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RAPID COMMUNICATIONS

Expression of Thy-1 Antigen by Murine Epidermal Cells

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We report on the occurrence of a cell population within the murine epidermis which, by both morphologic and surface property criteria, is distinct from all other epidermal cell types known so far. These previously unrecognized cells are evenly distributed within the epidermis, display a primarily dendritic shape, exhibit a lobulated nucleus, contain large amounts of vimentin type intermediate-sized filaments, but lack desmosomes, melanosomes, Merkel cell granules, and Birbeck granules. As opposed to melanocytes, these cells fail to display tyrosinase activity. Surface marker analysis reveals these cells to uniformly express the Thy-1 antigen and to lack I-A and I-E/C antigen specificities. A major portion of these Thy-1-bearing cells are reactive with a monoclonal antibody to the Ly-5 determinant whereas attempts to demonstrate Lyt-1,2,3 antigens consistently yield negative results. These findings strongly suggest that Thy-1$^+$ epidermal cells originate from the bone marrow; however, their precise relationship to distinct members of the hemopoietic differentiation pathway remains to be established.

The mammalian epidermis is a heterogeneous epithelium which is composed of ontogenetically and functionally diverse cell populations. In this report, we will morphologically and phenotypically characterize a hitherto undescribed dendritic cell population within the murine epidermis that is distinct from keratinocytes, Langerhans cells, melanocytes, and Merkel cells. Its most prominent phenotypic feature is the expression of Thy-1 antigen [1], whose occurrence within the epidermis had been previously suggested on the basis of cytotoxicity studies [2].

MATERIALS AND METHODS

Animals

Six-to eight-week-old AKR/J (Thy-1.1), C3H/He (Thy-1.2), C3H/He nu/nu (Thy-1.2), C57Bl/6 (Thy-1.2), and BALB/c (Thy-1.2) mice were obtained from the Zentralanstalt für Versuchstiere, Hannover, F.R.G.

Epidermal Specimens

Epidermal sheets from different body regions were obtained by EDTA- or NH_4SCN-separation procedures [3]. Single epidermal cell (EC) suspensions were prepared by standard trypsinization procedures [4].

List of Immunoreactants

See Table I.

Immunofluorescence Procedures

For the visualization of Thy-1-bearing epidermal cells (Thy-1$^+$ EC), acetone-fixed epidermal sheets and/or freshly isolated single EC suspensions were reacted with appropriate dilutions of monoclonal anti-Thy-1 reagents (or control ascitic fluid) in either direct or indirect immunofluorescence procedures as previously described [5,6]. For a more detailed phenotypic analysis of Thy-1$^+$ EC, double-staining procedures were performed using commercially obtained monoclonal reagents directed against Ia-, Lyt-1,2,3-, and Ly-5-specificities (see Table I) and an antivimentin monoclonal antibody (4C2, kindly provided by Dr. W. Aberer, University of Vienna) at appropriate working dilutions.

Immunoelectron Microscopic Studies

Single EC suspensions prepared from nonirradiated and UVA-irradiated [7] C3H/He ear skin by standard trypsinization procedures were incubated stepwise (30 min, 4°C) with appropriate dilutions of mouse IgM antimouse Thy-1.2 reagent and horseradish peroxidase (HRPO)-labeled sheep F (ab)'$_2$ antimouse Ig. After termination of the incubation

TABLE I. *Antibodies used for immunofluorescence and immunoelectron microscopic studies*

Antibody	Conjugate	Dilution[a]
1. Monoclonal mouse antimouse Thy-1.2 (IgM) (NEN)	None	1:100
2. Monoclonal mouse antimouse Thy-1.1 (IgM) (NEN)	None	1:100
3. Monoclonal rat antimouse Thy-1.2 (IgG_{2b}) (BD)	FITC	1:100
4. Monoclonal mouse antimouse Ia^k (IgG_{2b}) (BD)	Arsanilate	1:100
5. Monoclonal mouse antimouse Lyt-1.1 (IgG_{2b}) (NEN)	None	1:40
6. Monoclonal mouse antimouse Lyt-2.1 (IgG_{2b}) (NEN)	None	1:40
7. Monoclonal mouse antimouse Ly-5.1 (IgG_{2a}) (NEN)	None	1:50
8. Monoclonal antivimentin (4C2)[b]	None	1:50
9. Monoclonal rat antimouse IgM (IgG_{2b}) (BD)	FITC	1:100
10. Rabbit anti-arsanilate (BD)	TRITC	1:50
11. Sheep F(ab)'$_2$ antimouse Ig (Amersham)	HRPO	1:10
12. Goat antimouse IgM (Tago)	TRITC	1:20
13. Goat antimouse IgG (Tago)	FITC	1:10

[a] Antibodies were diluted in phosphate buffered saline, pH 7.4, containing 2% bovine serum albumin and 0.1% sodium azide.

[b] 4C2 reagent was kindly provided by W. Aberer, M.D., (University of Vienna)

Abbreviations: NEN = New England Nuclear; BD = Becton Dickinson; FITC = fluorescein isothiocyanate; TRITC = tetramethylrhodamine isothiocyanate; HRPO = horseradish peroxidase

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Abbreviations:
EC: epidermal cell(s)
FITC: fluorescein isothiocyanate
HRPO: horseradish peroxidase
PBS: phosphate-buffered saline
TRITC: tetramethylrhodamine isothiocyanate

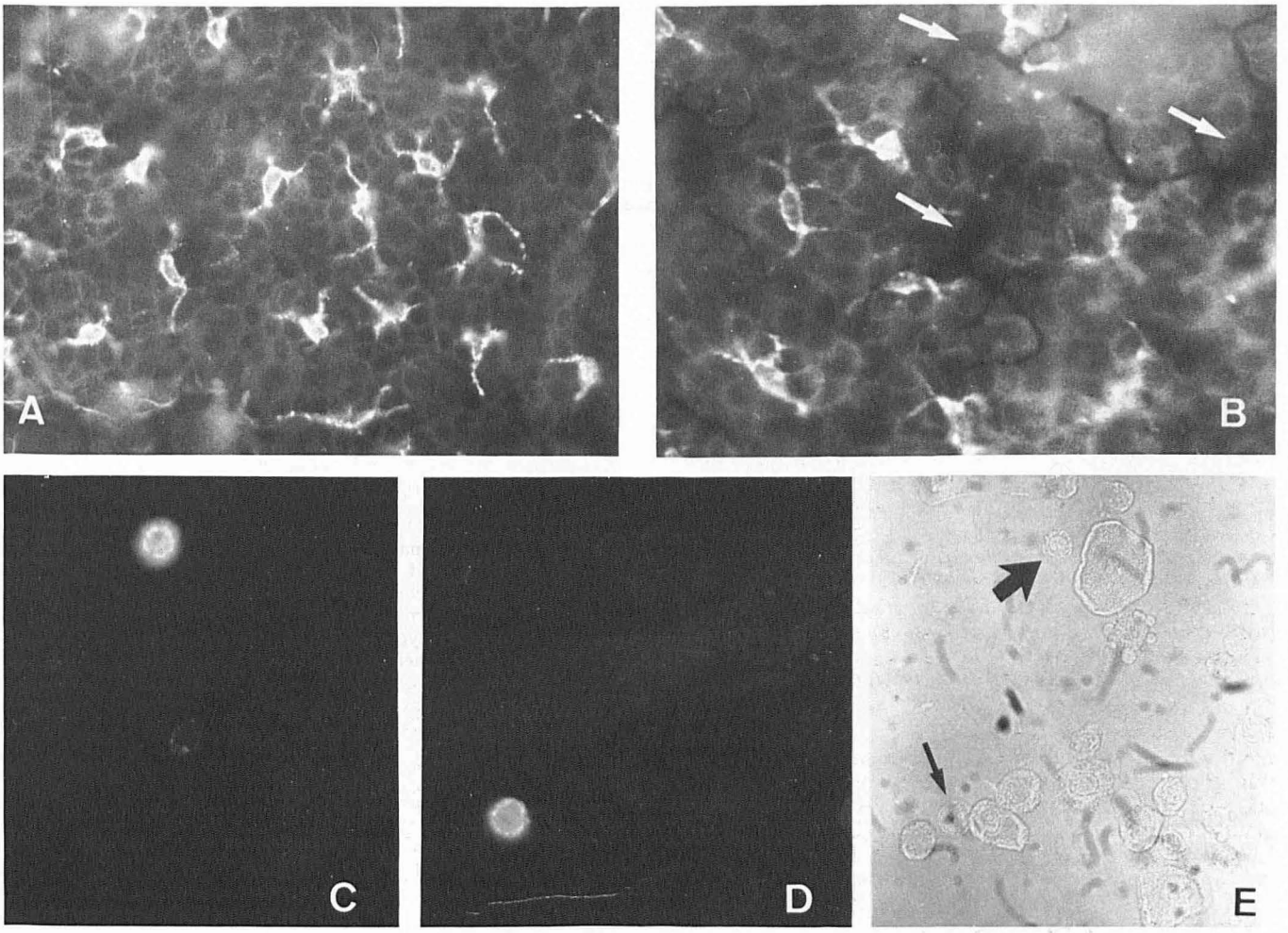

FIG 1. Identification of murine Thy-1$^+$ EC by immunofluorescence. *A and B*, Sheets of C3H/He ear epidermis [3] were fixed with 2% paraformaldehyde (0.1 M cacodylate buffer, 0.017 M $Cacl_2$) for 20 min at room temperature. After 3 PBS washes, sheets were exposed to a 1:100 dilution of a FITC-labeled rat IgG_{2b} antimouse Thy-1.2 monoclonal antibody for 16 h at 4°C (*A*). In a few experiments, specimens were subjected to the dopa reaction [11] for the demonstration of tyrosinase activity before being exposed to the immunofluorescence procedure (*B*). Slides were then examined with a Leitz-Ortholux incident light fluorescence microscope (Leitz, Wetzlar, F.R.G.). Anti-Thy-1.2-reactive EC appear evenly distributed over the entire specimen and exhibit a highly dendritic shape (*A,B*). Tyrosinase-positive melanocytes (*B, arrows*) represent a separate cell population. Magnification × 400. *C, D, and E*, Single cell suspensions from C3H/He epidermis were first reacted for 30 min at 4°C with a 1:100 dilution (PBS-azide) of an arsanilate-conjugated mouse IgG_{2b} anti-Ia^k monoclonal antibody followed by a 30-min incubation with a 1:50 diluted TRITC-labeled rabbit anti-arsanilate at 4°C. After PBS washes, cells were incubated with a 1:100 dilution of a FITC-labeled rat IgG_{2b} antimouse Thy-1.2 monoclonal antibody and, after final washes, the cell suspension was examined for fluorescence under a petroleum jelly-lined coverslip. A particular field was photographed by plane light microscopy (*E*), TRITC (*D*), and FITC (*C*) filter setting. Ia-positive EC (*D, E, small arrow*) and Thy-1.2$^+$ EC (*C, E, large arrow*) represent mutually exclusive cell populations. Magnification × 400.

chain, ultrastructural demonstration of HRPO-reactive cells was performed as described elsewhere [8].

RESULTS AND DISCUSSION

Exposure of murine epidermal sheets to either monoclonal anti-Thy-1.1 (AKR/J) or monoclonal anti-Thy-1.2 (C3H/He, C3H/He nu/nu, C57Bl/6, BALB/c) using immunofluorescence techniques results in the visualization of primarily dendritic cells (Fig 1*A*). Allelic exclusion [1] was confirmed by the finding that monoclonal antibodies against one Thy-1 allelic determinant failed to react with EC from mouse strains displaying the other allele. Shape and number of Thy-1$^+$ EC varied considerably among different mouse strains and body regions studied. In all strains, ear and trunk epidermis contain densely and evenly distributed dendritic cells, whereas only small numbers of less dendritic and more rounded Thy-1$^+$ EC are encountered in the tail region (Table II). C57Bl/6 animals exhibit the highest density of Thy-1$^+$ EC followed by C3H/He, AKR/J, BALB/c, and C3H/He nu/nu mice (Table II). A direct comparison between C3H/He and C3H/He nu/nu mice reveals a 6- to 9-fold numerical decrease of Thy-1$^+$ EC in the latter strain as determined on ear and trunk epidermis, whereas density values obtained from tail skin are almost equal (Table II). Frequency analysis on single EC suspensions revealed that, depending upon strain and body region investigated, strongly Thy-1$^+$ cells comprised 0.8–2.7% of all EC. As opposed to epidermal sheet analysis, an extremely faint membrane fluorescence was discernible on approximately 20–30% of residual EC, which was not seen in any of the control specimens (control ascites, normal mouse serum).

Immunoelectron microscopic studies using HRPO-labeled antibodies demonstrated that Thy-1$^+$ EC represent a morphologically distinct nonkeratinocyte EC subpopulation. When studied in single EC suspensions, they acquire a rounded shape and a slightly villous surface covered with the HRPO reaction product (Fig 2*a,b*). The nucleus appears lobulated and the surrounding cytoplasm has a dense appearance and contains abundant intermediate-sized filaments (Fig 2*b*). These fila-

TABLE II. *Thy-1-positive epidermal cells: strain and body region distribution*

	Thy-1.2				Thy-1.1
	C57Bl/6	BALB/c	C3H/He	C3H/He nu/nu	AKR/J
Ear	721 ± 141	116 ± 125	538 ± 125	61 ± 67	471 ± 256
Trunk	612 ± 77	305 ± 98	568 ± 88	93 ± 93	639 ± 254
Tail	131 ± 40	24 ± 29	52 ± 26	46 ± 37	31 ± 31

NH_4SCN-separated sheets of ear, trunk and tail epidermis from animals bearing either the Thy-1.2 or the Thy-1.1 allele were acetone-fixed for 20 min at room temperature. After PBS washes, sheets were treated with 1:100 dilutions of either anti-Thy-1.2 or anti-Thy-1.1 monoclonal antibodies for 16 h at 4°C. After further washes, antibody reactivity was visualized by means of a 1:100 diluted FITC-antimouse IgM monoclonal antibody. Epidermal cells (EC) were mounted on glass slides, coverslipped, and viewed under a fluorescence microscope. Using a rectangular ocular grid, 10 fields within each body region were randomly chosen and the density of Thy-1^+ EC was determined and expressed as the number of cells (± SD) per mm^2 of skin surface.

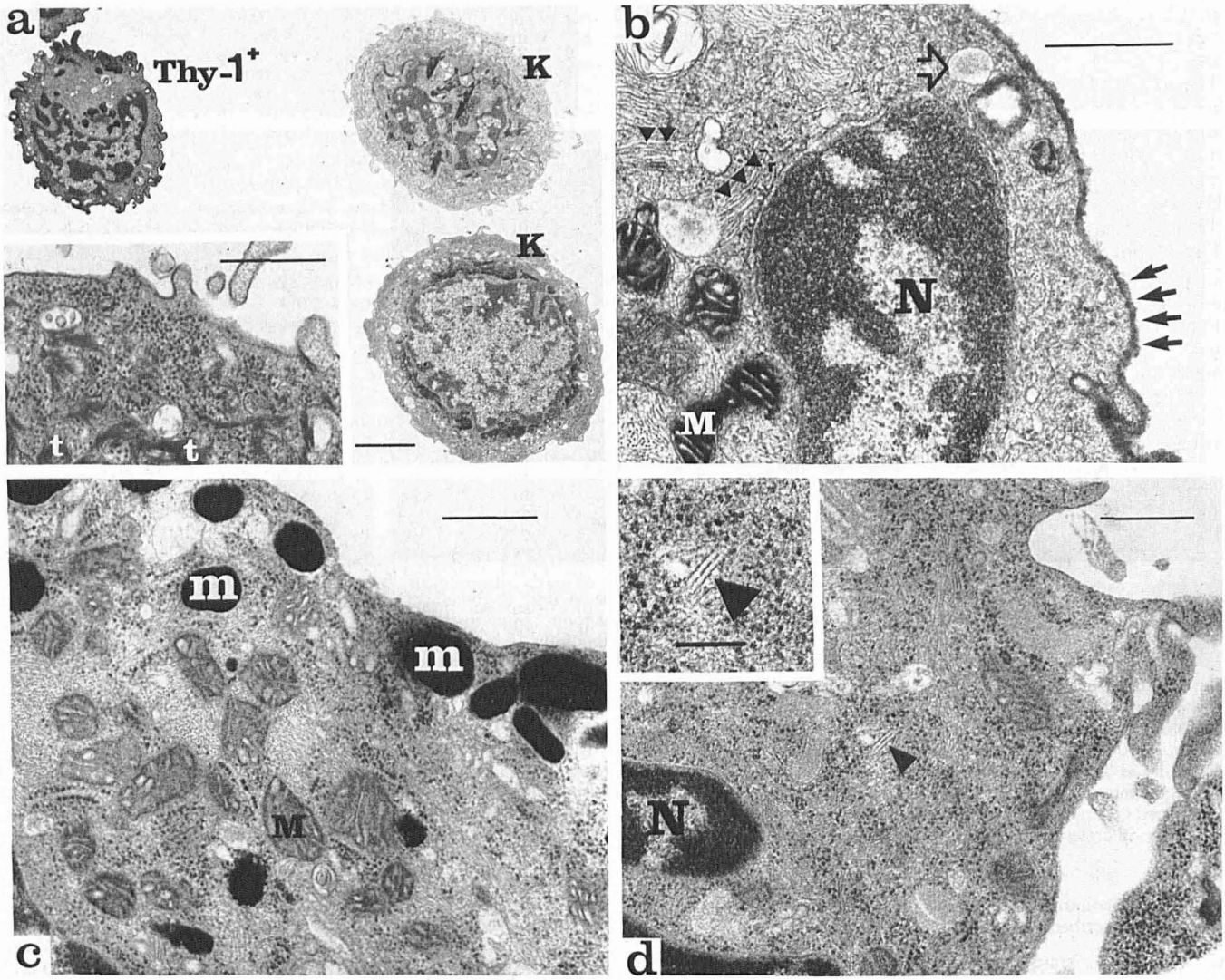

FIG 2. Immunoelectron microscopic identification of Thy-1-bearing murine EC. Nonirradiated and irradiated (not shown) Thy-1^+ EC as evidenced by deposition of HRPO reaction product onto the cell membrane (2*a*; 2*b*, *arrows*) exhibit a villous surface, a slightly lobulated nucleus, and a dark cytoplasm (2*a*, *bar* = 2 μm). Higher magnification (2*b*, *bar* = 0.5 μm) reveals abundant intermediate-sized filament bundles (*arrowheads*) and several cytoplasmic vesicles bound by a unit membrane and containing a central, granular core (*open arrow*). Keratinocytes (*K*) (2*a*) identified by tonofilament bundles (*t*) (2*a*, *inset*, *bar* = 0.5 μm) as well as Langerhans cells (2*d*; *bar* = 0.5 μm; *inset*, *bar* = 0.2 μm) characterized by Birbeck granules (*arrowheads*) are devoid of HRPO reaction product. Melanocytes (2*c*; *bar* = 0.5 μm) from irradiated epidermis which can be easily identified by the presence of singly dispersed, stage IV melanosomes (*m*), also lack anti-Thy-1.2 reactivity. *N* = nucleus; *M* = mitochondria. Control specimens (replacement of anti-Thy-1.2 monoclonal antibody by control ascites or normal mouse serum) were consistently negative.

ments proved to be of the vimentin type [9] as evidenced by their reactivity with a monoclonal antivimentin antibody. Keratin filaments, desmosomes, melanosomes, Birbeck granules, and Merkel cell granules were never found within these cells which, at least on morphologic grounds, sets them apart from keratinocytes, mature melanocytes, Langerhans cells, and Merkel cells. Indeed, none of these cell populations displayed anti-Thy-1 reactivity by immunoelectron microscopy (Fig 2*a*,*c*,*d*). This is also true for the so-called "indeterminate cells" which are generally considered to belong to the Langerhans cell line-

age [10]. The possibility that Thy-1$^+$ EC may represent a unique, hitherto undescribed EC population was strengthened by double-staining immunofluorescence procedures performed on both epidermal sheet preparations and single cell suspensions, which showed that Ia-positive EC and Thy-1-bearing EC represented mutually exclusive cell populations (Fig 1*C,D,E*). Since Ia antigens within the murine epidermis are predominantly expressed on Langerhans cells [6] these data, together with the ultrastructural studies, prove that Thy-1$^+$ EC do not include Langerhans cells, but do not definitely rule out an ontogenetic relationship between these two cell populations.

When epidermal sheets of C57Bl/6 and C3H/He mice were simultaneously tested for tyrosinase activity [11] and Thy-1.2 expression, it became apparent that dopa-positive dendritic EC and Thy-1$^+$ EC were separate cell populations which were located at different levels within the epidermis (Fig 1*B*). To exclude the possibility that Thy-1$^+$ EC or, at least, a portion thereof might represent melanocyte precursors or inactive melanocytes (lacking morphologic and enzymatic markers of melanogenesis) and that Thy-1 might thus represent a differentiation marker that is lost during the process of melanocyte maturation or activation, we irradiated C3H/He mice with UVA according to an established protocol [7]. The rationale of this experiment was that transformation of the (hypothetical) Thy-1$^+$, dopa-negative melanocyte precursors into mature Thy-1$^-$, dopa-positive melanocytes would result not only in a reciprocal quantitative change in the respective cell populations but also in the emergence of dendritic cells bearing both the Thy-1 and tyrosinase marker. Although dopa-positive cells were highly increased and Thy-1$^+$ EC were slightly decreased in number after irradiation, both tyrosinase-positive and Thy-1$^+$ EC were again mutually exclusive in that despite careful sampling a cell bearing both markers was never found. In keeping with these results, immunoelectron microscopic studies of UVA irradiated ear epidermis revealed that cells containing singly dispersed melanosomes (i.e., melanocytes) were consistently Thy-1$^-$ (Fig 2*c*). We therefore consider it highly unlikely that Thy-1$^+$ EC are related to the melanocyte lineage.

Although expression of Thy-1 antigen is not limited to T cells in cultures of mouse hemopoietic cells [12], there is an intimate in vivo and in vitro association between Thy-1 expression and T-cell ontogeny and differentiation [1,13]. Thus the foregoing results, in particular the finding that athymic mice—which are known to exhibit highly decreased numbers of Thy-1$^+$ cells in spleen and lymph node preparation [14]—have only few Thy-1$^+$ EC (Table II), suggested to us that Thy-1$^+$ EC represent thymus-dependent lymphocytes at a particular stage of development. However, the additional finding that monoclonal reagents against Lyt-1,2,3 antigens failed to react with Thy-1$^+$ EC but readily stained lymphoid cells from various lymphoid tissues argues against a thymocytic or peripheral T-lymphocytic nature of Thy-1$^+$ EC since both thymocytes and peripheral T cells express, at least, one of the Lyt antigenic determinants [15]. Further experiments revealed that the vast majority ($\geq$ 90%) of Thy-1$^+$ EC bore the Ly-5- specificity whose expression appears to be restricted to hemopoietic cells [16, 17]. This finding strongly suggests that Thy-1$^+$ EC belong to a certain lineage of bone marrow-derived hemopoietic cells and renders the possibility rather unlikely that they are related to ontogenetically different cell types which are also known to express the Thy-1 antigen, e.g., fibroblasts [18], neurons [19], and mammary myoepithelial cells [20].

Although the exact nature and, more importantly, the functional role of Thy-1$^+$ EC escapes us at the present time, the mere presence of a novel dendritic epidermal cell population—most likely originating from the bone marrow—will necessitate a reevaluation of the cellular origin of established epidermal cell functions and will open up a new dimension of research concerned with the elucidation of the biologic role of the epidermis.

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