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A Comparison of Murine Epidermal Langerhans Cells with Spleen Dendritic Cells

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To establish if epidermal Langerhans cells (LC) are related to spleen dendritic cells, we have considered the morphology, phenotype, and function of the 2 cell types in culture. Cultured LC could be partially enriched (up to 50%) on the basis of 2 simple physical properties: nonadherence to plastic, and low buoyant density in dense albumin columns. The morphology of cultured LC and spleen dendritic cells were similar. In particular both cell types had many cell processes and/or veils, and cultured LC lost their distinguishing Birbeck granules. Freshly isolated LC exhibited nonspecific esterase and ATPase, as well as the F4/80 (α-macrophage) and 2.4G2 (α-Fc receptor) antigens. However all these traits were lost in culture, while Ia and Mac-1 antigens persisted. As a result, the cytochemical and antigenic phenotype of LC became similar to spleen dendritic cells. The one exception was that LC lacked the 33D1 dendritic cell antigen.

The function of LC at first differed from spleen dendritic cells in that fresh LC were weak stimulators of T cell proliferation in the mixed leukocyte reaction and in sodium periodate-induced mitogenesis. However, stimulatory activity per cell increased at least 30 fold in culture so that by 2–3 days, LC were 3–10 times more potent than dendritic cells. Maturation of LC function was radioresistant and was accompanied by a small increase in cell surface Ia antigens. Although LC have been likened both to lymphoid dendritic cells and to macrophages, our data suggest a different conclusion. LC seem to be dendritic cell precursors and are immunologically immature. Possibly, lymphoid dendritic cells are in general derived from substantial pools of precursors in nonlymphoid tissues, such as epidermal LC.

Dermatologists and immunologists are well aware of the important studies in the 1970's that intensified interest in epidermal Langerhans cells (LC; reviewed in [1]). It was noted that LC might be important in inducing contact sensitivity [2], expressed Ia antigens as well as Fc and C3 receptors [3–7], were bone-marrow derived [8], and could present antigens to T cells [9–13]. However, relatively little is known about the properties of LC in tissue culture. We have recently completed a study of LC that were isolated from mouse ear skin, and have noted marked changes in phenotype and function during culture [14]. Of considerable interest are the data that LC are immature in immunologic terms but acquire, in culture, potent stimulatory capacity for primary T cell proliferative responses. Cultured LC come to resemble quite closely the dendritic cells (DC) that were first described in mouse spleen (reviewed in [15,16]). The purpose of this article is to further illustrate some of these recent experiments, and to compare the features of mouse LC in situ and in vitro with mouse spleen DC.

"PHYSICAL" PROPERTIES

Empirical properties such as adherence to tissue culture surfaces, buoyant density in dense media (albumin, Percoll, metrizimide), and radioresistance have been very useful in the identification and purification of lymphoid DC. Mouse spleen DC adhere firmly to glass or plastic, but they lose the capacity to adhere [15,16]. DC in many other sites are nonadherent immediately after isolation from the animal (reviewed in [15]). We have noted that mouse epidermal LC also are nonadherent, and remain so after several days in culture [14].

DC, wherever they have been studied, have a low buoyant density (reviewed in [15]). This is also true of cultured LC. About 70% of freshly isolated LC are found in the high density fraction, but these LC develop a low density when maintained in culture [14].

It has long been known that the function of accessory cells in inducing T cell responses is radioresistant. This is true of DC in that 1000–1500 rads of ionizing irradiation does not inhibit stimulatory capacity [17]. However, in unpublished experiments, it has been noted that enriched populations of mouse spleen and human blood DC die and lose functional activity within a day of being irradiated. LC are clearly radioresistant in culture [14]. Both the number and function of LC are largely unaffected for up to 3–4 days following a dose of 1500 rads ^{137}Cesium. Rat dendritic cells also are radioresistant [18,19].

ENRICHMENT TECHNIQUES

DC have been enriched to > 60% purity from a number of sites. Mouse spleen is still the best source, and populations consisting of > 90–95% DC are readily obtained [15]. In spleen, the approach is to make low density adherent cells and then deplete the macrophages after overnight culture. Macrophage depletion is accomplished by removing cells that rosette antibody-coated erythrocytes (Fc receptor positive) or that readhere to plastic/glass surfaces. These and other approaches have been used to deplete macrophages and enrich DC from additional tissues and species [15]. Macrophages and lymphocytes can also be removed with cell-specific monoclonal antibodies [20]. Of interest is the fact that DC will cluster with responding T lymphocytes. The clusters can be isolated and the T cells eliminated, leaving behind enriched DC [21].

LC usually have been enriched by positive selection using rosetting, panning or fluorescence-activated cell sorter (FACS) approaches directed to Fc receptors, Ia antigens or the OKT6 marker (e.g., [9,22–25]). These current positive selection approaches are time consuming and could alter function.

In our recent work, we have used a negative selection procedure to partially enrich LC from 2–4 day cultures of murine

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Abbreviations:
DC: dendritic cell(s)
LC: Langerhans cell(s)
HRP: horseradish peroxidase

epidermis [14]. We take advantage of our observations that LC have a low buoyant density and are nonadherent, in contrast to basal keratinocytes. Therefore nonadherent epidermal cells, depleted of basal keratinocytes, are floated on dense albumin columns providing low density fractions with ~30–50% LC. We suspect that further purification will be possible, e.g., by removing contaminating cells with anti-thy 1 antibody [26].

Although these low density fractions are only partially enriched, they still prove to be suitable for many studies. For example, the morphology of the cultured LC is so distinctive that one can perform cytochemical and endocytosis experiments and identify LC in smears or in the electron microscope without any further labeling [12]. In functional studies, we have found no evidence that the contaminating cells alter the accessory function of the Ia^+ LC [14].

MORPHOLOGY

The discovery of DC in spleen has in part depended upon an awareness of their distinctive cell shape and cytologic features (reviewed in [16]). The cell processes take on many forms ranging from small pseudopods and dendrites to very long processes and cytoplasmic veils [16]. The processes are especially striking in the living state where they extend, bend, and retract. Similar observations have been made with DC in several other species and tissues [15,16]. Phase contrast microscopy of live preparations or cell smears reveals that cultured LC and spleen DC have a similar appearance (Fig 1, 2). In practice, simply observing one's cultures with an inverted phase contrast microscope provides a good estimate of the frequency of LC.

Cultured macrophages exhibit a different type of surface activity. The cell surface forms ruffles and pinocytic vesicles. Cell shape does not change significantly in culture except over large periods, or during the time that the cell attaches and spreads on a tissue culture surface.

The ultrastructural features of interest in mouse spleen DC (Fig 3) include the paucity of lysosomes, phagosomes, and polysomes (either free or membrane-bound) particularly when compared to the abundance of these organelles in macrophages. The nucleus is irregular in shape and the nucleolus is small. Mitochondria are common, and smooth surfaced vesicles are few to moderate in number. The matrix includes a few bundles of intermediate filaments.

The cytologic features of LC resemble those of spleen DC (Fig 4, 5). The one exception is the Birbeck granule, but the granules are no longer apparent after culture. LC vary from one another in two respects. First, the surface processes can take the form of thin veils or bulbous protrusions. Second, the number of electron-lucent smooth vesicles can differ considerably. The function of these vesicles has yet to be determined. One could postulate an endocytic role, but LC do not exhibit active endocytosis by other criteria (see below).

CYTOCHEMISTRY

LC in situ as well as freshly isolated LC clearly exhibit nonspecific esterase and membrane ATPase [27]. LC lose both esterase and ATPase activities in culture (Fig 6). Neither enzyme is detectable in spleen DC [14]. In contrast mononuclear phagocytes in most tissues are rich in both nonspecific esterase and ATPase.

ANTIGENIC PROFILE

An extensive study of the phenotype of epidermal LC in situ has been completed [27]. Sheets have been studied by indirect immunofluorescence, particularly with double immunofluorescence to distinguish Ia^+ LC from thy-1^+ cells [28,29]. Immunoelectron microscopy also has been used for certain critical markers. The conclusion of this work is that all Ia^+ LC express low amounts of 3 distinct antigens—F4/80 anti-macrophage [30]; M1/70 anti-Mac-1 or C3bi receptor [31]; and 2.4G2 anti-Fc receptor [32]. The monoclonal that is specific for lymphoid DC, 33D1 [33] does not stain epidermal LC.

Freshly isolated LC have the same antigenic profile as LC in situ [14] (Fig 7, Table I). However when LC are placed in culture, the staining intensity with anti-Ia antibodies typically increases, while Mac-1 remains constant (Fig 7). F4/80 and 2.4G2, however, become undetectable. The dendritic cell marker 33D1, does not appear. Except for the latter finding, the phenotype of the cultured LC fully resembles spleen DC (Table I). Indirect immunofluorescence can also be used to demonstrate that LC lack keratin and do not incorporate BrdU (Fig 7).

The level of Ia has been quantitated on LC [14] and found to be very high—perhaps the highest ever seen on leukocytes. Early in culture, LC have twice as much Ia as spleen DC (3–4 vs $1.5–2 \times 10^6$ binding sites per cell for the B21-2 monoclonal antibody). The amount increases another 50–100% during 3 days of culture reaching levels of $4–6 \times 10^6$ binding sites per LC (Table II). Adding 10–100 units/ml of recombinant γ-interferon does not increase the levels of Ia on LC (Table II) or spleen DC (unpublished data).

ENDOCYTIC ACTIVITY

There is evidence that LC in situ are not actively endocytic [36] i.e., in contrast to keratinocytes, LC accumulate very little of the tracers, horseradish peroxidase (HRP), ferritin, or thorotrast. Likewise, LC rarely contain phagosomes [36]. LC seem to have little endocytic activity after a period in culture. First morphologic studies revealed little evidence for phagocytosis (vacuoles with particulate contents) even though the LC have

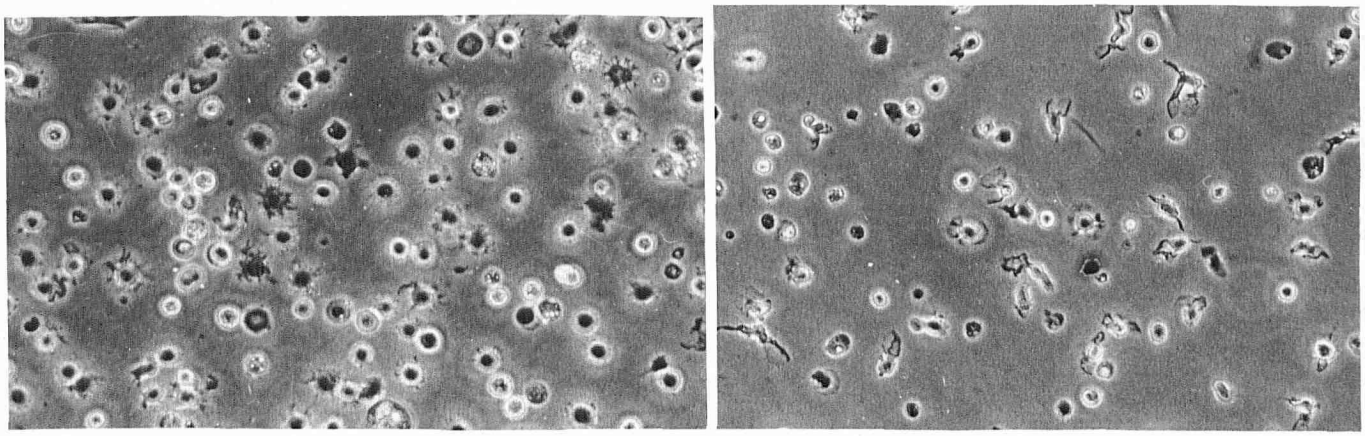

FIG 1. Cultures of enriched epidermal LC (*left*) and spleen dendritic cells (*right*) as viewed with an inverted phase contrast microscope. Many cells with spiny processes and veils are evident. × 150.

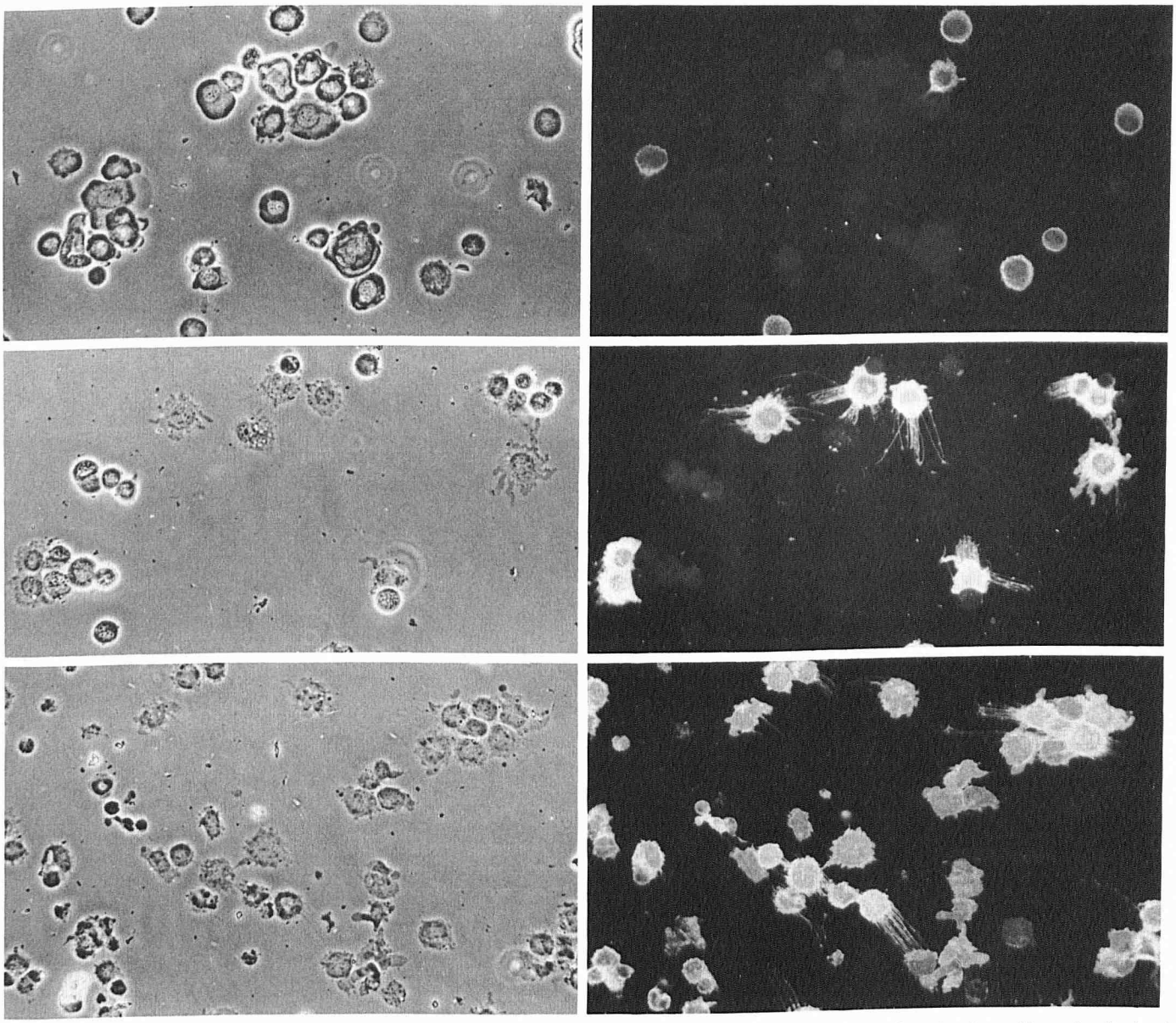

FIG 2. Phase contrast (*left*) and immunofluorescence microscopy (*right*) of cytospin preparations. Fresh low density epidermal cells (*top*) have several round Ia^+ LC, while cultured (3 days) suspensions (low density fractions, *middle*) have many large irregularly-shaped LC. Spleen dendritic populations (*bottom*) have many Ia rich cells. × 400.

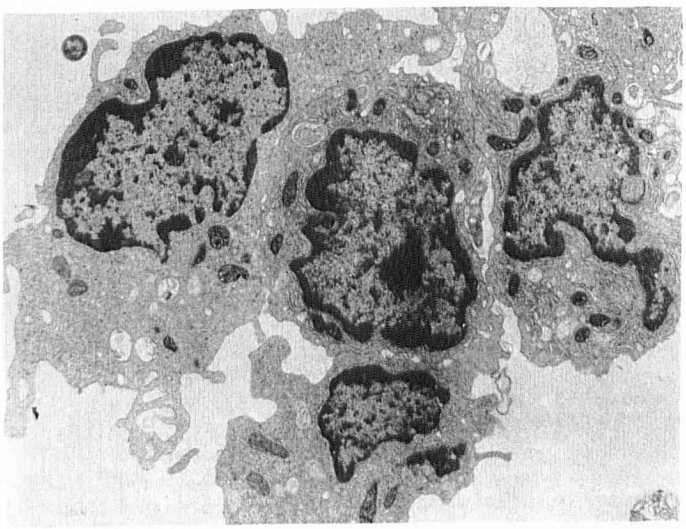

FIG 3. Spleen dendritic cells. × 9600.

been bathed for days among dead cells and debris [14]. Second, cultured LC failed to accumulate HRP when it is applied to the culture (Fig 6). The apparent lack of endocytic activity again is reminiscent of the situation with DC [16]. Third, both cultured LC and DC exhibit a striking lack of coated pits and vesicles, the morphologic hallmarks of adsorptive uptake.

It is to be stressed that the tracer studies to date have looked for accumulation of bulk markers. Little has been done with ligands and anti-cell surface antibodies. For example, freshly isolated guinea pig LC can phagocytose some *Staphylococcus aureus* particles that have been coated with α-Ia antibody (unpublished data). There is also the possibility that endocytosis occurs but that most content is recycled, or that LC are capable of endocytosis at restricted points in their life span.

STIMULATORY FUNCTION FOR T CELL PROLIFERATIVE RESPONSES

We find that fresh epidermal suspensions are very weak stimulators of T cell proliferative responses [14] (Tables II–V). The assays we use are the primary mixed leukocyte reaction

and the mitogenesis of periodate-modified T cells. The periodate system is dendritic cell-dependent and provides a rapid (2 day) quantitative assay of stimulatory function [37]. During 3 days of culture, LC stimulatory activity progressively and markedly increases so that on a per cell basis, LC become 3–10 times more active than spleen DC (Table III, Fig 8). Exogenous γ-interferon does not further enhance LC stimulatory activity (Table II). If epidermal cells are irradiated (1500 rads) prior to culture, stimulatory function still "matures," (Table IV). When we add indomethacin, to block potential immunosuppression by prostaglandins, there is some increase in T cell proliferative responses, but the marked differences between fresh and cultured LC persist (Table V). Addition of epidermal cell-conditioned medium also does not significantly enhance stimulatory function (Table V).

We are concerned about the possibility that freshly isolated LC are in some way damaged or inhibited, but we believe that this is not the case. The initial epidermal suspensions have excellent viability—> 80% by trypan blue exclusion—and the basal keratinocytes efficiently adhere and begin growing [14]. The enzymes we use to dissociate the skin (trypsin, and in a few cases dispase) have no inhibitory effect on cultured LC or spleen DC [14]. Mixing experiments show that fresh epidermal suspensions do not inhibit stimulation by spleen DC or cultured LC (Table V) [14]. Given the fact that LC undergo selective changes in phenotype (loss of F4/80 and 2.4G2, Birbeck granules, ATPase, and nonspecific esterase, but no loss of surface Ia, Mac-1, and other ultrastructural features), we suspect that LC are indeed maturing in culture rather than slowly recovering from the insult of epidermal dissociation.

We have yet to evaluate highly purified, cultured LC. We do know that the active stimulator cell is Ia^+, as is its precursor in the initial cell suspension [14]. Also the Ia^- contaminating cells neither enhance nor inhibit stimulatory function [14]. There still is a need to study the possible effects of keratinocytes and thy-1^+ epidermal cells [28,29] on the viability and/or maturation of LC in culture.

We do not yet understand why the Ia-rich epidermal LC fails to act as an active stimulator cell shortly after isolation. It may be important to note that freshly isolated LC do not form aggregates with responding T cells. Cultured LC (Fig 9) and spleen DC efficiently cluster the lymphocytes they stimulate [15,21]. Perhaps stimulatory function requires the capacity to form clusters, and this capacity requires more than expression of Ia.

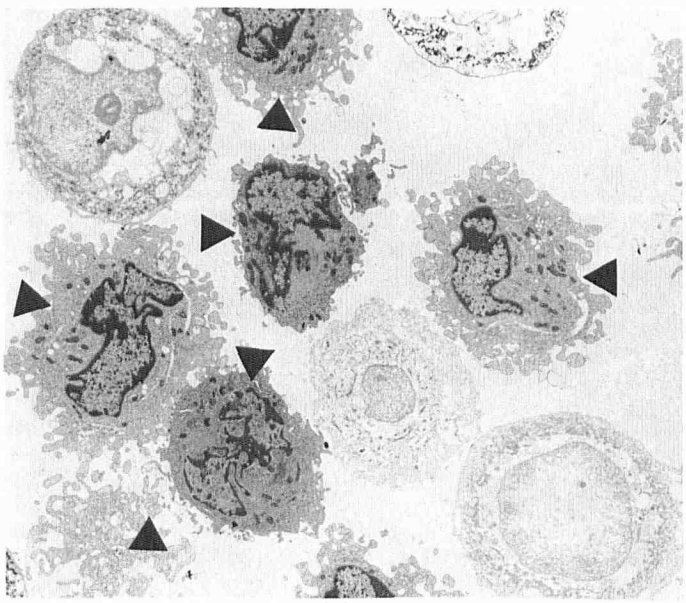

FIG 4. Cultured epidermal LC. The EM contains many LC (*arrowheads*), which were enriched as described in the text, as well as scattered keratinocyte profiles. × 4400.

DISCUSSION

The relationship of LC to macrophages and lymphoid DC has been unclear. A significant unknown is the behavior and features of LC in culture. Our observations on phenotype and function suggest a fascinating conclusion, namely that LC are immature precursors of lymphoid DC. The properties that are considered to be macrophage-like—such as Fc receptors, F4/80 antigen, and membrane ATPase and nonspecific esterase—are all lost when LC are cultured. Such changes have not been observed with cultured mononuclear phagocytes isolated from many tissues and species. Several features of cultured LC are actually very different from macrophages. LC are nonadherent, are not actively endocytic, and express very high levels of Ia. Whereas γ-interferon enhances expression of Ia on macrophages, it does not increase Ia on LC (Table II) or spleen DC (unpublished data). Combined with extensive morphologic similarity, the properties of cultured LC are virtually identical to spleen DC.

The situation with the F4/80 antigen is especially intriguing since this marker, whose physiologic function is unknown, is

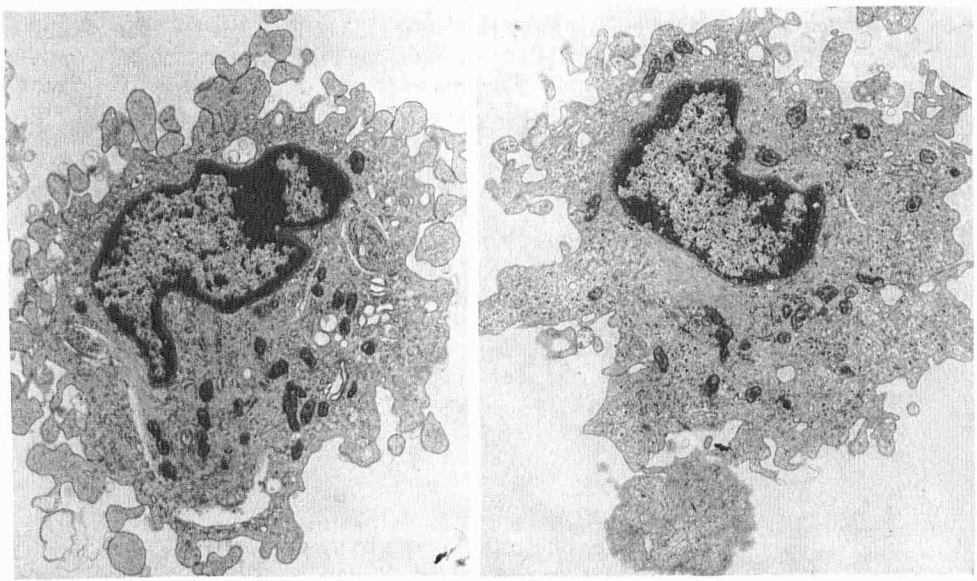

FIG 5. Cultured epidermal LC. Note that the cell surface is covered with bulbous processes and that the features of both nucleus and cytoplasm are similar to that of spleen dendritic cells. × 9600.

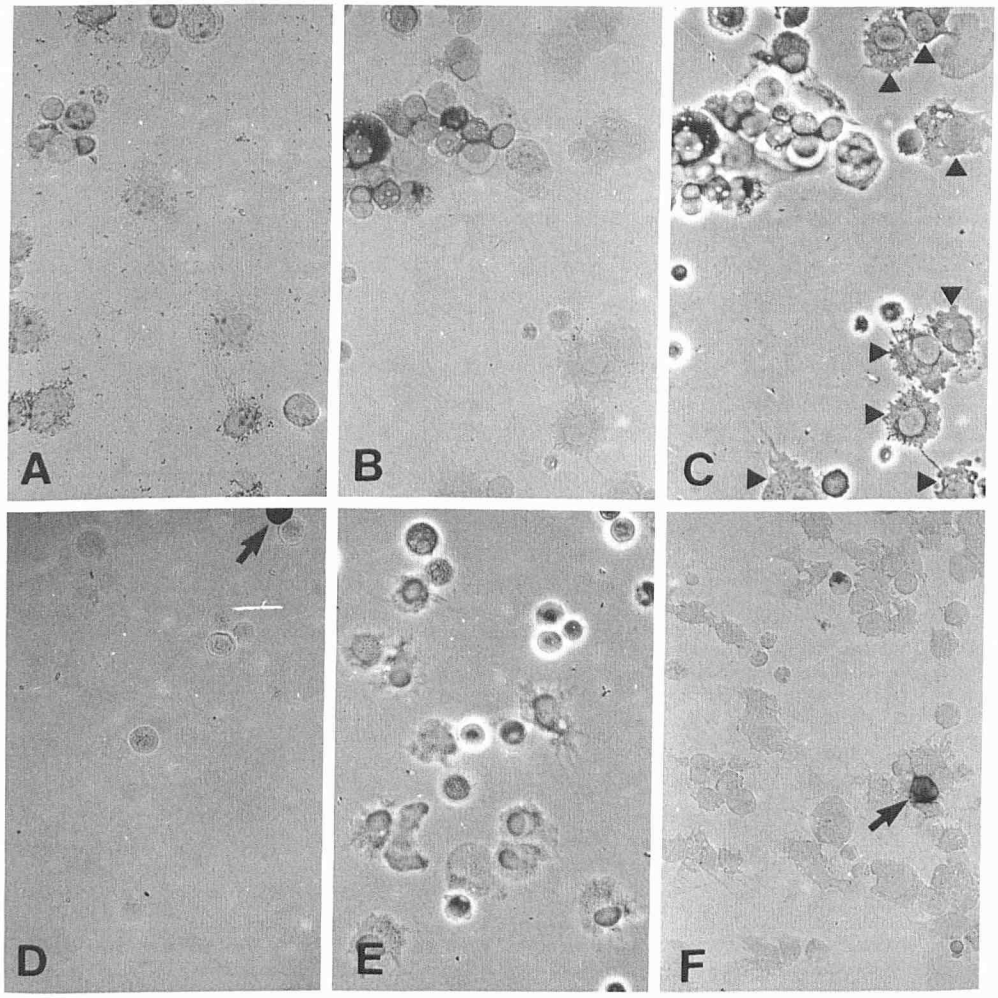

FIG 6. Cytochemistry of LC and spleen dendritic cells. × 180. *A*, Bright field of low density cultured epidermal cells stained for ATPase. The LC are negative. *B,C*, Bright field and phase contrast of low density epidermal cells stained for nonspecific esterase. The LC (*arrowheads*) are negative. *D,E*, Bright field and phase contrast of low density epidermal cells exposed to 1 mg/ml horseradish peroxidase and stained for peroxidase to identify pinocytosed enzyme. Only a rare epidermal cell is pinocytic (*arrow*). *F*, Nonspecific esterase of enriched spleen dendritic cells. A rare contaminating macrophage is stained (*arrow*).

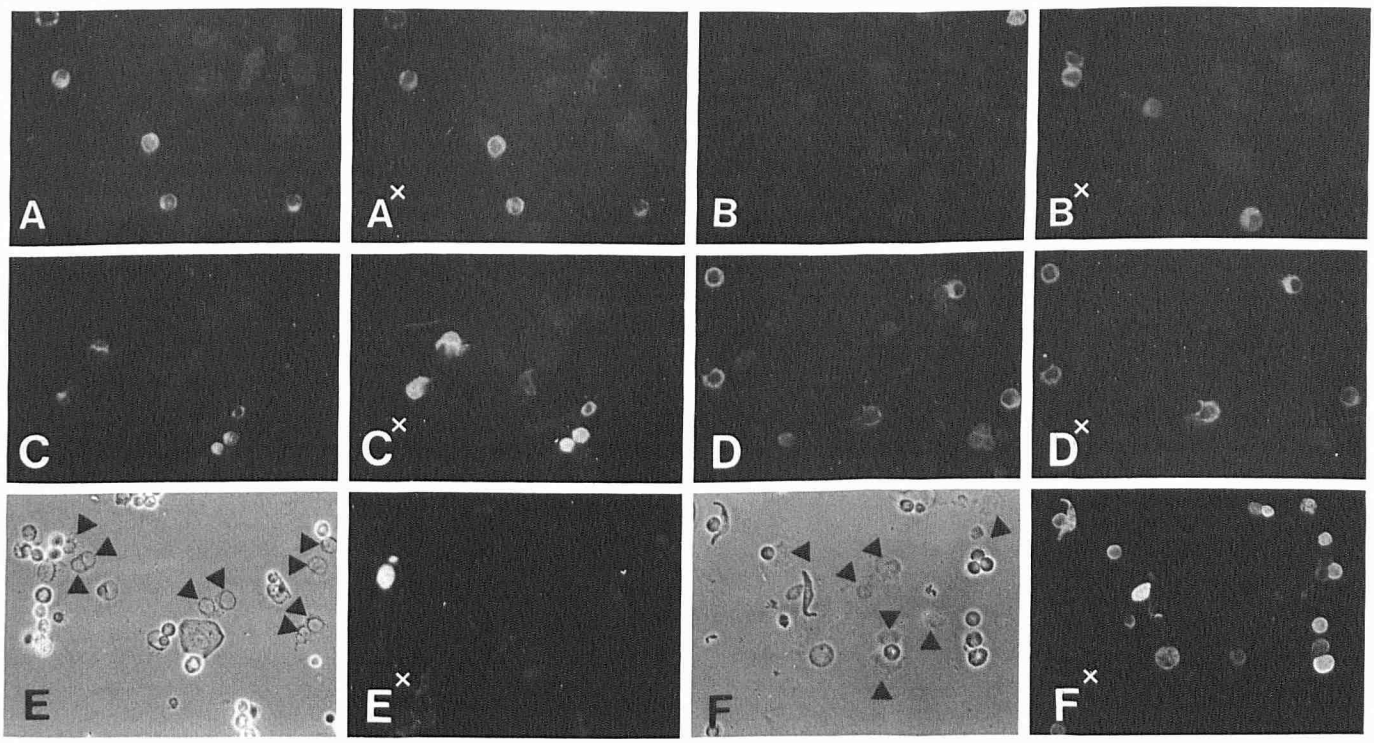

FIG 7. Phenotyping of epidermal LC. Panels A–D: double label immunofluorescence staining with 2 monoclonal antibodies was described [14]. Each field has about 20 profiles. × 180. *A*, A^x, Low density epidermal cells. Ia (*left*) and Fc receptor (*right*). *B*, B^x, Same as A, but thy-1 (*left*) and Fc receptor (*right*). *C*, C^x, Same as A, but Ia (*left*) and Mac-1 (*right*). *D*, D^x, Day 2 epidermal cells. Ia (*right*) and Mac-1 (*left*). *E*, E^x, Low density epidermal cells that have been exposed to BrdU and stained with monoclonal α-BrdU antibody. Only a rare keratinocyte is labeled. The cytologic appearance of LC (*arrowheads*) is poor because of the pretreatment (NaOH, Tween) necessary for α-BrdU staining. *F*, F^x, Low density epidermal cells that have been stained for keratin. The LC (*arrowheads*) are negative.

TABLE I. *A comparison of mouse epidermal Langerhans cells (LC) with spleen dendritic cells (DC)*

Antigens as detected with monoclonal	LC in situ	Isolated LC (0 h)	Isolated LC (48–72 h)	Spleen DC (20–50 h)
B21-2; 10-2.16: α-Ia	+	+	+	+
F4/80: α-macrophage	+	+	−	−
2.4G2: α-FcR	+	+	−	−
M1/70: α-C3biR, Mac-1	+	+	+	+[a]
33D1: α-spleen DC	−	−	−	+
TIB 145, 146: α-B cell	−	−	−	−
B5-3, 53-7.3, 53-6.7 (αthy-1, αlyt-1, αlyt-2)	−	−	−	−
Cytochemistry				
Nonspecific esterase	+	+	−	−
Membrane ATPase	+	+	−	−
Myeloperoxidase	−[b]	−	−	−
Exogenous HRP (pinocytosis)	−	ND[c]	−	−

[a] Mac-1 previously was not detected [34] on dendritic cells, but a triple-layer staining method has now identified this antigen [14].

[b] LC do have a rough ER peroxidase [35], but it is sensitive to fixation, unlike myeloperoxidase.

[c] Not determined.

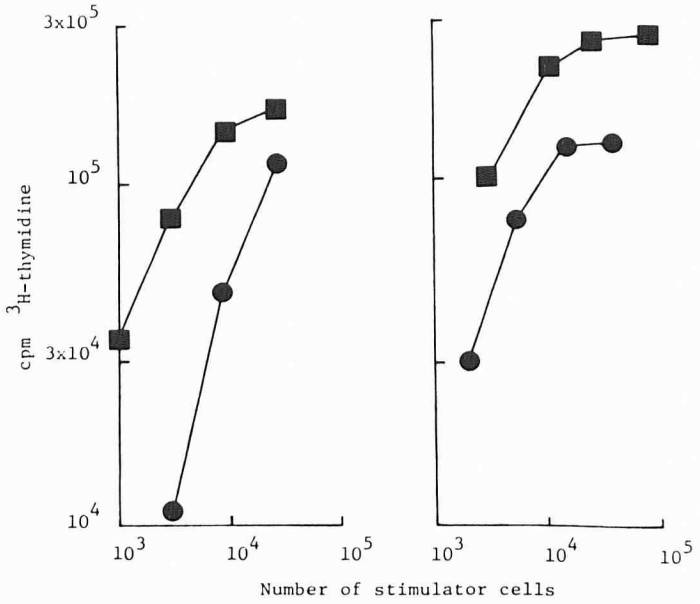

FIG 8. The relative efficacy of enriched populations of cultured LC (30% Ia⁺; ■———■) and spleen dendritic cells (< 90% Ia⁺; ●———●) as stimulators of oxidative mitogenesis.

TABLE II. *Immune interferon does not enhance the level of LC Ia antigens or stimulatory function*

A. Amount of $Ia^{b,d}$: binding of ^{125}I-B21-2 monoclonal antibody

Experiment	INF	Binding sites/cell
1	−	419,000
	+	478,000
2	−	518,000
	+	536,000

B. Stimulatory activity: oxidative mitogenesis of T cells

Low density stimulators	% Ia⁺ cells	cpm ³H-TdR, graded doses of stimulators			
		6.2×10^4	2.1×10^4	6.9×10^3	2.2×10^3
day 0 epidermal cells	17	17,213	7,490	5,722	5,182
		1.5×10^4	5×10^3	1.7×10^3	0.6×10^3
day 4, no γ-interferon	24	100,654	45,909	16,920	8,916
day 4, + γ-interferon	23	69,384	31,114	13,746	7,872

In part A, low density fractions of cultured epidermal cells (+/− 100 units of recombinant *E. coli*-derived γ-interferon (kindly provided by Genentech, San Francisco, California) were exposed to saturating levels of ^{125}I-B21-2 (α-$Ia^{a,d}$) as described [14].

In part B, cells were tested for stimulatory activity using periodate-modified T cells as responders. T cells only incorporated 4867 cpm.

TABLE III. *The capacity of epidermal cells to stimulate T-cell proliferative responses increases during culture*

Stimulator Cells	% Ia⁺	cpm ³H-thmidine at graded doses of stimulators				
		3×10^5	1×10^5	3.3×10^4	1.1×10^4	3.3×10^3
Experiment 1: B6XD2F1						
Spleen adherent cells	N.T.	—	120,000	52,780	21,916	6,000
Epidermis, day 0, low density	13.2	11,942	5,648	1,720	754	687
Epidermis, day 0, high density	2.8	1,925	1,700	1,478	1,025	1,052
Mix, 2 parts low 1 part high	—	4,915	3,395	1,611	1,205	748
Epidermis, day 3, low density	32	—	268,440	260,942	213,620	100,397
Epidermis, day 3 high density	0.3	5,091	3,013	1,968	1,796	—
Mix 10^5 high with low density			256,154	271,112	200,386	107,562
		6×10^4	2×10^4	7×10^3	2.3×10^3	
Spleen dendritics	>90	130,521	132,430	79,809	29,561	
		1×10^5	3.3×10^4	1×10^4	3×10^3	
Experiment 2: B6.H-2k						
Epidermis, day 0 (trypsin)	5	1,485	660	424	347	
Epidermis, day 3 (trypsin)	30	—	122,533	73,489	32,852	
Epidermis, day 0 (dispase)	3	2.174	1,067	517	276	
Epidermis, day 3 (dispase)	14	131,325	77,017	36,025	12,797	

In experiment 1, fresh epidermal cells were compared to spleen adherent cells, and 3-day cultured epidermal cells were compared to enriched spleen dendritic cells. Stimulatory activity was assessed in the oxidative mitogenesis system, i.e., the polyclonal proliferation of periodate-modified T cells at 24–40 h.

In experiment 2, epidermal cells were released from sheets using trypsin or dispase. Low density cells were prepared at day 0 or day 3, and tested as stimulators.

Thymidine uptakes by T cells or stimulator cells only were < 800 cpm in all experiments.

TABLE IV. *Irradiation (1500 rads) does not reduce the number or the stimulating activity of cultured LC*

Irradiation prior to culture	% Ia cells	cpm ^{3}H-thymidine with graded doses of stimulators					
		oxidative mitogenesis			allogeneic MLR		
		2×10^4	6.7×10^3	2.2×10^3	2×10^4	6.7×10^3	2.2×10^3
No	32	192,747	134,347	62,824	133,791	69,300	15,655
Yes	30	184,364	151,741	55,596	131,014	64,275	13,507

Epidermal cell suspensions (CXD2 F1) were exposed to 1500 rads ^{137}Cs, or not treated. The cells were cultured for 1 day; nonadherent cells were removed; and, 2 days later, low density cells were isolated. The latter were stained with α-Ia. Aliquots were used to stimulate-periodate modified T cells (oxidative mitogenesis; cpm ^{3}H-thymidine measured at 24–40 h) or allogeneic T cells [B10T(6R),H-2^{9qd}]; cpm at 72–88 h).

MLR = mixed leukocyte reaction.

TABLE V. *Low density cells, which are partially enriched in LC, exhibit a marked increase in stimulatory capacity during culture*

Stimulator cells	Irradiation	Indomethacin	Conditioned medium	Top dose Ia$^+$ cells	cpm ^{3}H-thymidine at graded doses of			
					1.2×10^5	4×10^4	1.3×10^4	4×10^3
Day 0[a]	−	−	−	1.1×10^4	33,000	8,164	2,874	1,841
	−	+	−		47,448	8,411	2,351	1,871
Day 0, culture 3 days[b]	−	−	−		179,786	103,182	14,817	3,185
	−	+	−		191,182	129,015	12,221	3,268
Day 0, culture 3 days, nonadherent[c]	−	−	−	2.1×10^3	203,687	70,634	21,614	7,968
	−	+	−		197,134	66,587	13,392	6,891
	−	−	+		134,176	57,872	22,597	9,858
	−	+	+		135,041	45,609	15,597	N.D.
	+	−	−	2.2×10^3	121,109	33,653	8,724	4,303
	+	+	−		136,726	41,723	10,308	5,094
	+	−	+		81,676	26,233	10,024	8,019
	+	+	+		109,808	26,355	10,015	5,965
Mix above and 4×10^4 day 0[d]	−	−	−		117,549	57,203	24,564	12,323
	−	+	−		178,460	79,249	33,874	14,283
	+	−	−		96,488	39,757	15,604	8,882
	+	+	−		139,505	48,842	19,217	8,350
Day 0[d]	−	−	−	1.5×10^4	26,989	7,090	3,343	2,973
	−	−	+		48,839	18,512	7,476	6,705
	−	+	−		34,146	12,037	4,528	3,559
	−	+	+		35,110	21,385	8,571	5,645

In this experiment low density cells were tested for stimulatory activity (oxidative mitogenesis assay) at day 0 or after 3 days of culture. In contrast to the experiments in Table II, the 3-day cells were cultured low density cells rather than epidermal cells cultured for 3 days and then floated on albumin columns.

3 variables were tested, irradiation with 1500 rads ^{137}Cs prior to culture; addition of 1 μg/ml indomethacin to cultures upon addition of responding T cells; and conditioned medium, i.e., the oxidative mitogenesis cultures were supplemented with 50% v/v medium from epidermal cultures.

[a] Fresh low density cells prepared and tested at the start of the experiment.

[b] Same as [a], but the cells were cultured in graded doses in microtest wells for 3 days prior to adding T cells.

[c] Same as [a], but the cells were cultured 3 days in dishes; then the nonadherent cells were harvested, washed, resuspended to half the *initial* cell volume and mixed with 50% fresh or conditioned medium. The top dose of viable cells was 7×10^3 vs 1.2×10^5.

[d] Same as [a], but the cells were prepared at day 3 of the experiment.

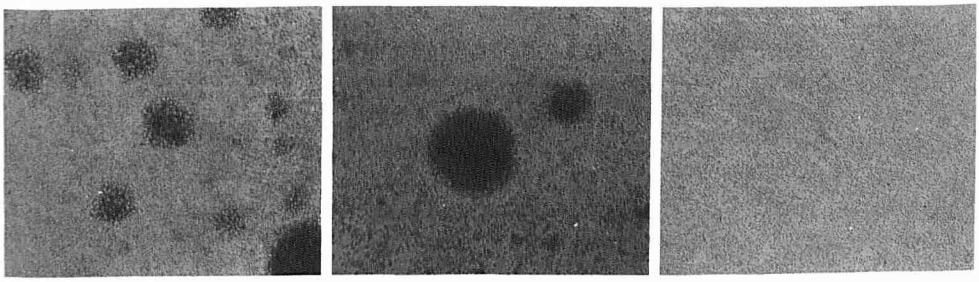

FIG 9. Clustering of dendritic cells and periodate-modified T cells during oxidative mitogenesis. The frames are from 40 h cultures of T cells only (*left*); T cells stimulated with spleen dendritic cells (*middle*); and T cells stimulated with LC (*right*). × 55.

the most restricted and widespread macrophage marker known to date [30]. F4/80 on LC really provides the only link between typical macrophages and DC. There is still no evidence that DC can develop in bone marrow cultures that are generating macrophages. The properties of DC are also thoroughly different from monocytes [20,34] which are the precursors of most tissue macrophages. Therefore if LC and lymphoid DC are related to macrophages, the lineages likely diverge early on in the marrow.

The development of LC immunostimulatory function in culture is also striking. Fresh LC have little stimulating activity for oxidative mitogenesis or for the mixed leukocyte reaction, but after 2–3 days in culture, LC are even more active than spleen DC [12] (Table III, Fig 8). Ia-rich, fresh LC thus resemble Ia-rich B lymphoblasts and lymphokine-treated macrophages in not being able to stimulate primary T-cell responses [38].

Taken together, our studies suggest that functioning DC, which are the principal stimulators for a variety of T-dependent immune responses [15], are derived from nonlymphoid tissues like skin and not immediately from marrow. In fact the skin of two mouse ears develops more stimulatory capacity than an entire spleen. A possible pathway for the production of DC would be skin to afferent lymph to node, since dendritic-type cells have been characterized in afferent lymph where they are usually called veiled cells [39–42]. A search for dendritic-type

cells in nonlymphoid tissues, other than skin, is warranted but suggestive immunocytochemical evidence certainly exist [43]. Recently, Faustman et al have used the 33D1 monoclonal to identify small numbers of DC in pancreatic islets of Langerhans [44]. They have shown that the DC are responsible for initiating graft rejection in a mouse allograft model. Conceivably islet DC are immature and acquire function when they move to the draining node. The epidermis may have solved the longstanding mystery of the origin of the active DC needed for the initiation of T-dependent immune responses. Clearly more work is needed on the mechanism of this maturation and its occurrence in situ, as well as in other species like human.

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