0022-202X/85/8501s-0085s$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 85:85s–90s, 1985

Vol. 85, No. 1 Supplement
Printed in U.S.A.

Origin and Function of Thy-1$^+$ Dendritic Epidermal Cells in Mice

PAUL R. BERGSTRESSER, M.D., SABRA SULLIVAN, B.S., J. WAYNE STREILEIN, M.D., AND ROBERT E. TIGELAAR, M.D.*

Departments of Dermatology and Internal Medicine (PRB, RET), and the Graduate Program in Immunology (SS), Dallas, Texas, and the Department of Microbiology and Immunology (JWS), University of Miami School of Medicine, Miami, Florida, U.S.A.

The epidermis of normal mouse skin incorporates a newly-recognized population of dendritic cells which express relatively large amounts of the cell surface glycoprotein, Thy-1 antigen. These cells, termed Thy-1$^+$dEC, are distinct from both epidermal Langerhans cells (LC) and melanocytes, and they populate cutaneous sites in surface densities which range to as high as 580 cells/mm^2, approximately two-thirds that of LC. Studies of lethally irradiated mice which were reconstituted with semiallogeneic bone marrow cells and mice which received grafts of semiallogeneic skin have demonstrated that some, if not all, Thy-1$^+$dEC are of bone marrow origin, and that they are capable of migrating into epidermis from a vascular source. Thy-1$^+$dEC expressed both asialo GM$_1$ and a cell surface determinant recognized by the monoclonal antibody 20-10-5S, further suggesting their functions will be included among those normally ascribed to lymphoreticular cells. Isolation of epidermal cells with the Fluorescence Activated Cell Sorter (FACS) was successful in producing relatively pure populations of Thy-1$^+$dEC and LC. Such technological advances as this should facilitate testing several hypotheses concerning the ultimate function of these cells, including the possibilities that they are antigen-presenting cells which selectively activate down-regulating signals, T lymphocytes, natural killer (NK) cells, or natural suppressor (NS) cells.

Five years ago we presented in the Salishan Symposium data which supported the relatively new hypothesis that dendritic epidermal Langerhans cells (LC) possessed several functional capacities which were of immunologic relevance [1]. That data and data collected subsequently comprise part of an expanding body of work from several laboratories which have characterized for LC a variety of functional properties, including the capacities of rapid migration [2] and antigen presentation [2,3], and a selective susceptibility to ultraviolet radiation [3]. In the last 2 years we have experienced déjà vu, confronted with a new dendritic cell in mouse epidermis [4,5], a cell rich with as yet unproven immunologic possibilities.

Work in several laboratories has been directed at identifying the biologic relevance of this unique dendritic cell which expresses relatively large amounts of the cell surface glycoprotein, Thy-1 antigen (Thy-1$^+$dEC) [4,5,6]. Our own bias in this investigation originates with 3 constraints: (1) a specific interest in immunologically based cutaneous investigation, (2) the identification of this cell through the presence of Thy-1 antigen, a differentiation antigen for T lymphocyte maturation in the mouse, and (3) the anatomic and morphologic similarities between Thy-1$^+$dEC and epidermal LC. This report reviews the place of Thy-1$^+$dEC within the epidermal economy, its several unique characteristics, and a method for its isolation. These studies, although not conclusive with respect to ultimate functions, lead to a variety of hypotheses concerning the immunologic relevance of these cells, hypotheses which may be examined by in vitro and in vivo tests of function.

THY-1 ANTIGEN-BEARING DENDRITIC CELLS (THY-1$^+$dEC) POPULATE NORMAL MOUSE EPIDERMIS

We and others observed in mouse skin a population of dendritic cells which superficially resembled epidermal LC [4,5]. Initial studies concerning this new cell population were conducted with inbred strains of mice which were congeneic either for Class II alloantigens or for Thy-1 antigen, allowing us to employ well-characterized monoclonal antibodies to differentiate them on the basis of cell surface markers from other epidermal cells [4]. Much of the interest in observing Thy-1 antigen in large amounts on a population of epidermal cells arose because this cell surface glycoprotein exhibits restricted tissue distribution. Thy-1 antigen has been found in high concentration on brain [7] as well as on fibroblasts [8], mammary epithelial cells [9], a high percentage of epidermal cells [10], natural killer (NK) cells [11] and natural suppressor (NS) cells [12]. Most importantly, Thy-1 is expressed as a differentiation antigen for T-lymphocyte maturation [13]. Thy-1 also exhibits considerable homology with the invariant regions of immunoglobulin [7], Class I alloantigens, and beta-2 microglobulin, suggesting that all of these cell surface proteins may be derived from a common and more primitive predecessor [14].

For the identification of Thy-1$^+$dEC, whole mounts of epidermis in acetone-fixed specimens were stained and then examined "en face" with the fluorescence microscope as described previously [4,15]. Employing either fluoresceinated monoclonal anti-Thy 1.2 or conjugated anti-Thy 1.2 followed by the appropriate secondary reagent (Table I), we observed regularly spaced and brightly stained cells in epidermis taken from ear, back, abdominal wall, and tail skin. Upon higher magnification, Thy-1$^+$dEC were observed to be mainly dendritic, although round, oval, and angular forms could also be observed [6]. Such nondendritic forms were observed infrequently in specimens which were prepared most rapidly and carefully, suggesting that this loss of dendrites represents an artifact of tissue preparation.

Thy-1$^+$dEC observed in whole mounts of epidermis contrasted with LC with their more irregular distributions and considerably greater variation in form. By contrast, Thy-1$^+$dEC were restricted in tail epidermis to interscale regions, corresponding with a similar restricted distribution of LC in the same tissue. An analysis of surface densities in several mouse

Supported in part by United States Public Health Service Grants AI-17363, CA-09082, and by a grant from Mary Kay Cosmetics, Inc.
* Dr. Tigelaar is the recipient of NIH Research Center Career Development Award AM-00940.
Reprint requests to: Paul R. Bergstresser, M.D., Department of Dermatology, University of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Abbreviations:
FACS: fluorescence activated cell sorter
LC: Langerhans cells
NK: natural killer
NS: natural suppressor
Thy-1$^+$dEC: dendritic epidermal cells which express Thy-1 antigen
UVB: ultraviolet radiation from spectrum B

TABLE I. *Reagents used to identify dendritic epidermal cells by immunofluorescence microscopy [4,15]*

Reagent	Conjugate
Monoclonal anti-Thy 1.2	None, biotin, fluorescein
Monoclonal anti-I-A^k	None, biotin
Antimouse immunoglobulin	Fluorescein
Avidin	Rhodamine, fluorescein

strains revealed significant variation among strains, with densities as high as 580 cells/mm^2 for the B10.A ear epidermis and as low as 5 cells/mm^2 observed in BALB/c ear (Table II). Although the biologic relevance of these differences among strains is not known, the observation does suggest that appropriate tests of function may lead to differences among these strains of mice.

THY-1$^+$dEC ARE DISTINCT FROM LC AND MELANOCYTES

Despite the morphologic, numerical, and anatomical differences between LC and Thy-1$^+$dEC it was important to determine whether cells would ever express both determinants simultaneously; that is, were investigators, despite these unusual patterns, simply observing a phenotypic marker which was expressed on some or all epidermal LC. To test this possibility specimens of epidermis from appropriate mice were first exposed to anti-Thy-1.2 followed by fluoresceinated antimouse immunoglobulin, and then to biotinylated anti-IA^k, followed by rhodaminated avidin. These reagents identified 2 different populations of dendritic epidermal cells in normal mouse skin. In photomicrographs made by double exposure, and employing light serially from different excitor systems, 2 distinct cell populations could be observed [4]. This study was repeated on multiple occasions for ear skin, abdominal wall skin, and tail skin, each with identical results. When abdominal wall epidermis was examined after disaggregation, as a single cell suspension, approximately 5% of cells exhibited red fluorescence (Ia$^+$ LC) or green fluorescence (Thy-1$^+$dEC), with no cell ever exhibiting both antigenic determinants [4].

Although melanocytes also possess an in vivo dendritic configuration, it was observed quite early that Thy-1$^+$dEC never contained the black granules of melanocytes in any of the specimens examined from 4 pigmented strains of mice [4]. This and the distinct ultrastructural features of Thy-1$^+$dEC led us and others to the conclusion that there were not 2 but 3 distinct dendritic cell populations in normal mouse epidermis [4,5].

This initial work led to several hypotheses concerning the identity of Thy-1$^+$dEC, that they were: (1) of T lymphocyte lineage, (2) perhaps a natural killer (NK) cell, or (3) perhaps a previously unrecognized structural or constitutive cell of epithelial origin. It was recognized in this regard that lymphoid cells may be distinguished from structural cells such as neurons, fibroblasts, and myoepithelial cells, through their marrow derivation, their capacity to migrate into nonlymphoid tissues via vascular routes, and their capacity to repopulate such tissues after ontogeny is complete. We therefore employed skin grafts and bone marrow grafts to determine whether Thy-1$^+$dEC possessed a bone marrow origin as had been suggested previously [5], or even whether they had the capacity to migrate into epidermis at all.

THY-1$^+$dEC ARE DERIVED FROM BONE MARROW PRECURSORS

Two experimental procedures were employed to test the hypotheses of bone marrow origin and migratory capacity. They made use of parental and F_1 hybrid tissue from 2 inbred strains (Table III). AKR mice possess the IA^k haplotype in the *I* region of the mouse major histocompatibility complex (MHC) and they express the Thy-1.1 haplotype at Thy-1. Conversely, DBA/2 mice are IA^{d+} and Thy 1.2$^+$ respectively. Obviously, the

TABLE II. *Surface densities of Thy 1$^+$dEC in whole mounts of ear and abdominal wall epidermis from 6 strains of mice [4]*

Strain	Cells/mm^2[a]	
	Abdominal wall	Ear
B10	440 ± 95[b]	550 ± 80
B10.A	480 ± 65	580 ± 110
C57/BL/6	340 ± 110	468 ± 80
C3H	540 ± 80	120 ± 120
A/J	340 ± 80	40 ± 5
BALB/c	170 ± 100	5 ± 10

[a] Cells identified with biotinylated monoclonal anti-Thy 1.2 and fluoresceinated avidin, followed by examination with a calibrated fluorescence microscope [4].

[b] Mean ± 1 SD in 10–50 contiguous fields of view.

TABLE III. *Ia and Thy-1 haplotypes of mouse strains employed to test the hypothesis that Thy-1$^+$dEC are of bone marrow origin*

Strain	Ia	Thy-1
AKR	k	1.1
DBA/2	d	1.2
(AKD2)F_1	k and d	1.1 and 1.2

F_1 hybrid of AKR and DBA/2, termed (AKD2)F_1, expresses both parental haplotypes at both loci (Table III). We employed well-characterized monoclonal reagents for the identification of cells which express each antigenic determinant, once again in EDTA-separated, acetone-fixed whole mounts of epidermis [15].

In the first experiments, AKR whole skin grafts of body wall skin were placed orthotopically on full thickness defects prepared on (AKD2)F_1 recipients. The migration of recipient-derived cells into these tolerated grafts was monitored by the appropriate reagents. From these experiments 4 observations deserve comment. First, IA^{d+} LC, obviously of recipient origin, infiltrated grafts of AKR skin to normal densities within 2 weeks. Secondly, the sharp transition between recipient and donor epidermis was maintained throughout, with no evidence for lateral migration of Thy-1$^+$dEC into epidermis from graft margins. Thirdly, Thy 1.2$^+$ cells, also of recipient origin, were also observed to migrate into the epidermis of the same grafts, although their migratory rates were substantially slower than that observed for LC. As late as 9 months after grafting, the surface density of Thy-1$^+$dEC had risen to as high as 100 cells/mm^2 in some specimens, a value which was still only 25% of their numbers in normal skin. The fourth observation, made with monoclonal anti-Thy 1.1, was that donor Thy-1$^+$dEC commonly failed to survive the grafting process [15]. We concluded from these studies that Thy-1$^+$dEC share with LC the capacity to migrate into tolerated grafts of semi-allogeneic skin, although they do so at rates far slower than that observed for LC. Moreover, it was possible to deplete skin of Thy-1$^+$dEC by the simple process of skin grafting. It is instructive in this regard that the macroscopic appearance of skin depleted of Thy-1$^+$dEC by grafting was indistinguishable from those skin grafts which had been partially repopulated, suggesting that their function is not related to the structural integrity of skin.

In the second set of experiments AKR mice were lethally irradiated and then reconstituted with 20×10^6 bone marrow cells from (AKD2)F_1 donors. Tissues from recipients which had been grafted successfully were then examined periodically. By 10 weeks, dendritic Thy 1.2$^+$ cells of bone marrow origin were observed in low numbers in the epidermis of ear skin, and at 4 months similar Thy-1$^+$dEC were seen in both ear and abdominal wall skin, but once again at low surface densities. By contrast, all epidermal LCs were IA^{d+} by ten weeks after grafting. By 5 months, Thy-1$^+$dEC, of donor origin, had populated all specimens of ear and abdominal wall skin, with highest densities reaching 100 cells/mm^2 in specimens taken from the ear. We as well as others [16] in independent experiments

concluded that some, if not all, Thy-1$^+$dEC in murine epidermis are derived from bone marrow precursors, and that these dendritic cells are distinct from constitutive cutaneous cells in their capacity to migrate into epidermis during life.

THY-1$^+$dEC EXPRESS AN UNUSUAL THY-1 DETERMINANT

Studies with a distinctive monoclonal antibody have provided additional insight into the nature of the Thy-1 determinant expressed on Thy-1$^+$dEC. Auchincloss, Ozato, and Sachs have characterized a monoclonal antibody which is directed against Thy-1 determinants in mice [17]. This antibody, identified as 20-10-5S, shows allospecificity for peripheral blood T-lymphocytes but no allospecificity on thymocytes. That is, 20-10-5S identifies peripheral T lymphocytes only in strains of the Thy-1.2 haplotype, but it identifies thymocytes in both Thy-1.1$^+$ and Thy-1.2$^+$ strains. Moreover, this antibody differs from conventional anti-Thy-1 reagents in that exhaustive absorption with murine brain tissue fails to deplete its cytotoxic activity against T-lymphocytes. One interpretation of the observations made with this reagent is that during early stages of T-lymphocyte differentiation (i.e., thymocytes) a specific determinant is held in common by both Thy-1.1 and Thy-1.2 haplotypes, but that with maturation this determinant is either lost or modified in the Thy-1.1 haplotype [17].

Since 20-15-5S antibody discriminates between the Thy-1 determinant expressed on tissue of the central nervous system and the Thy-1 determinant expressed by lymphoid cells, we employed this reagent to determine whether Thy-1$^+$dEC from Thy-1.2$^+$ mice would express the determinant detected by 20-10-5S, thereby distinguishing them from cells of neural origin. By fluorescence microscopy, employing 20-10-5S and appropriate secondary reagents, dendritic cells were seen within the epidermis of Thy-1.2$^+$ mice. The distribution and morphologic appearance of these cells was indistinguishable from that observed previously with the conventional anti-Thy-1 reagents. To determine whether 20-10-5S and conventional anti-Thy 1.2 did identify the same cell populations, epidermal specimens were treated in sequence with 20-10-5S, fluoresceinated anti-mouse Ig, biotinylated monoclonal anti-Thy 1.2, and then rhodaminated avidin. By fluorescence microscopy, all Thy-1.2$^+$ cells were invariably labeled with 20-10-5S. In specimens treated with biotinylated anti-I-A^k rather than anti-Thy-1.2, it was observed that no cell possessed both Ia antigens and the Thy-1 antigen identified with 20-10-5S. We concluded from this study that Thy-1$^+$dEC cells resemble lymphoreticular cells in their capacity to express this determinant.

The second important characteristic of monoclonal antibody 20-10-5S is the failure to discriminate between Thy-1.2$^+$ and Thy-1.1$^+$ thymocytes. To determine whether 20-10-5S would discriminate between the Thy-1 determinants on epidermal cells in Thy-1.1$^+$ and Thy-1.2$^+$ strains, specimens from abdominal wall skin from AKR and C3H mice were stained with serial dilutions of 20-10-5S; additional control specimens from both strains were stained with conventional anti-Thy-1.2 or anti-Thy-1.1. Anti-Thy-1.2 identified dendritic epidermal cells in C3H but not AKR mice, whereas anti-Thy-1.1 identified dendritic epidermal cells in AKR but not C3H mice. By contrast, 20-10-5S identified dendritic cells in both strains of mice. Moreover, as judged by serial dilutions, the density of the Thy-1 determinant detected by 20-10-5S on these epidermal cell populations appeared to be similar in both strains. We concluded that Thy-1$^+$dEC resemble thymocytes more closely than peripheral T-lymphocytes in their failure to exhibit allospecificity for the Thy-1 determinant detected by 20-10-5S. However, it is important to recognize that Thy-1$^+$dEC have not been found to express detectable amounts of other cell surface alloantigens commonly expressed by thymocytes or peripheral T cells, such as Lyt1, Lyt2, L3T4 or TL ([7], and our laboratory, unpublished observations).

THY-1$^+$dEC EXPRESS THE GLYCOLIPID ASIALO GM$_1$

Asialo GM$_1$ is a cell surface glycolipid [18] of limited tissue distribution, having been found on a variety of cells with immunologic relevance, including natural killer (NK) [19] cells, natural suppressor (NS) [12] cells, certain T cells [20], as well as certain cells of monocyte/macrophage lineage [21]. Employing a polyclonal rabbit antiserum against asialo GM$_1$ [22] in a two-step immunofluorescence assay, we examined mouse epidermis for this determinant. In specimens of epidermis from back, abdominal wall, tail, and ear skin from both Thy 1.1$^+$ and Thy 1.2$^+$ mouse strains, this cell surface determinant was observed on a dendritic cell population which exhibited a distribution and morphology similar to that of Thy-1$^+$dEC. By 2-color immunofluorescence we observed asialo GM$_1$ to occur exclusively on Thy-1$^+$dEC rather than on Ia$^+$ LC or melanocytes. Although this observation obviously does not identify the function of Thy-1$^+$dEC as being that of NK cells, it does suggest that they may exhibit some biologic features in common; furthermore, the presence of asialo GM$_1$ offered an additional distinctive manner of identifying their presence.

NOT ALL DENDRITIC EPIDERMAL ASIALO GM$_1^+$ CELLS EXPRESS THY-1 ANTIGEN IN GRAFTS OF MOUSE SKIN

Several AKR skin grafts which had been in residence on (AKD2)F$_1$ recipients for as long as 9 months were available for examination for asialo GM$_1^+$ cells. Grafts were examined simultaneously for Thy-1.2$^+$dEC or IA^{d+} LC and then for the presence of asialo GM$_1$. In 3 consecutively examined grafts, considerably more asialo GM$_1^+$ cells than Thy-1$^+$dEC were observed. In 1 representative region the mean ± 1 SD for Thy-1.2$^+$dEC was 15 ± 20 cells/mm^2 and for asialo GM$_1^+$ dendritic cells 170 ± 60 cells/mm^2. By 2-color fluorescence microscopy every Thy-1.2$^+$ cell was also asialo GM$_1^+$. Different portions of each graft were examined simultaneously for I-A^{d+} and asialo GM$_1^+$ cells; I-A^{d+} LC were seen in normal numbers and they were invariably asialo GM$_1^-$. We conclude from these observations that there exists a substantial discordance between the expression of asialo GM$_1$ and Thy-1.2 on those cells in skin grafts of adult skin which had been placed on adult recipients, suggesting that at least in the adult, those precursor cells which migrate into epidermis express little or no Thy-1 antigen and that only after arrival in the epidermis does the expression of Thy-1 antigen increase to an amount sufficient to be seen by fluorescence microscopy.

ONTOGENY OF THY-1$^+$dEC

To assess the appearance of Thy-1$^+$dEC in the epidermis during the perinatal period, appropriate monoclonal reagents were used to stain epidermal whole mounts at specific times before and after birth. This study also included a comparison during ontogeny between LC and Thy-1$^+$dEC, and therefore the data concerning the appearance of Ia$^+$ and ATPase$^+$ cells in epidermis is included as well. Note that the appearance of Thy-1$^+$dEC during this period was observed to be unrelated to either Ia$^+$ cells or to ATPase$^+$ cells (Table IV). Thy-1$^+$dEC were occasionally present before and at the time of birth, and increased to greater than 140 cells/mm^2 by day 2, contrasting with the relative absence of cells expressing Ia antigens at that time. Following day 2 there was a gradual increase in the number of Thy-1$^+$dEC to approximately 50% of adult values by day 9. These data are similar to that observed in tail, back, and abdominal wall skin (data not shown).

It is of particular interest that dendritic ATPase cells were present in numbers as high as 400/mm^2 even 2 days before birth. By contrast, cells bearing Ia antigens were not found until 4 days later and they did not rise to substantial numbers for 2 more days. The most likely conclusion to be drawn from

TABLE IV. *Surface densities of dendritic cell populations in C3H mouse ear epidermis during the perinatal period*

Day	Thy-1$^+$ dEC[a]	Langerhans cells[b]	
		ATPase$^+$	I-A^{k+}
−2	<20	307 ± 21[c]	<20
0	58 ± 17	—	<20
2	128 ± 7	416 ± 13	<20
4	149 ± 15	512 ± 64	446 ± 83
9	147 ± 24	960 ± 64	887 ± 41
Adult female	330 ± 20	980 ± 70	

[a] Thy 1.2$^+$dEC by fluorescence microscopy.
[b] LC identified by ATPase and I-A^k by fluorescence microscopy.
[c] Mean ± 1 SD in 10 contiguous fields of view.

this observation is that Ia antigen in amounts necessary for visualization by fluorescence microscopy are not expressed on LC until sometime after migration into the epidermis.

In a separate study, specimens of skin were examined for asialo-GM$_1^+$ cells on the day of birth (Table V). We observed a clear discordance between asialo GM$_1^+$ and Thy-1$^+$ dendritic cells, suggesting that the appearance or at least a large increase in the amount of Thy-1 antigen may occur only after the entry of cells into the epidermis. These results from the neonatal study complement the above mentioned study with skin grafts; both are consistent with the possibility that epidermis has the capacity to increase substantially the expression of Thy-1 antigen on some, if not all, Thy-1$^+$dEC.

THY-1$^+$dEC ARE SEPARABLE FROM LC AND OTHER EPIDERMAL CELLS WITH THE FLUORESCENCE ACTIVATED CELL SORTER (FACS)

Although the need to obtain purified populations of epidermal cells has been perceived for some time, efforts to isolate mouse epidermal cell populations in our laboratories as well as in the laboratories of others have been relatively unsuccessful in comparison with what has been obtained for other species [23–25]. In the interim, we have employed indirect strategies to identify the function of LC. We have worked with skin which was relatively depleted of functioning LC, first by using skin sites which were deficient naturally, as in cornea, hamster cheek pouch, and mouse tail [26], and then by using sites which were deficient artificially such as occurs after cellophane tape stripping [2]. Moreover, work with skin sites which are depleted of normally functioning LC as after UVB irradiation [27], has allowed us to assign to LC a central role for antigen presentation in contact hypersensitivity and for the immunization with skin grafts of transplantation alloantigens [28]. It is also of interest in these studies that in the absence of normally functioning LC a significant trend toward down-regulation was observed [29]. This work with immunizing protocols in vivo is consistent with the experiments of Sauder et al [30] and of Granstein et al [31], which strongly suggested that other populations of epidermal antigen-presenting cells may exist and that these cells are capable of initiating competing, down-regulating responses. Despite the success of these indirect experiments concerning the function of epidermal subpopulations of cells, the need to isolate these cells persisted.

The path to success in isolating dendritic cells from mouse epidermis began by solving the problem of the stratum corneum debris which would accompany the epidermal cell suspension obtained from trypsin-disaggregated epidermis. This debris with its capacity to bind immunoreagents nonspecifically led to considerable contamination during isolation procedures such as panning or cell separation with the FACS. Consequently, centrifugation through Ficoll was employed to remove debris prior to such separation procedures. Surprisingly, we observed for hamster epidermis that not only had most cellular debris been removed, but that we had enriched LC to as high as 40% [32]. In this procedure, as it is now used, cells from the Ficoll-medium interface are recovered, washed, and then exposed to the appropriate concentration of either monoclonal anti-IA^k or monoclonal anti-Thy 1.2, followed by washing and the appropriate exposure to a fluorescein-conjugated F(ab)'2 rabbit antimouse immunoglobulin [33].

TABLE V. *Surface density of epidermal cells in newborn mouse skin (C3H) using 3 cell surface determinants*[a]

Skin Site	Thy 1.2	Asialo GM$_1$	IA^k
Abdomen	25 ± 10[b,c]	190 ± 20	40 ± 15[d]
Back	30 ± 10	100 ± 15	95 ± 15
Ear	60 ± 15	120 ± 15	50 ± 10
Tail	20 ± 10	130 ± 10	10 ± 5

[a] All cells enumerated by fluorescence microscopy.
[b] Mean ± 1 SD in 10 contiguous fields.
[c] By 2-color immunofluorescence all Thy-1.2$^+$ cells were asialo GM$_1^+$.
[d] IA^{k+} cells were invariably asialo GM$_1^-$.

TABLE VI. *Isolation and recovery of dendritic cells from mouse epidermis (CBA)*

Stage	Cell number[a]	Percent positive		Cell recovery	
		IA$^+$	Thy 1$^+$	IA$^+$	Thy 1$^+$
Excision	300 × 10^6	3%	2%	100%	100%
Disaggregation	80 × 10^6	2%	5%	18%	66%
Ficoll separation	8 × 10^6	10%	40%	8%	53%
FACS (IA$^+$)	0.5 × 10^6	97%		5%	
(Thy-1$^+$)	0.8 × 10^6		95%		11%

[a] Cell number represents the total number of cells removed after each stage of the isolation procedure.

Data from FACS sorting of Ia$^+$ and Thy-1$^+$ epidermal cells have been reported previously [33], and the data for recoveries in a typical experiment are tabulated in Table VI. In one experiment, skin from 10 mice was excised for disaggregation, representing approximately 15,000 mm^2 of skin surface and therefore approximately 300 × 10^6 epidermal cells (20,000 cells/mm^2) [34]. After disaggregation, 80 × 10^6 cells were recovered, although in other experiments it ranged up to 120 × 10^6. In general, the percentage of Ia$^+$ and Thy-1$^+$ cells in such "crude" disaggregates were approximately 2 and 3% respectively, and recoveries from the original in vivo population were calculated at 18 and 66%. Although Ficoll separation was associated with a 10-fold loss in total cells, declining from 80 to 8 × 10^6, there was only a relatively small loss of dendritic cells with a substantial enrichment for both. The percentage of LC increased from 2–10% and Thy-1$^+$dEC cells from 5–40%. The percentage of LC increased from 2–10% and Thy-1$^+$dEC cells from 5–40%. These populations were then subjected to cell sorting.

By cell surface fluorescence for Thy-1 antigen, the cells recovered from the Ficoll-medium interface exhibited two discrete cell populations before sorting. When the data were displayed as a dot plot, with coordinates representing cells size (light scatter) and Thy-1 antigen (fluorescence intensity), a clear separation between highly positive and other cells was seen. A similar separation occurred for Ia$^+$ LC as well. After sorting for Thy-1$^+$ cells, the recovered cells were 95% pure upon reanalysis, and for Ia$^+$ cells, 97% purity was achieved. Final numerical recoveries were calculated as 5% and 11% respectively. By electron microscopy the majority of Ia$^+$ cells exhibited characteristic Birbeck granules.

DISCUSSION

The studies reported in this paper outline several distinctive features of the newly recognized population of dendritic epidermal cells in mouse skin. Thy-1$^+$dEC are derived, at least in part, from bone marrow precursors and they migrate into epidermis from a vascular route. They possess a distinctive phenotype, including an unusual Thy-1 determinant which links them more closely to thymocytes than to peripheral T

lymphocytes. Moreover, studies with the cell surface glycolipid, asialo GM_1, suggest that Thy-1 antigen is expressed in large amounts only after cellular migration into epidermis during both the perinatal period and after grafting with semiallogeneic skin.

The ability to isolate subpopulations of epidermal cells in relatively pure numbers with the FACS as outlined above will permit investigators to address more accurately questions concerning the biologic function of Thy-1$^+$dEC, particularly in relation to the functions of LC and keratinocytes. Previous work in our own laboratories has helped to identify roles for LC in allograft recognition and during the induction of contact hypersensitivity [27]. One line of investigation concerned the capacity of relatively low doses of ultraviolet from spectrum B (UVB) to convert an otherwise immunizing regimen of hapten painting for CH into one which is tolerogenic [29]. This work demonstrated that tolerogenic signals also may originate in the skin, and furthermore it posed the testable hypothesis that an antigen-presenting cell for suppressor cells resides in skin. These in vivo studies were complemented by the work of Sauder et al [30] who observed that while derivatized epidermal cells normally lead to an immunogenic response when injected subcutaneously, cells which are first treated with UVB radiation induce a tolerogenic rather than an immunogenic response. Granstein and his associates [31] subsequently demonstrated that there exists an IJ$^+$ and UVB-resistant epidermal cell which is required for the induction of suppressor cells for delayed type hypersensitivity to the azobenzenearsonate (ABA) hapten. This indicated once again the presence in epidermis of an antigen-presenting cell for suppression. Most recently, studies from our own laboratories using FACS-purified populations of epidermal cells derivatized with the hapten trinitrobenzene sulphonate (TNBS) demonstrate that the i.v. inoculation of small numbers of Thy-1$^+$dEC (but not LC or keratinocytes) results in significant down-regulation of the CH response to trinitrochlorobenzene (Sullivan et al, unpublished results). The obvious and testable hypothesis which integrates all of the above studies is that the phenotypic characteristics of one or more epidermal cells necessary for the induction of down regulatory (tolerogenic) signals includes UVB resistance, as well as the expression of IJ and Thy-1 determinants.

The identification of Thy-1$^+$dEC as a second population of immigrant bone marrow-derived cells in mouse epidermis also emhasizes the need for a new model of epidermal function. Most conventional models of the physiologic processes which occur in epidermis have been constructed with the assumption that epidermis is largely a tissue of proliferation, maturation, and differentiation. Implicit in this view of epidermis as a proliferative population of cells is the notion that the identity of those cells which reside in epidermis is relatively constant; resident epidermal cells are simply descendants from a proliferative pool of precursor cells. Although epidermal cell proliferation is indeed dynamic in this sense, the new model must incorporate the realization that epidermal cellular composition is also dynamic.

The observation that bone marrow-derived cells occur in normal mouse epidermis leads to the conclusion that resident keratinocytes provide a potential space which is highly attractive to them. Although earlier studies employing radiation chimeras demonstrated LC to be bone marrow-derived [35,36], our more recent work with tape-stripped skin grafts demonstrated that LC have the capacity to repopulate regenerating epidermis with striking rapidity [2]. Thus, one must assign to epidermis and perhaps to keratinocytes within that structure not only the elaboration of important cytokines which modulate the function of lymphocytes (including directional movement) [37–39], but also the elaboration of signals which lead to the attraction and retention of blood-borne epidermal LC precursors. A similar set of signals must now be postulated to account for the observations that Thy-1$^+$dEC are also of bone marrow origin and also migrate into epidermis, albeit at substantially lower rates than LC. The recognition that epidermis is thus dynamic in this compositional sense implies that the functional attributes of skin replete with such cells will be altered when they are absent. This issue is not trivial, since our studies with UVB radiation [27] demonstrate that an immunologic signal perceived in the absence of normally functioning LC leads to a relatively permanent change in systemic immunologic function, even though the skin is subsequently replenished with normal numbers of such cells. This capacity for immunologic memory means that the timing of physiologic events is critical and thus adds the dimensions of time and circumstance to the analysis of cutaneous processes. Obviously, skin depleted of Thy-1$^+$dEC should be deficient in function in the same fashion.

One of the most intriguing results from the identification of Thy-1$^+$dEC has been the extension of the analogy between thymus and skin. This analogy, which was developed more than a decade ago [40] links several important relationships, beginning with their common embryologic origin from a complex of epithelial and mesenchymal tissues. Both organs possess a keratinizing [41], stratified squamous epithelium which is the source of hormones which can alter both the functional activity of lymphocytes and their state of differentiation [37–39, 42–45]. Our data suggests that during residence within the epidermis, Thy-1$^+$dEC may develop unique phenotypic characteristics, including the expression of large amounts of Thy-1 antigen. The work of Haynes et al [46] in humans supports this hypothesis that the phenotypic expression of immigrant lymphoid cells may be altered once they gain entry into epidermis. Working with 2 patients with Sezary cell leukemia they observed that the circulating peripheral blood leukemic cells were Thy-1$^-$, whereas the leukemia cells infiltrating the epidermis were Thy-1$^+$. This data supports the notion that skin may be an important site of extra-thymic T cell maturation and which promotes phenotypic changes in T cells and/or pre-T cells which migrate there [44,45].

The majority of studies conducted to date on the identity of Thy-1$^+$dEC have focused on its tissue of origin and its cell surface and cytoplasmic phenotype. Such studies are obviously important, since by analogy with phenotypically similar cells of the immune system whose functional capabilities have been studied, they can suggest reasonable hypotheses concerning biologically relevant methods of testing their function. We are currently in a position to begin to test several such nonexclusive hypotheses, including that they are: (1) antigen-presenting cells which selectively activate down-regulatory signals, (2) thymic-independent cells with natural killer (NK) and/or natural suppressor (NS) functions, (3) thymic-dependent T lymphocytes with unique phenotypic characteristics conferred upon them by virtue of their intraepidermal residence, and (4) cells which have developed functional and phenotypic properties of T lymphocytes as a result of cutaneous, rather than thymic, influence.

We wish to thank Ms. Dolores Juarez for her expert technical assistance and Mrs. Betty Janes for her continuous administrative support. Dr. David Sachs graciously provided the 20-10-5S monoclonal antibody.

REFERENCES

1. Bergstresser PR, Toews GB, Streilein JW: Natural and perturbed distributions of Langerhans cells: responses to ultraviolet light, heterotopic skin grafting and dinitrochlorobenzene sensitization. J Invest Dermatol 75:73–77, 1980
2. Streilein JW, Lonsberry LW, Bergstresser PR: Depletion of epidermal Langerhans cells and Ia immunogenicity from tape-stripped mouse skin. J Exp Med 155:863–871, 1982
3. Elmets CA, Bergstresser PR, Tigelaar RE, Wood PJ, Streilein JW: Analysis of the mechanism of unresponsiveness produced by haptens painted on skin exposed to ultraviolet radiation. J Exp Med 158:781–794, 1983
4. Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW: Thy-1

antigen-bearing dendritic cells populate murine epidermis. J Invest Dermatol 81:286–288, 1983
5. Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K, Stingl G: Expression of Thy-1 antigen by murine epidermal cells. J Invest Dermatol 81:282–285, 1983
6. Cohen RL, Heiss MA, Alves MEAF, Chambers DA: Heterogeneity of Thy-1$^+$ cells in Balb/c mouse epidermis (abstr). J Invest Dermatol 82:416, 1984
7. Williams AF, Gagnon J: Neuronal cell Thy-1 glycoproteins: homology with immunoglobulins. Science 216:696–703, 1982
8. Stern PL: Theta alloantigen on mouse and rat fibroblasts. Nature 246:76–78, 1973
9. Lennon V, Unger M, Dulbecco R: Thy-1: a differentiation marker of potential mammary myoepithelial cells in vitro. Proc Natl Acad Sci USA 75:6043–6047, 1978
10. Scheid M, Boyse EA, Carswell EA, Old LJ: Serologically demonstrable alloantigens of mouse epidermal cells. J Exp Med 135:938–955, 1972
11. Herberman RB, Nunn ME, Holden HT: Low density of Thy-1 antigen on mouse effector cells mediating natural cytotoxicity against tumor cells. J Immunol 121:304–309, 1978
12. Hertel-Wulff B, Okada S, Oseroff A, Strober S: *In vitro* propagation and cloning of murine natural suppressor (NS) cells. J Immunol 133:2791–2796, 1984
13. Cantor H, Weissman I: Development and function of subpopulations of thymocytes and T lymphocytes. Prog Allergy 720:1–64, 1976
14. Mansour MH, Cooper EL: Purification and characterization of *Rana Pipiens* brain Thy-1 glycoprotein. J Immunol 132:2515–2523, 1984
15. Bergstresser PR, Tigelaar RE, Streilein JW: Thy-1 antigen-bearing dendritic cells in murine epidermis are derived from bone marrow precursors. J Invest Dermatol 83:83–87, 1984
16. Breathnach SM, Katz SI: Thy-1$^+$ dendritic cells in murine epidermis are bone marrow-derived. J Invest Dermatol 83:74–77, 1984
17. Auchincloss H, Ozato K, Sachs DH: A monoclonal antibody detecting unusual Thy-1 determinants. J Immunol 128:1584–1588, 1982
18. Shimamura K, Habu S, Fukui H, Akatsnka A, Okunnra K, Tamaoki N: Morphology and function of ganglio-n-tetraosylceramide-positive lymphocyte mediators of natural killer activity. JNCI 68:449–455, 1982
19. Kasai M, Iwamori M, Nigai Y, Okuranura K, Tada T: A glycolipid on the surface of mouse natural killer cells. Eur J Immunol 10:175–180, 1980
20. Beck BN, Gillis S, Henney CS: Display of the neutral glycolipid ganglio-n-tetraosylceramide (asialo GM_1) on cells of natural killer and T lineages. Transplantation 33:118–122, 1982
21. Akagawa KS, Tokunaga T: Appearance of a cell surface antigen associated with the activation of peritoneal macrophage in mice. Microbiol Immunol 26:831–842, 1982
22. Charley MR, Mikhael A, Bennett M, Gilliam JN, Sontheimer RD: Prevention of lethal minor-determinant, graft-versus-host disease in mice by the *in vitro* administration of anti-asialo GM_1. J Immunol 131:2101–2103, 1983
23. Scheynius A, Klareskog L, Forsum U, Matsson P, Karlsson L, Peterson PA, Sundström C: Enrichment of epidermal Langerhans cells: studies with a monolayer technique and flow cytometry sorting. J Invest Dermatol 78:452–455, 1982
24. Schuler G, Aubock J, Linert J: Enrichment of epidermal Langerhans cells by immunoadsorption to *Staphlococcus aureus* cells. J Immunol 130:2008–2010, 1983
25. Morhenn VB, Wood GS, Engleman EG, Oseroff AR: Selective enrichment of human epidermal cell subpopulations using monoclonal antibodies. J Invest Dermatol 81 (suppl):127s–131s, 1983
26. Bergstresser PR, Fletcher CR, Streilein JW: Surface densities of Langerhans cells in relation to rodent epidermal sites with special immunologic properties. J Invest Dermatol 74:77–80, 1980
27. Toews GB, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–453, 1980
28. Streilein JW, Wood PJ, Lonsberry LW, Bergstresser PR: Hapten-derivatized skin grafts induce H-2 restricted contact hypersensitivity. Evidence that the immunologic signal includes H-2 determinants derived from the skin. Transplantation 37:195–201, 1984
29. Elmets CA, Bergstresser PR, Tigelaar RE, Wood P, Streilein JW: Analysis of the mechanism of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet irradiation. J Exp Med 158:781–794, 1983
30. Sauder DN, Tamaki K, Moshell AN, Fujiwara H, Katz SI: Induction of tolerance to topically applied TNCB using TNP-conjugated ultraviolet light-irradiated epidermal cells. J Immunol 127:261–265, 1981
31. Granstein RD, Lowy A, Green MI: Epidermal antigen-presenting cells in activation of suppression: identification of a new functional type of ultraviolet radiation resistant epidermal cell. J Immunol 132:563–565, 1984
32. Sullivan S, Bergstresser PR, Streilein JW: Intravenously injected, TNP-derivatized, Langerhans cell-enriched epidermal cells induce contact hypersensitivity in Syrian hamsters. J Invest Dermatol 84:249–252, 1985
33. Sullivan S, Bergstresser PR, Tigelaar RE, Streilein JW: FACS purification of bone marrow-derived epidermal populations in mice Langerhans cells and Thy 1$^+$ cells. J Invest Dermatol 84:491–495, 1985
34. Bergstresser PR, Chapman SL: Maturation of normal human epidermis without an ordered structure. Br J Dermatol 102:641–648, 1980
35. Frelinger JG, Hood L, Hill S, Frelinger JA: Mouse epidermal molecules have a bone marrow origin. Nature 282:321–323, 1979
36. Katz SI, Tamaki K, Sachs DH: Epidermal Langerhans cells are derived from cells which originate in bone marrow. Nature 282:324–326, 1979
37. Sauder DN, Carter C, Katz SI, Oppenheim JJ: Epidermal cell production of thymocyte activating factor (ETAF). J Invest Dermatol 79:34–39, 1982
38. Luger TA, Stadler BM, Luger M, Matheson BJ, Mage M, Schmidt JR, Oppenheim JJ: Murine epidermal cell derived thymocyte activity factor resembles murine interleukin-1. J Immunol 128:2147–2152, 1982
39. Sauder DN, Monick M, Hunninghake G: Human epidermal cell derived thymocyte activating factor is a potent T cell chemoattractant (abstr). J Invest Dermatol 82:399, 1984
40. Gaudecker B, Schmale EM: Similarities between Hassal's corpuscles of the human thymus and epidermis. Cell Tissue Res 151:347–363, 1974
41. Sun TT, Shih C, Green H: Keratin cytoskeletons in epithelial cells of internal organs. Proc Nat Acad Sci USA 76:2813–2817, 1979
42. Goldstein G: The isolation of thymopoietin. Ann NY Acad Sci 249:177–189, 1975
43. Low TLK, Goldstein AL: Thymosins: structure, function, and therapeutic applications. Thymus 6:27–42, 1984
44. Rubenfeld MR, Silverstone AE, Knowles DM, Halper JP, DeSostoa A, Fenoglio CM, Edelson RL: Induction of lymphocyte differentiation by epidermal cultures. J Invest Dermatol 77:221–224, 1981
45. Chu AC, Patterson JA, Goldstein G, Berger CL, Takezaki S, Edelson RL: Thymopoietin-like substance in human skin. J Invest Dermatol 81:194–197, 1983
46. Haynes BF, Hensley LL, Jegasothy BV: Differentiation of human T lymphocytes: II. Phenotypic difference in skin and blood malignant T-cells in T-cell lymphoma. J Invest Dermatol 82:323–326, 1982