skin sites were examined in 6 strains (Table II). Three strains, B10, B10.A, and C57Bl/6, exhibited high densities of Thy-1$^+$ cells, with values approaching those of epidermal LC. For comparison, surface densities of Ia-positive LC were observed in parallel studies with both B10.A and BALB/c mice to be greater than 700 cells/mm$^2$ in the abdominal wall and ear epidermis. By contrast, densities of Thy-1$^+$ cells in ear skin of A/J and BALB/c mice were 10- and 100-fold less, respectively. In tail skin, Thy-1$^+$ cells assumed a restricted distribution which resembled that of LC [3]. Both cell types occurred in a gridlike pattern with restriction to orthokeratotic, hair-bearing areas. Specimens from all 3 skin sites in strains AKR/J and PL/J, which possess the Thy-1.1 allele, were consistently negative. This specificity argues against both nonspecific cell surface adherence of secondary reagents and the binding of primary or secondary reagents via cell surface Fc receptors.

A common anatomic characteristic of LC and melanocytes is their in vivo dendritic configuration, and we were therefore concerned that Thy-1 antigen might be a marker for either of these cells. By immunofluorescence microscopy, melanin contained within melanocytes appears black, and the dendritic form of melanocytes appears as an absence of background stain in epidermal specimens treated with fluoresceinated reagents. Our conclusion that Thy-1 antigen does not occur on mature melanocytes is based on observations made in 4 pigmented strains: B10, B10.A, C3H, and C57BL/6. First, the black negative image of scattered melanocytes was clearly visible in ear epidermis, and these cells were invariably Thy-1$^-$. Secondly, numerous melanocytes were observed in parakeratotic regions of tail epidermis, whereas Thy-1$^+$ cells occurred predominantly in orthokeratotic regions. Where these two regions met, both cell types could be seen, clearly demonstrating their distinctiveness.

To determine whether one cell type expressed both Thy-1 and Ia antigens, epidermal specimens from a B10.BR mouse were first exposed to biotinylated anti-Ia$^k$ followed by rhodaminated avidin and then to fluoresceinated anti-Thy-1.2. By fluorescence microscopy these monoclonal reagents identified two different populations of dendritic cells. Fig 2 was obtained by photographing a single field, first using the rhodamine excitor (Fig 2*a*), then using the fluorescein excitor (Fig 2*b*). It illustrates that both antigens never occurred simultaneously on one cell. This study was repeated with identical results, first in a different strain (B10.A), and then using different reagents: biotinylated anti-Ia$^k$, fluoresceinated avidin, and rhodaminated anti-Thy-1.2. Finally, B10.A abdominal wall epidermis was disaggregated into a single cell suspension with trypsin, cytocentrifuged onto glass slides, and then fixed. These cells were stained with biotinylated anti-Ia$^k$ followed by rhodaminated

avidin, and then with fluoresceinated anti-Thy-1.2. Approximately 5% of cells exhibited red fluorescence ($Ia^+$) or green fluorescence ($Thy\text{-}1^+$). No disaggregated cell ever exhibited both antigenic determinants. Thus, although their dendritic configuration first suggested that the $Thy\text{-}1^+$ cells might be LC or melanocytes, these possibilities are unlikely since Thy-1 and Ia antigens were never observed on the same cell, and since melanin-containing epidermal melanocytes failed to express Thy-1 antigen.

By electron microscopy, employing monoclonal anti-Thy-1.2 and an immunoperoxidase technique on trypsin-disaggregated epidermal cells, $Thy\text{-}1^+$ cells were observed to possess numerous intermediate filaments and no Birbeck granules, distinguishing them from Langerhans cells (Fig 3).

TABLE I. *Immunoreagents with titers employed for cell identification*

| Reagent | Conjugate | Dilution[a] |
|---|---|---|
| 1. Monoclonal antimouse Thy-1.2 (BD)[b] | fluorescein | 1:20 |
| 2. Monoclonal antimouse Thy-1.2 (NEN) | none | 1:20 |
| 3. Monoclonal antimouse Thy-1.2 (M-Y) | rhodamine | 1:40 |
| 4. Monoclonal antimouse $Ia^k$ (2) (BD) | biotin | 1:20 |
| 5. Avidin (BD) | fluorescein | 1:100 |
| 6. Avidin (BD) | rhodamine | 1:200 |
| 7. Goat antimouse Ig(7S) (M) | fluorescein | 1:10 |
| 8. Rabbit antimouse IgG (C) | none | 1:20 |
| 9. Mouse peroxidase-antiperoxidase (J) | none | 1:50 |

[a] Reagents diluted in 0.05 M phosphate-buffered saline.

[b] Manufacturer: BD = Becton-Dickinson Monoclonal Center, Inc.; C = Cappel Laboratories; J = Jackson Immunologic Research; M = Meloy Laboratories, Inc.; M-Y = Miles-Yeda, Ltd; NEN = New England Nuclear.

## DISCUSSION

Thy-1 antigen is a cell surface glycoprotein of restricted tissue distribution which has been studied most extensively in its capacity to serve in mice as a differentiation antigen for T-lymphocyte maturation [6–8]. The distribution of Thy-1 antigen is by no means limited to cells of the immune system; neuronal cells [9], fibroblasts [10], and mammary myoepithelial cells [11] have been observed to express this antigen. Most relevant to the present study is the work of Scheid et al [12] in which Thy-1 antigen on the surface of greater than 50% of tail epidermal cells was claimed on the basis of a direct cytotoxicity assay. By contrast, we have demonstrated Thy-1 antigen in substantial amounts only on a minor subpopulation of epidermal cells.

At least two nonexclusive possibilities exist for the functional identity of $Thy\text{-}1^+$ dendritic epidermal cells: (1) a novel resident epidermal cell, or (2) T lymphocytes. Although $Thy\text{-}1^+$ epidermal cells may represent previously unrecognized resident epidermal cells, this possibility does not exclude their sharing a common lineage with either LC or melanocytes. The second possibility is that at least some if not all $Thy\text{-}1^+$ cells represent

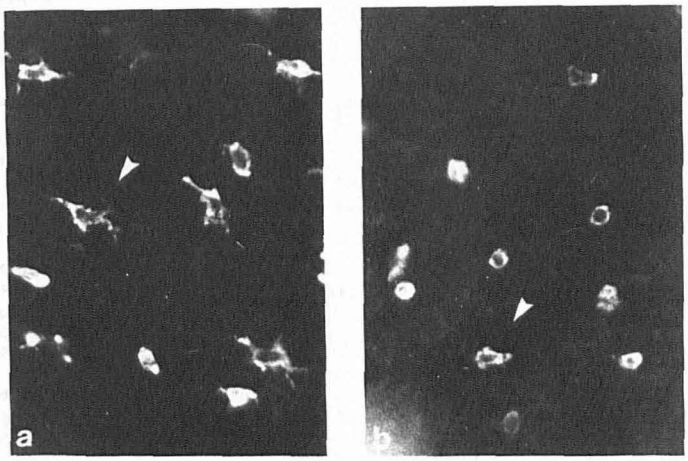


FIG 1. Thy-1.2-bearing cells demonstrated in whole mounts of epidermis from normal abdominal wall skin of a BALB/c mouse. *a*, Regularly spaced, dendritic cells predominate. *b*, Round and angular cells occur frequently.

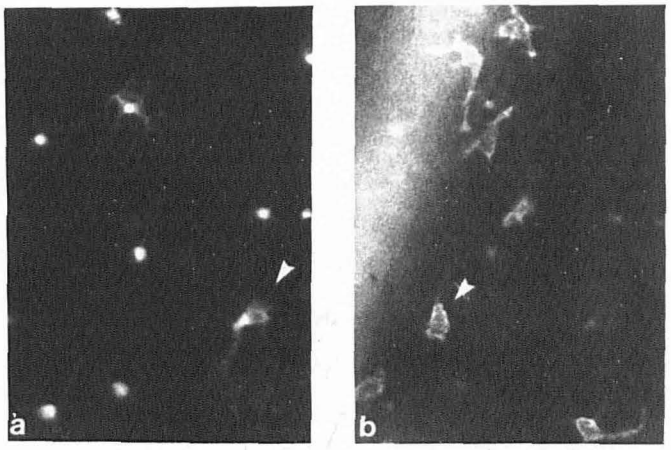


FIG 2. A single epidermal specimen from the abdominal wall of a B10.BR mouse was exposed first to biotin-conjugated monoclonal anti-Ia antibody followed by rhodaminated avidin and then to directly fluoresceinated monoclonal anti-Thy-1.2 antibody. *a*, Rhodamine excitor demonstrates Ia-positive LC (*arrow*). *b*, Fluorescein excitor demonstrates Thy-1.2-positive cells (*arrow*). No cell ever exhibited both antigenic determinants.

TABLE II. *Surface densities and percent of Thy-1.2-positive cells[a] which are dendritic in murine epidermis*

| Strain | Skin site | | | | | |
|---|---|---|---|---|---|---|
| | Ear | | Abdomen | | Tail[b] | |
| | Cells/mm² | % Dendritic | Cells/mm² | % Dendritic | Cells/scale | % Dendritic |
| B10 | 550 ± 80[c] | 85% | 440 ± 95 | 80% | 60 ± 15 | 80% |
| B10.A | 580 ± 110 | 80% | 480 ± 65 | 50% | 50 ± 6 | 80% |
| C57BL/6 | 460 ± 80 | 85% | 340 ± 110 | 60% | 25 ± 10 | 45% |
| C3H | 120 ± 120 | 90% | 540 ± 80 | 75% | 4 ± 4 | 20% |
| A/J | 40 ± 50 | 15% | 340 ± 80 | 90% | 5 ± 2 | 0% |
| BALB/c | 5 ± 10 | 80% | 170 ± 100 | 70% | 7 ± 5 | 20% |

[a] Cells identified in acetone-fixed whole mounts of epidermis, stained with fluoresceinated monoclonal anti-Thy-1.2 antibody.

[b] Because of difficulty in differentiating orthokeratotic from parakeratotic regions in tail epidermis by fluorescence microscopy, tail densities are reported as cells per scale rather than cells/mm². Since each scale and surrounding orthokeratotic region occupies approximately 0.1 mm², densities in tail skin are similar to those in other sites.

[c] Mean ± 1 SD; numbers represent mean and SD of 15–30 consecutive fields in 2 specimens from 2 different mice.

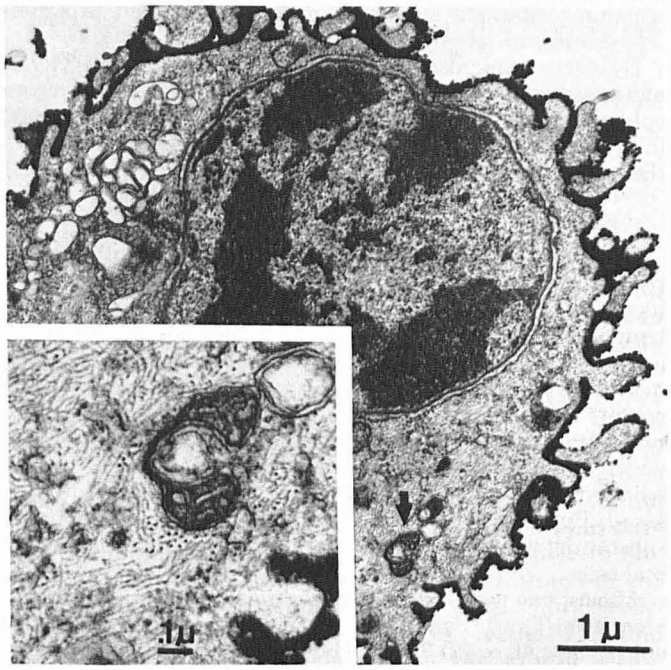


FIG 3. Immunoperoxidase labeling of a Thy-1.2-positive cell from a trypsin-disaggregate- epidermal cell suspension. Cells identified in this way possess an irregular nuclear outline and numerous intermediate filaments. No Birbeck granules were observed. *Arrow* identified location of insert.

intraepidermal T lymphocytes. In fact, there exists some evidence that T lymphocytes do occur in normal mammalian epidermis. Andrew and Andrew first identified lymphocytes histologically in human and rodent epidermis [13]. Recently, Thomas et al [14] described by immunofluorescence the presence of small numbers of cells bearing T-cell surface markers within the epidermis of sections of normal human skin. Further evidence for the obligate presence of immunocompetent effector lymphocytes in murine skin comes from the work of Barker and Billingham [15] in which parental skin induced significant local graft-vs-host reactions in $F_1$ recipients. Since the only source of effector T lymphocytes was the grafted skin, a cutaneous origin of these cells was affirmed, although their anatomic location (epidermis and/or dermis) was not identified. It is intriguing to speculate that the dendritic and round (angular) forms may be of separate lineage, the former representing a novel resident cell, the latter representing T lymphocytes. The truth to these possibilities will emerge only with further studies of phenotypic and functional attributes of these cells.

The observation that unperturbed murine epidermis possesses substantial numbers of Thy-$1^+$ cells provides support for an important role for epidermis in cutaneous immune processes. Epidermis may provide a microenvironment which promotes the acquisition of Thy-1 antigen and/or an environment which facilitates the immigration of circulating Thy-$1^+$ cells. In either event, the examination of this issue with murine skin may provide further insight into the nature and complexity of skin-associated lymphoid tissue [16] and into the specific affinity of T lymphocytes for the epidermis which occurs in many cases of cutaneous T-cell lymphoma [17].

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