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THE THY-1-BEARING CELL OF MURINE EPIDERMIS

A Distinctive Leukocyte Perhaps Related to Natural Killer Cells

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It has been known for more than five years that epidermal Langerhans cells (LC)[1] are Ia^+ leukocytes that can provide accessory cell function for T lymphocyte responses (reviewed in 1). Recent studies (2) of LC in culture have established a close relationship to the immunostimulatory dendritic cells within lymphoid organs. Specifically, LC behave as immature or precursor elements in the dendritic cell family.

In 1983, we and others (3, 4) simultaneously encountered a previously unrecognized cell within murine epidermis that was Ia^-, dendritic, lacked the Birbeck granules of LC, and had large amounts of Thy-1 antigen (in contrast to the low amounts on most keratinocytes; 3, 5). Like LC, these $Thy-1^+$ epidermal cells ($Thy-1^+$ EC) carried the Ly-5 leukocyte common antigen (3) and were bone marrow derived (6, 7). The question then arose whether $Thy-1^+$ EC are precursors to Ia^+ LC, particularly since Thy-1 is an antigen found on immature cells in several lymphoid and myeloid lineages (8, 9), or do they represent a different lineage?

Here we have used electron microscopy (EM), cytochemistry, and a large panel of monoclonal antibodies (mAb) to show that $Thy-1^+$ cells are thoroughly different from LC. In fact, Thy-1 cells resemble only natural killer (NK) cells and no other known class of leukocyte. The phenotyping we describe should provide a means for their enrichment and functional characterization.

Materials and Methods

Mice. C3H/He, C57BL/6, and B6.H-2k mice of both sexes, 6–12 wk old, were from The Trudeau Institute, Saranac Lake, NY or the Bundesanstalt for Versuchstieranstalt, Himberg, Austria. For selected purposes, we used CBA, C57BL/6 (bg/+), and C57BL/6 (bg/bg) mice from The Jackson Laboratory, Bar Harbor, ME; WBB6 F1/J (W/Wᵛ), AKR,

and SJL from Olac, 1976 Ltd., Bicester, England; BALB/cAn from The Rockefeller University; and A/J from Dr. Mazuzko, Institut für Krebsforschung, Vienna, Austria.

Preparations of Mouse Ear Epidermis. Epidermal sheets were used to immunostain leukocytes in situ, rather than 8-μm frozen sections, which were much less sensitive (see Results). Sheets were peeled from mouse ear skin treated with ammonium thiocyanate (10), rinsed in phosphate-buffered saline (PBS), fixed in acetone at room temperature for 20 min, rinsed again, and stained with antibodies. Alternatively (see below), epidermal-dermal separation was achieved with dispase (11).

Semithin cryosections were used also. Ear skin was prefixed in Nakane's fixative (12) for 60 min and soaked in 2.3 M sucrose in PBS for 60 min, and 200-nm frozen sections were cut at −100°C with an ultramicrotome equipped with an FC-4 cryoattachment (Ultracut; Reichert, Vienna). Sections were processed as described (13) and stained for Thy-1 and Ia with a three-step immunofluorescence technique (see below). EC suspensions were prepared by a trypsinization method (2) or, for selected purposes, with dispase (2).

Immunolabeling Techniques. Our antibody panel is described in Table I. All antibody dilutions and washes were in PBS–3% bovine plasma albumin–0.04% sodium azide except that azide was omitted for peroxidase-labeled reagents. Antibodies were tested at saturating or supersaturating levels and, if need be, positive controls were run on single-cell suspensions of spleen, peritoneal cells, bone marrow, and thymus. Negative controls included replacement of the first antibody with unrelated or unreactive antibodies of the same isotype, e.g., anti-Ia^{b,d} on H-2k mice. Antibodies were considered unreactive only if staining was not observed using all immunolabeling techniques, with both epidermal

TABLE I
Description of Primary (First-Step) Antibodies

Specificity*	Clone	Ig‡	Reference	Source§	Specificity*	Clone	Ig‡	Reference	Source§
Thy-1.2	5a-8	mIgG2b	—	AS	Ia^k	11-5.2	mIgG2b	27	BD
Thy-1.2	30-H12	rIgG2b	25	BD	Ia^{k,s}	10-2.16	mIgG2b	28	ATCC
Thy-1.2	B5-3	rIgG2b	26	RS	Ia^{b,d}	B21-2	rIgG2b	26	RS
Lyt-1	53-7.3	rIgG2a	35	ATCC	H-2D^d	B25-1	rIgG2a	26	RS
Lyt-1.1	—	mIgG2b	—	NEN	H-2K^k	—	mIgG2a	27	BD
Lyt-1.2	—	mIgG2b	—	NEN					
Lyt-2	53-6.7	rIgG2a	25	BD	LCA	Ly-5.1	mIgG2a	—	NEN
Lyt-2.1	—	mIgG2b	—	NEN	LCA	MI/84	rIgG2a	29	HT
Lyt-2.2	—	mIgM	—	NEN					
Lyt-3.2	—	mIgM	—	NEN	Macrophage	F4/80	rIgG2b	37	JA
L3T4	GK 1.5	rIgG2b	30	FF	FcR	2.4G2	rIgG	38	JU
TL	TL.m4	mIgG2a	—	NEN	C3biR	1.21J	rIgG2a	26	JU
Il-2 R	7D4	rIgM	31	ES	C3biR	MI/70	rIgG2b	39	ATCC
IL-2 R	3C7	rIgG	31	ES	DC	33D1	rIgG	40	RS
T, thymus	H-11	rIgG2b	25	HM	asGM₁	—	rabbit	41	GS
lgp100	M5/113	rIgG2a	25	HT	NK1.1	PK136-3-6	mIgG2b	42	GK
T, B	H9/25	mIgM	43	SL	S100	—	rabbit	—	Dako
NK, T	Qat-4	mIgM	32	CLS	HSA	MI/69.16	rIgG2b	44	HT
NK, T	Qat-5	mIgM	32	CLS	I-J^k	WF8.C.12.8	mIgG	45	CW
Lym-10.1	S3.214	mIgM	33	UH	I-J^k	—	alloAb	—	AS
Lym-18.1	S8.261	mIgM	34	UH	B cell	TIB145	rIgM	46	ATCC
Lym-19.2	K10.6	mIgG2b	35	UH	B cell	TIB146	rIgM	47	ATCC
Lym-22.2	T28.45	mIgG2b	36	UH	Mast cell	B23.1	rIgM	48	PL

* C3biR, C3bi receptor; DC, spleen dendritic cell; FcR, Fc receptor; asGM₁, asialo GM₁; HSA, heat-stable antigen; IL-2R, interleukin 2 receptor; LCA, leukocyte common antigen.

‡ r, Rat; m, mouse.

§ Commercial sources: AS, Accurate Chemical & Scientific Corp. (Cedarlane); ATCC, American Tissue Culture Collection; BD, Becton, Dickinson & Co.; CLS, Camon Labor Service; HT, Hybritech Inc.; NEN, New England Nuclear; SL, Sera Labs. Individual sources: CW, C. Waltenbaugh, University of Chicago; ES, E. Shevach, NIH, Bethesda, MD; FF, F. Fitch, University of Chicago; GK, G. Koo, Merck & Co., Rahway, NJ; GS, G. Schwarting, Waltham, MA; HM, H. Morse, NIH, Bethesda, MD; JA, J. Austyn, Oxford University; JU, J. Unkeless, The Rockefeller University; PL, P. LeBlanc; University of Alabama at Birmingham; RS, R. Steinman, The Rockefeller University; UH, U. Hammerling, Sloan-Kettering Cancer Institute, New York.

suspensions and sheets (acetone fixed or unfixed; prepared with dispase or with ammonium thiocyanate).

For staining of sheets, the first antibody was applied for 16 h at 4°C with constant shaking. The sheets were washed and exposed 90–180 min at 37°C with one of the following: affinity-purified F(ab')$_2$ goat anti–mouse IgG and IgM; fluorescein or rhodamine isothiocyanate (FITC, RITC) conjugates (Grub antibodies; Scandic, Vienna) at 20 µg/ml; FITC–goat F(ab')$_2$ anti–rat IgG at 1:20 (U.S. Biochemical Corp., Cleveland, OH); Texas Red–labeled, affinity-purified goat F(ab')$_2$ anti–rat IgG at 10 µg/ml (Jackson Immunoresearch Lab, Inc., Avondale, PA). For three-step staining, the first rat or mouse mAb was followed by an affinity-purified, polyclonal mouse anti–rat Ig (5–10 µg/ml; provided by Dr. E. Havell and Dr. G. Spitalny, The Trudeau Institute) or rat anti–mouse Ig (1:100; Jackson Immunoresearch Lab, Inc.), and then FITC or RITC conjugates (90 min, 37°C), as above. Double labeling with anti-Thy-1 or anti-Ia and a second mAb was performed as described (2). For anti-Thy-1/anti–asialo GM$_1$ double labeling, we used FITC–anti-Thy-1.2 (Becton, Dickinson & Co., Oxnard, CA) at 1:100, followed by affinity-purified rabbit anti–asialo GM$_1$ at 1:100 and species-specific, Texas Red–labeled donkey anti–rabbit Ig at 1:50 (Amersham Corp., Arlington Heights, IL). Sheets were mounted in PBS/glycerol/azide, dermal side up, and viewed with Leitz Ortholux II or Nikon Optiphot fluorescence microscopes.

EC suspensions were stained on ice, 30–60 min per step, using the same immunofluorescence reagents as for sheets. For anti-S100, B6.H-2k cells in suspension were exposed to biotin anti-Thy-1 or anti-Ia^k (1:50; Becton, Dickinson & Co.) followed by Texas Red–streptavidin (1:100; Amersham Corp.). The cells were cytospinned, fixed in acetone for 5 min, and incubated with rabbit anti-S100 at 1:200 (Dako Corp., Santa Barbara, CA) followed by species-specific, FITC-labeled donkey anti–rabbit Ig at 1:40 (Amersham Corp.). Controls used normal rabbit serum and omitted anti–S100.

Two immunoperoxidase techniques gave similar results. For peroxidase antiperoxidase (PAP) (14), sheets were exposed to 1% normal goat serum for 30 min at room temperature; primary antibody for 16 h at 4°C; goat anti–mouse IgG (1:10; Tago Inc., Burlingame, CA) or goat anti–rat IgG (1:20; Nordic Labs, Tilburg, Holland) as link antibodies for 90 min at 37°C; and finally the respective PAP complex for 90 min at 37°C (mouse PAP, 50 µg/ml, Arnel, New York; rat PAP, 50 µg/ml, Accurate Chemical & Scientific Corp., Westbury, NY). After washing, sheets were stained with diaminobenzidine-H$_2$O$_2$ (15) for 30 min at room temperature in the dark followed by 0.5% CuSO4 enhancement for 5 min at room temperature. Sheets were mounted in PBS-glycerol. For the second avidin-biotin-peroxidase complex technique (16), we used a Vectastain kit as prescribed by the manufacturer (Vector Laboratories, Inc., Burlingame, CA).

For immuno-EM of unfixed EC suspensions, we used two-step methods (2) with either peroxidase-labeled, F(ab')$_2$ goat anti–mouse IgG (Thy-1) or goat anti–rabbit IgG (anti–asialo GM$_1$), both from Tago, Inc. To label EC in situ, dispase sheets (11) were fixed with periodate-lysine-paraformaldehyde (12).

Cytochemistry. Sheets were stained for ATPase (17). EC suspensions were used for EM demonstration of α-naphthylbutyrate (Sigma Chemical Co., St. Louis, MO) esterase (18); acid phosphatase (19) (sodium β-glycerophosphate as substrate; grade I, Sigma Chemical Co.); and arylsulfatase B (20) with Goldfischer's reaction medium (21) and p-nitrocatechol sulfate (Sigma Chemical Co.) as substrate. For some experiments, EM cytochemistry was combined with anti-Thy-1 immunogold labeling. Cells were exposed for 60 min on ice with mouse mAb anti-Thy-1.2 (1:500; Cedarlane Laboratories Limited, Toronto, Canada) followed by goat anti–mouse IgG/20 nm colloidal gold conjugate (1:20; Janssen Life Sciences, Beers, Belgium) either before (acid hydrolase) or after (nonspecific esterase) the cytochemical procedure.

Results

Light and Electron Microscopy of Thy-1$^+$ EC

Epidermal sheets rather than cryostat sections proved to be much more sensitive for the localization of Thy-1 and Ia antigens. Both antigens were visualized using directly labeled mAb or two-step indirect immunofluorescence. In cryostat sections, triple-layer methods failed to identify most Thy-1$^+$ and Ia$^+$ cells, although these cells were readily apparent if sheets were stained first and then cryostat sections made (not shown). After indirect immunofluorescence or peroxidase techniques, epidermal sheets contained a regular system of Thy-1$^+$ and Ia$^+$ cells, each with a robust array of dendrites (Fig. 1). The Thy-1 and Ia markers never overlapped, but both were distributed in a homogenous pattern, usually adjacent to one another (see below). A few (<5%) of the LC stained very strongly with anti-Ia, sometimes in clusters (Fig. 1); by immuno-EM, the typical features of LC (1) were apparent (not shown). Thy-1$^+$ EC were found in normal numbers in epidermal sheets from mast cell–deficient W/W^v mice (22).

By immuno-EM, all Thy-1$^+$ EC (Fig. 2) lacked Birbeck granules and instead exhibited distinctive, membrane-bound granules that had an electron-dense core surrounded by less electron-dense material, occasionally including small vesicles. All mouse strains, including C57BL/6 bg/+, had similar granules, but beige (bg/bg) Thy-1 cells had only a few very large granules (Fig. 2). Typically, the
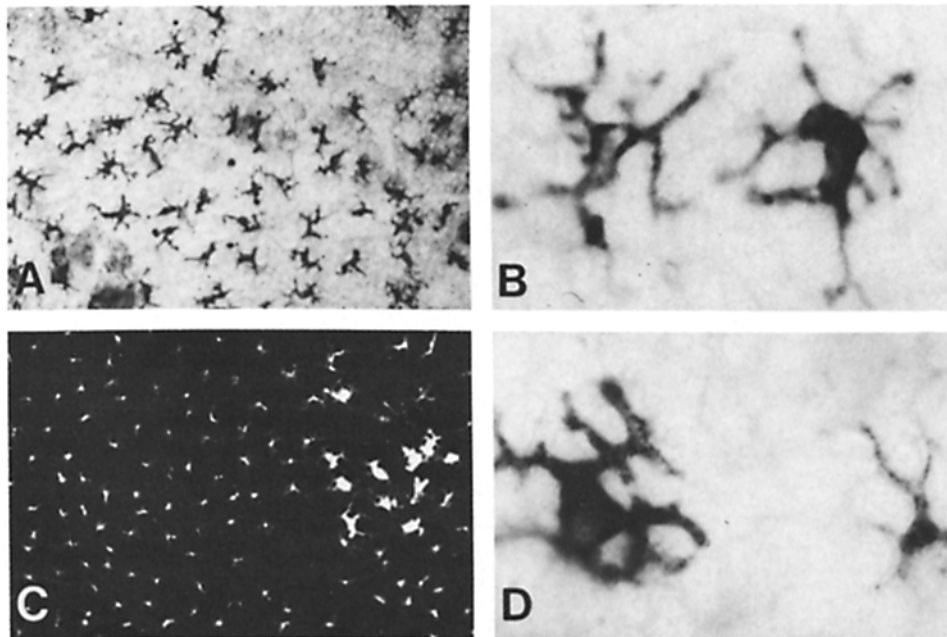

FIGURE 1. Immunolocalization of Thy-1 and Ia in murine ear epidermal sheets: (*A, B*). Thy-1$^+$ EC. The highly dendritic shape is seen if sensitive techniques are used (PAP; C57BL/6 mice). (*A*) × 140. (*B*) × 900. (*C, D*) Ia$^+$ EC. At low power a subfraction of strongly Ia$^+$ cells, here in a cluster, is on the right (indirect immunofluorescence, C3H mice) while, at higher power, the strongly Ia$^+$ cell is on the left (ABC technique, C57BL/6 mice). (*C*) × 140. (*D*) × 900.
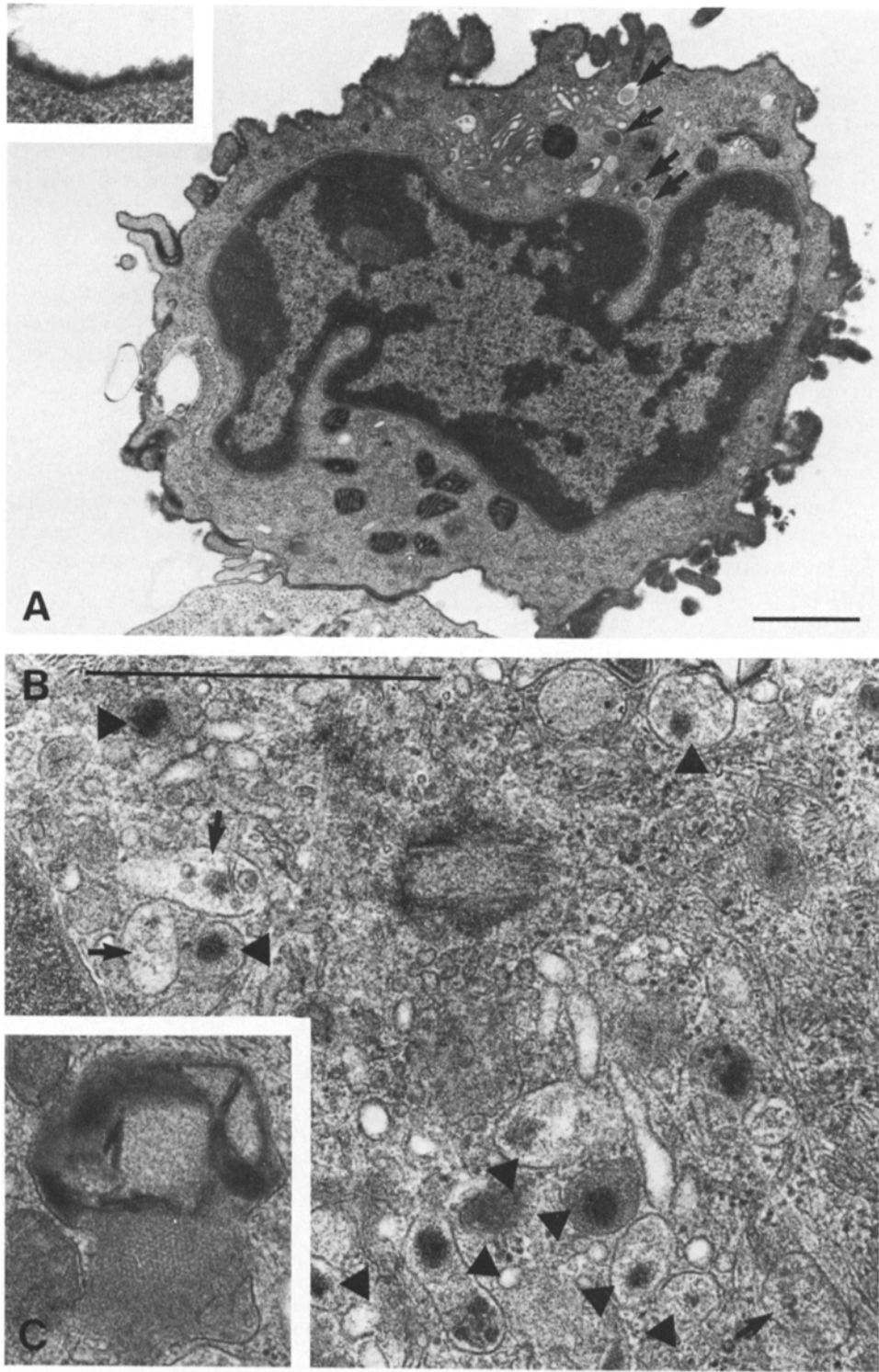
lysosomes of bg/bg mice are enlarged in many cell types, including NK cells, so the granules of Thy-1⁺ cells may be lysosomes (23).

Immunofluorescence of semithin sections showed that the Thy-1⁺ EC, like LC, were located in the basal layer (Fig. 3). By immuno-EM, small amounts of Thy-1 were seen on most keratinocytes, especially in lower layers (Fig. 3).

Cytochemistry

Double-labeling experiments indicated that Thy-1⁺ EC did not overlap with the ATPase⁺ LC network (1). Acid phosphatase was found in the Golgi of Thy-1⁺ EC, but was absent from most (>90%) of the granules (data not shown). Another hydrolase, arylsulfatase, was found in most granules (Fig. 4). Both enzymes were present in the Golgi and a few lysosomes of LC, as described for man (24).
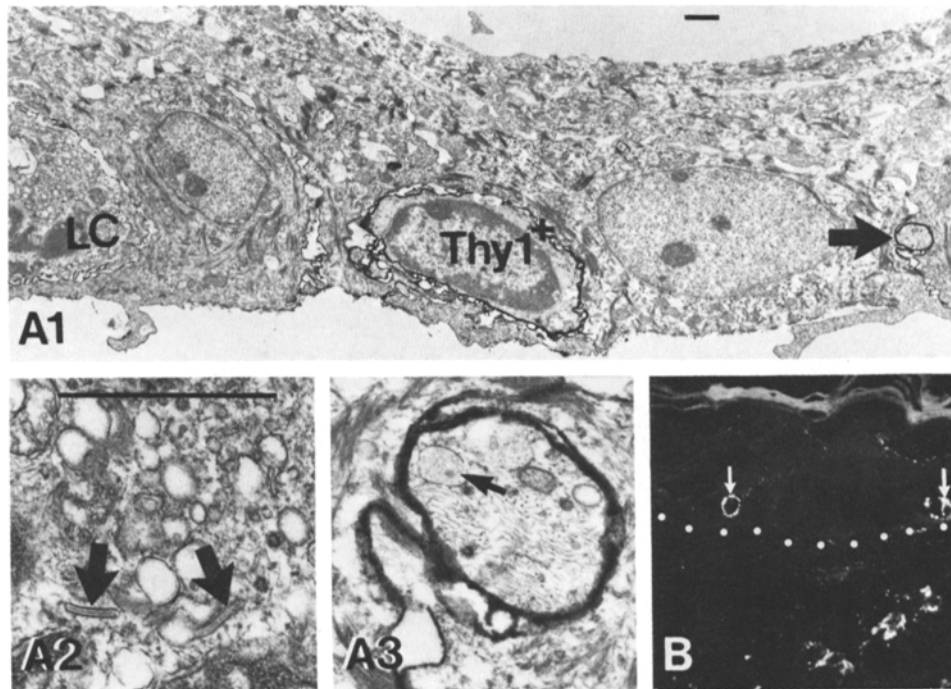
FIGURE 3. Intraepidermal localization of Thy-1⁺ EC (C3H). (*A*) Immunoperoxidase at the EM level of dispase sheets. At low power (*A1*) the Thy-1⁺ cell and a process (arrow) stain strongly and are localized to the basal layer along with LC. At higher magnification, the typical granules of LC (*A2*, arrows) and Thy-1⁺ EC (*A3*) are evident. × 4,600 (*A1*); × 28,500 (*A2, 3*); scale bar = 1 μm. (*B*) Semithin cryosection stained for Thy-1 by a triple layer, fluorescence method. Dotted line marks the epidermal-dermal junction. Two Thy-1⁺ EC (arrows) are seen in the basal layer as well as Thy-1⁺ fibroblasts in the dermis. × 360.

FIGURE 2. Ultrastructure of Thy-1⁺ EC: Thy-1⁺ cells (C3H) were identified in EC suspensions using anti-Thy-1 mAb followed by peroxidase F(ab′)₂ goat anti–mouse IgG and diaminobenzidine cytochemistry. Bar = 1 μm. (*A*) Overview of a Thy-1⁺ cell (inset shows the immunocytochemical reaction product) to show the villous surface, lobulated nucleus, dense cytoplasm, and characteristic granules (arrows). × 14,900. Inset, × 51,000. (*B*) Golgi region of another Thy-1⁺ cell to demonstrate the distinctive membrane-bound granules that have an electron-dense core (arrowheads) and/or content of vesicles (arrows). × 51,000. (*C*) Enlarged granules occur in Thy-1⁺ EC from beige mice (bg/bg). Same magnification as in *B*, × 51,000.
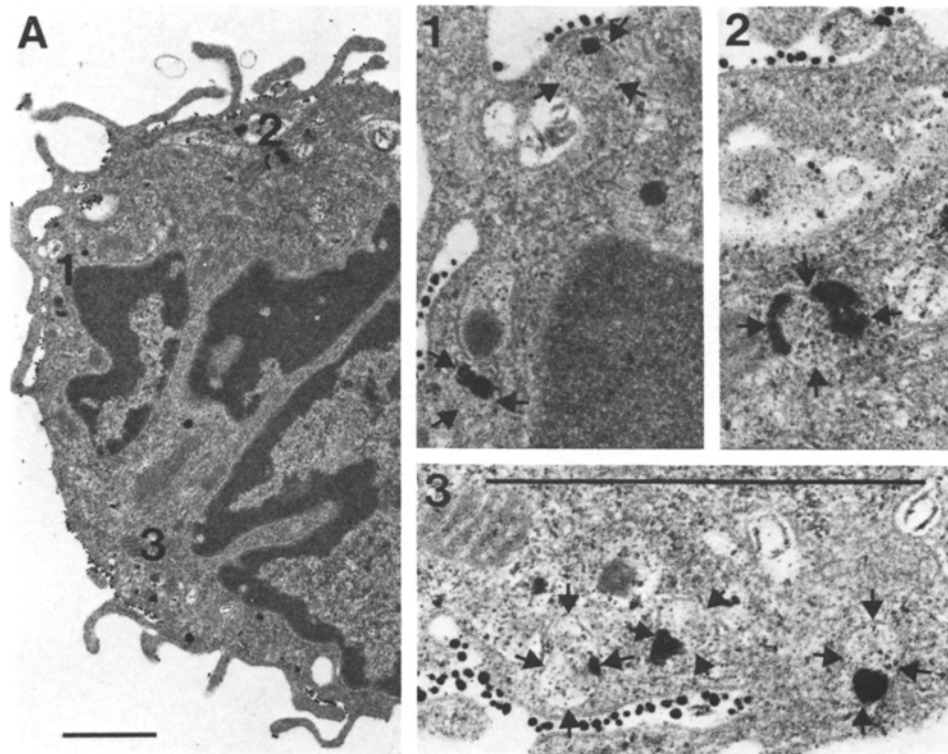
FIGURE 4. EM arylsulfatase combined with Thy-1 immunogold labeling (C3H) (A) Gold granules mark the Thy-1 surface antigen and dark spots the cytoplasmic arylsulfatase reaction product. × 12,600. Areas 1, 2, and 3 are shown at higher power in the respective insets. × 58,000. Most cytoplasmic granules (arrows) contain arylsulfatase, especially at the granule periphery.

α-Naphthylbutyrate esterase (sodium fluoride inhibitable), was found on the cell surface of LC (Fig. 5) but not on any Thy-1⁺ EC. Esterase was also noted on the surface of most keratinocytes, by EM, and in cytocentrifuge preparations by light microscopy. Fixation-resistant, endogenous peroxidase was not observed in any EC. We conclude that Thy-1⁺ and Ia⁺ epidermal leukocytes differ markedly from one another in cytologic features.

Immunolabeling

We next applied a large panel of antibodies (Table I) to sheets and to EC suspensions. When reactive reagents were identified, we did double labeling and immuno-EM to distinguish binding to LC or Thy-1⁺ EC. Both cell types expressed class I major histocompatibility antigens and the leukocyte common antigen, but otherwise their phenotypes were different (Table II).

T Lymphocyte Reagents. Lyt-1, 2, 3 antigens were not detectable when we used mice reared under specific pathogen–free conditions and tested them within a week of shipment. Conventionally reared mice had a few Thy-1⁺ cells that also expressed either Lyt-1 and/or Lyt-2, but these likely were passenger T cells rather than resident Thy-1⁺ EC. No staining was observed with L3T4, anti-
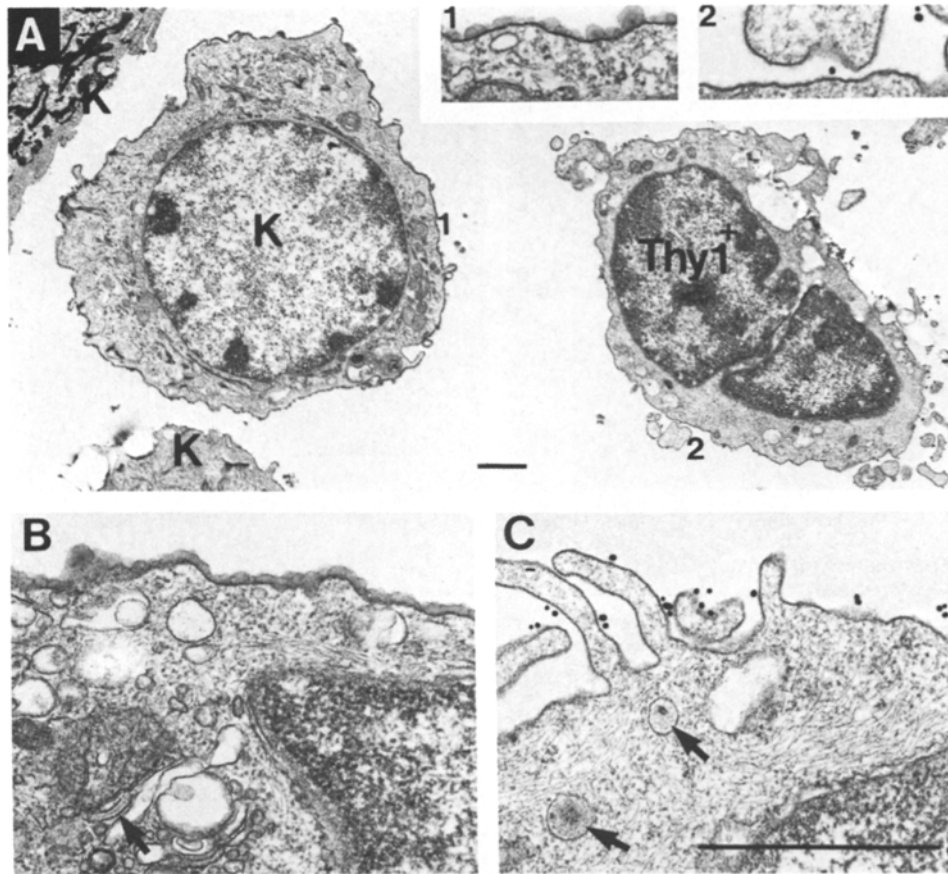
FIGURE 5. EM α-napthylbutyrate esterase combined with Thy-1 immunogold (C3H). (A) Two of three keratinocytes (K) have small amounts of esterase reaction product (area 1 is shown at higher power in insert 1), whereas Thy-1$^+$ EC are unreactive (area 2, insert 2). $\times$ 6,600 and $\times$ 34,000. (B) LC (arrow, LC granule) show surface esterase reaction product. $\times$ 32,000. (C) Thy-1$^+$ EC (gold labeled for Thy-1) are esterase negative on the cell surface and in the cytoplasmic granules (arrows). $\times$ 32,000.

interleukin 2 receptor, Qat-4 and 5, and four recently introduced T cell alloan-tibodies, Lym-10, 18, 19, and 20. The thymus leukemia antigen (TL), originally detected on thymocytes and recently in epidermis (49), was only found on LC and not Thy-1$^+$ EC (data not shown).

Mononuclear Phagocyte and Dendritic Cell Markers. Previous studies (50, 51) identified the F4/80 anti-macrophage and 2.4G2 anti–Fc receptor antigens, but not 1.21J and M1/70 anti-C3biR, on epidermal dendritic cells. We visualized all three antigens, but only with sensitive three-step techniques, and we verified with double labeling that the antigens were expressed by all LC (Fig. 6). A few Thy-1$^+$ EC reacted strongly with 2.4G2. These were typically found in groups close to hair follicles (Fig. 6), but were not T cells since replicate sheets lacked Lyt-1$^+$ or Lyt-2$^+$ cells. 33D1, an mAb specific for lymphoid dendritic cells, did not stain any EC.

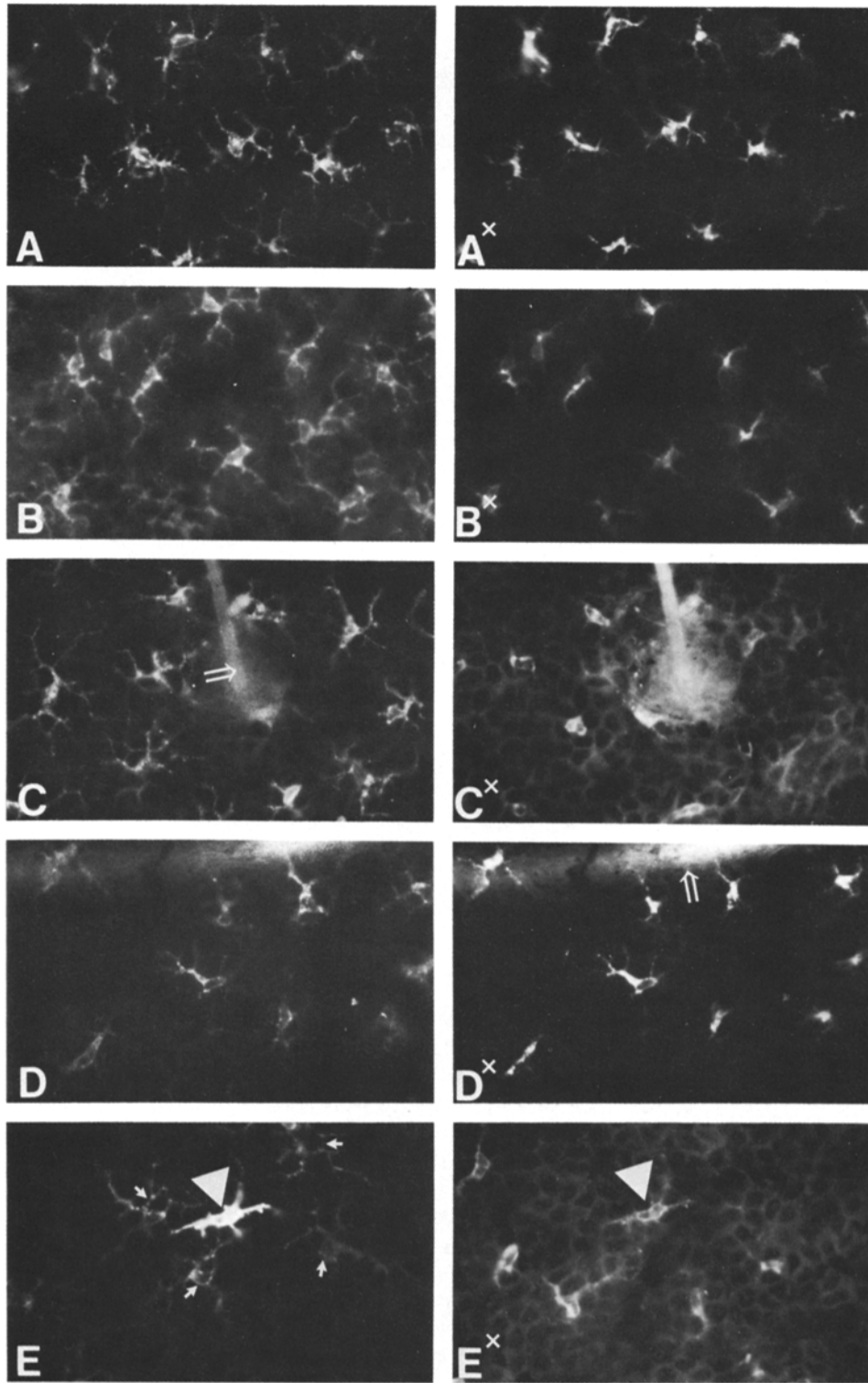*NK Cells.* Rabbit anti–asialo GM$_1$, which reacts strongly with NK cells (52,

TABLE II

Phenotype of Epidermal Langerhans Cells, ⁻hy-1⁺ Cells, and Keratinocytes

B or T cell markers	Thy-1	L3T4; Lyt-123	Lym-10, 18, 19, 22	H-11	TIB 145, 146	Ig	H9.25 lgp100	TL	IL-2R
Langerhans cell	−	−	−	−	−	−	−	+	−
Thy-1 cell	+	−	−	−	−	−	−	−	−
Keratinocyte	tr	−	−	−	−	−	−	−	−

Diverse markers	Ia	FcR	F4/80 C3biR	M1/ 69.16	asGM₁	S100	Qa-4, 5; NK-1	33D1	I-J, B23.1
Langerhans cell	+	+	+	+	−	+*	−	−	−
Thy-1 cell	−	+/−	−	−	+	−	−	−	−
Keratinocyte	−	−	−	tr	−	−	−	−	−

* 60–70% positive; +/−, <5% positive; tr, trace; asGM₁, asialo GM₁.

53), stained Thy-1⁺ EC strongly but not Ia⁺ LC (Fig. 7). In suspension, 1–2% of EC expressed asialo GM₁ and, by immuno-EM, all had the cytologic features of Thy-1⁺ EC (Fig. 7). Qa-4 and 5, and NK-1, which react with most but not all NK populations (54, 55), were not found on Thy-1⁺ EC.

Diverse Markers. Anti–B cell, anti–mast cell, H-11 (thymocytes), lgp100 (most lymphocytes), and H9/25 (some lymphocytes) did not stain any EC. M1/69.16, which reacts with a heat-stable antigen on most leukocytes, except for peripheral T cells, reacted strongly with LC, weakly with most keratinocytes, but not with Thy-1⁺ EC. Anti–S100 (56) stained many LC but no Thy-1⁺ EC. Functional studies (57) have suggested that epidermis contains I-J⁺ cells, but we could not kill Thy-1⁺ EC with anti-I-J and complement. Also, we could not find I-J by immunofluorescence and EM, even after neuraminidase treatment (which enhanced I-J-mediated killing in control T cells as reported; 58).

Discussion

We have presented two types of evidence showing that Thy-1⁺ murine EC are substantially different from LC. At the light microscopy level, Thy-1⁺ and Ia⁺ cells each express a distinct group of antigens (Table II). The experiments included double-labeling approaches, which are necessary because the epidermis contains two types of dendritic leukocytes and two types of dendritic nonleukocytes (melanocytes and Merkel cells). The fine structure of LC and Thy-1 cells also differs, particularly with respect to distinctive granules. LC have Birbeck granules and Thy-1⁺ EC have membrane-bound granules with an electron-dense core and/or small internal vesicles. All of these properties are apparent in intact epidermal sheets and in isolated suspensions.

NK cells are the only known class of leukocyte resembling Thy-1⁺ EC. All Thy-1⁺ cells contain granules that are similar in structure and in their content

FIGURE 6. Double immunofluorescence of epidermal sheets (B6.H-2k mice) × 250. MAC-1 (*A*) and Ia (*A**) antigens are found on the same cells. F4/80 (*B*) and Ia (*B**) antigens overlap. F4/80 (*C*) and Thy-1 (*C**) are found on different cells (arrow, hair follicle). 2.4G2 (*D*) and Ia (*D**) antigens stain the same cells (arrow, hair). 2.4G2 (*E*) stains several cells weakly (small arrows) and a single cell strongly; the latter (large arrowhead) bears Thy-1 (*E**). Note that Thy-1 stains keratinocytes in a honeycomb pattern (see also *C**).
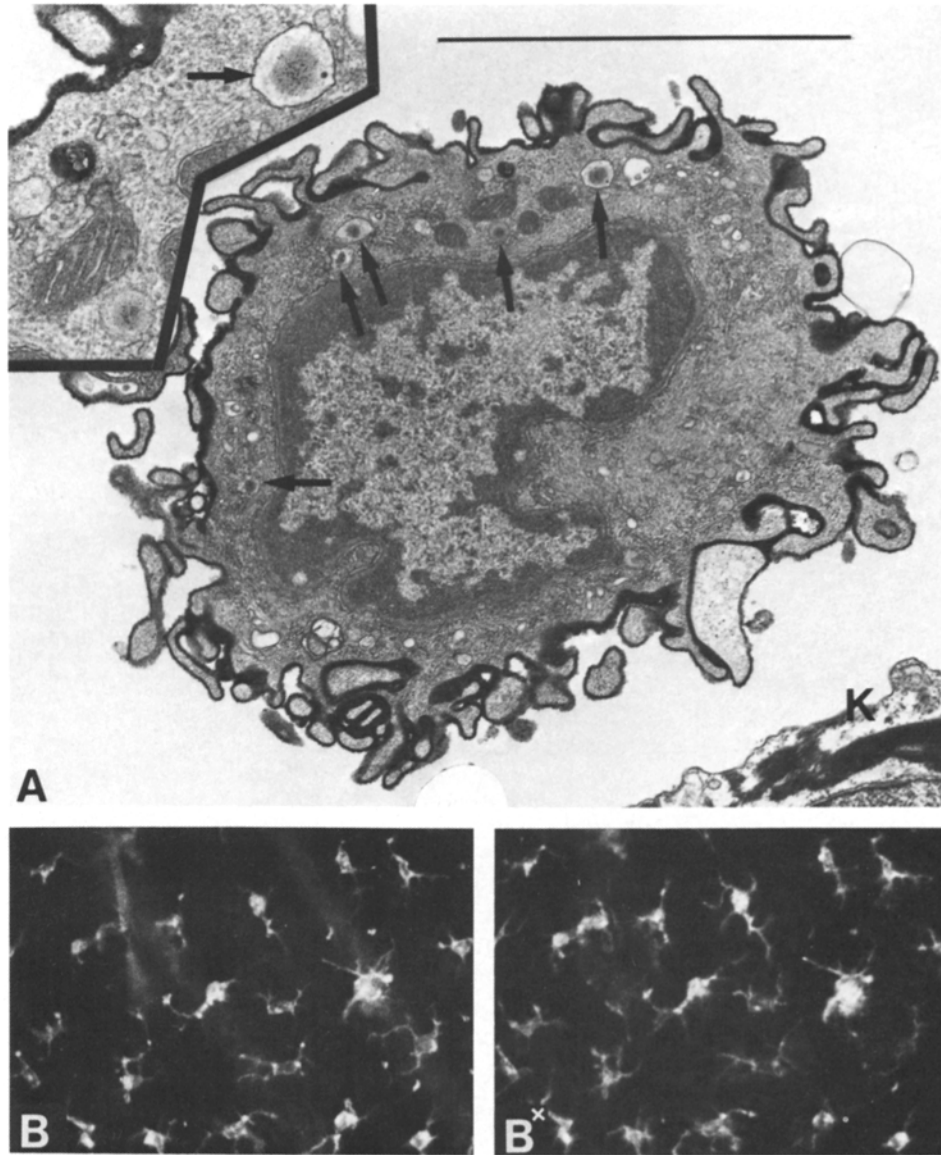
FIGURE 7. Thy-1⁺ EC (C3H) express asialo GM_1: (*A*) Immuno-EM (two-step peroxidase method) showing that Thy-1 cells, with their characteristic granules (arrows), express asialo GM_1, whereas keratinocytes (*K*) do not. × 15,200; insert, × 55,000. Scale bar = 1 μm. Double immunofluorescence of C3H mice stained for Thy-1 (*B*) and asialo GM_1 (*B**). The stains overlap. × 270.

of aryl sulfatase to those found in large granular lymphocytes enriched for NK cells and in certain NK and cytolytic T cell lines and tumors (59–63). Small amounts of Thy-1 are found on subsets of spleen NK cells (55), with larger amounts on immature NK cells (64) and certain tissue NK cells (65, 66). Both NK (52, 53) and Thy-1⁺ EC have readily detectable asialo GM_1, but this marker

is also found on some T cells (41) and macrophages (67). We did not detect epidermal killing of the NK target cell line, YAC, but, for that purpose, studies should be performed after stimulation with interferon or interleukin 2 and/or enrichment procedures (only 0.5–2.0% of EC express Thy-1). The distinctive phenotype of Thy-1$^+$ cells (Table II) should be useful in devising techniques for their isolation from LC and keratinocytes.

Summary

Bone marrow–derived leukocytes of murine epidermis can express two phenotypes: typical Langerhans cells, which are Ia$^+$ and Thy-1$^-$, and a recently discovered second population that is Thy-1$^+$ and Ia$^-$. To verify that these phenotypes are expressed by two different cell types, and to help understand their lineage and function, we have studied morphology and reactivity with a large panel of antibodies. Dual antibody immunofluorescence combined with electron microscopy showed that Thy-1$^+$ and Ia$^+$ cells were each distributed in a regular fashion and formed adjacent dendritic systems in or close to the basal layer. Double-labeling studies with anti-Ia and a second monoclonal antibody revealed that all Langerhans cells expressed F4/80 (macrophage), Mac-1 (C3bi receptor), and 2.4G2 (Fc receptor), as well as the thymus leukemia (TL) and heat-stable (M1.69/16) antigens. A large fraction expressed S100 and all exhibited membrane ATPase and nonspecific esterase. In contrast, Thy-1$^+$ cells lacked all these features of Langerhans cells, except that a minority were strongly reactive with 2.4G2. Thy-1$^+$ cells also lacked differentiation antigens of most other types of leukocytes, except they were rich in asialo GM$_1$. By electron microscopy, Thy-1$^+$ cells had cytoplasmic granules that were similar in structure and in their aryl sulfatase content to those previously described in natural killer cells. The granules were enlarged in beige mice, suggesting a lysosomal origin, and were present in mast cell–deficient W/W^v mice, indicating no relation to mast cells. We conclude that Thy-1$^+$ epidermal cells are thoroughly distinct from Langerhans cells. On the basis of morphology and phenotype, they may represent a type of tissue natural killer cell.

Thy-1$^+$ natural killer cells are now being identified in several nonlymphoid sites, such as gut epithelium (66) and the livers of mice given adjuvants (65). If Thy-1$^+$ epidermal cells prove to be natural killer cells, it is noteworthy that they represent a resident population regularly distributed in the basal layer of all mouse strains. The notion that Thy-1$^+$ epidermal cells are immature natural killer cells is intriguing in light of recent evidence that Ia$^+$ Langerhans cells are also immature with respect to accessory cell function. The epidermis may not have the functional capacities of a lymphoid organ, but it could contribute immature cells important for both natural and acquired resistance.

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