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Migration and Maturation of Langerhans Cells in Skin Transplants and Explants

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Summary

The behavior of Langerhans cells (LC) has been examined after skin transplantation and in an organ culture system. Within 24 h (and even within 4 h of culture), LC in epidermal sheets from allografts, isografts, and explants dramatically increased in size and expression of major histocompatibility complex class II molecules, and their numbers were markedly decreased. Using a new procedure, dermal sheets were then examined. By 24 h, cells resembling LC were found close to the epidermal-dermal junction, and by 3 d, they formed cords in dermal lymphatics before leaving the skin. In organ culture, the cells continued to migrate spontaneously into the medium. These observations establish a direct route for migration of LC from the epidermis into the dermis and then out of the skin. These processes are apparently induced by a local inflammatory response, and are independent of host-derived mediators. The phenotype of migratory cells was then examined by two-color immunocytochemistry and FACS analysis. The majority of migratory leukocytes were Ia^+ LC, the remainder comprised Thy-1^+, $CD3^+$, $CD4^-$, $CD8^-$ presumptive T cell receptor γ/δ^+ dendritic epidermal cells, which clustered with the LC, and a small population of adherent Ia^-, $FcRII^+$, $CD11a/18^+$ macrophages. In contrast to the cells remaining within the epidermis of grafted skin at 1 d, the migratory cells were heterogeneous in phenotype, particularly with respect to F4/80, FcRII, and interleukin 2 receptor α expression, which are useful markers to follow phenotypic maturation of LC. Moreover, cells isolated from the epidermis of grafts at 1 d were more immunostimulatory in the allogeneic mixed leukocyte reaction and oxidative mitogenesis than LC isolated from normal skin, though less potent than spleen cells. The day 1 migratory cells were considerably more immunostimulatory than spleen cells, and day 3–5 migratory cells even more so, suggesting that functional maturation continues in culture. Thus, maturation of LC commences in the epidermis and continues during migration, but the cells do not need to be fully mature in phenotype or function before they leave the skin. In vivo, the migration of epidermal LC via the dermis into lymphatics and then to the draining nodes, where they have been shown previously to home to T areas, would provide a powerful stimulus for graft rejection.

The generation of cell-mediated immune responses against foreign antigens requires the participation of both antigen-specific T cells and APC. While a wide variety of cell types can present antigens to activated T cells, the ability to activate resting T cells (immunostimulation) is possessed by a more select group of cell types (1).

Members of the dendritic cell (DC)[1] lineage have potent immunostimulatory properties and are widely distributed throughout the body (2). They play an important role in the initiation of immune responses in vivo and are thought to be critical "passenger leukocytes" that trigger graft rejection (reviewed in reference 3). Evidence is accumulating that DC function by transporting antigens from peripheral nonlymphoid tissues via the lymphatics or bloodstream into lymphoid tissues for presentation to resting T cells (4–9). For example, antigen-bearing DC can be isolated from draining LN after skin painting with contact allergens (10), alloantigen-bearing DC are evident within the host's spleen after transplantation of fully vascularized cardiac allografts (6), and

[1] *Abbreviations used in this paper:* DC, dendritic cells; DTH, delayed-type hypersensitivity; EC, epidermal cells; LC, Langerhans cells; GM, granulocyte/macrophage.

afferent lymph DC carry protein antigens that have been administered intradermally (9).

Langerhans cells (LC) are the DC of the epidermis and likely present antigens that breach the epidermal barrier. In vitro experiments with cell suspensions have demonstrated that freshly isolated LC are poorly stimulatory for unprimed T cells but process and present native protein antigens to T cell clones (11, 12). After culture, however, particularly in the presence of granulocyte/macrophage (GM)-CSF, LC undergo a maturation process characterized by a number of changes in phenotype and the development of immunostimulatory activity for resting T cells (13). These cultured LC closely resemble DC from spleen and other lymphoid organs.

Because lymphatics must be intact for optimal sensitization to skin transplants (14, 15) and to contact allergens (16), it can be proposed that epidermal LC may gain access to lymph and thereby to draining LN, where they sensitize host T cells to epidermal antigens. However, there has been little information on the behavior of DC in nonlymphoid tissues during the early stages of an immune response. Here, we have examined LC in epidermal and dermal sheets prepared at various times after transplantation of skin. We observed extensive changes in their phenotype, distribution, and function, and describe how these changes occur in skin that has been grafted to syngeneic recipients or maintained in organ culture.

Materials and Methods

Mice

Male C57BL/10 (H-2^b), BALB/c (H-2^d), CBA/Ca (H-2^k), and C3H/He (H-2^k) mice were obtained from Olac Ltd. (Bicester, Oxon, UK). CD2 F_1 (H-2^d), CBA/J (H-2^k), C57BL/6 (H-2^b), C57BL6H-2^k, C3H/HeJ, and B6D2 F1(H-2^{bxd}) mice were obtained from the Trudeau Institute (Saranac Lake, NY).

Skin Grafting

Ear skin was used for grafting because of its high density of LC and the ease with which epidermal sheets can be prepared. The ears were rinsed in 70% ethanol and split with forceps into dorsal and ventral halves. The dorsal halves, which contained little or none of the cartilage of the ear, were grafted to the trunk of the recipients and protected with a nonadherent dressing covered by a plaster cast.

Organ Culture

Skin from mouse ears was prepared as for grafting, and both halves were cultured on filter paper (Millipore, Peterborough, UK) (pore size, 4.5 or 0.8 μm) atop spongostan rafts (Ferrosan, Denmark; supplied by Tridas Medical Products Ltd., Langley, Berks, UK) floating in complete culture media (RPMI 1640, 10% FCS, 2 mM glutamine, 45 μg/ml penicillin, 45 μg/ml streptomycin, and 90 μg/ml kanamycin), similar to the method used for fetal thymus organ culture (17).

Antibodies

The following were used as primary mAbs for immunocytochemistry or FACS analysis: 25-9-3S (HB38, anti-Ia^b), 10.2-16 (TIB 93, anti-Ia^k), and MKD6 (HB3, anti-Ia^d), which are mouse mAbs; M1/70 (TIB 128 anti-iC3bR), 2.4G2 (HB197, anti-FcRII, [18]), NLDC-145 (anti-LC and veiled cells [19]), GK1.5 (TIB 207, anti-CD4), 53-6.72 (TIB 105, anti-CD8), F4/80 (20) (HB198, recognizes an antigen on macrophages and LC in situ), B21-2 (TIB 229, anti-I-A^d), and PC 61 5.3 (TIB 222), 7D4 (CRL 1968), and 3C7 (21), (the latter three are anti-IL2R p55), which are rat anti-mouse mAbs.

Second stage antibodies were peroxidase conjugates of goat anti-mouse IgM, goat anti-rat IgG (Sigma Chemical Co., St. Louis, MO), and mouse anti-rat IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN); 10-nm gold-conjugated goat anti-rat IgG (Janssen, ICN Biomedicals Ltd., High Wycombe, Bucks, UK); FITC-conjugated mouse anti-rat IgG (Boehringer Mannheim Biochemicals), FITC-conjugated goat anti-mouse IgM (Sigma Chemical Co.), and PE-conjugated goat anti-rat IgG (SeraLab).

Immunocytochemistry

Epidermal and Dermal Sheets. Dorsal halves of split ears (fresh, cultured, or after skin grafting) were floated dermal side down in a petri dish containing 0.5 M ammonium thiocyanate for 20 min at 37°C (22); epidermis was separated from dermis with fine forceps and stained immediately. Epidermal and dermal sheets were cut into ~3 × 3-mm sections, fixed in acetone for 20 min, and then rehydrated in PBS. They were incubated with primary antibodies for ~16 h at 4°C, washed three times in PBS containing 1% FCS and 0.01% NaN_3, and then incubated in FITC-conjugated mouse anti-rat IgG at 1:100 for 90 min at 37°C. After three washes, the sheets were mounted in aquamount (BDH Ltd., Poole, UK) and evaluated under a UV microscope (Ortholux II; E. Leitz Inc., Wetzlar, FRG). Dermal sheets were handled exactly as for epidermal sheets; evaluation of the dermis in this manner has not been previously described. Counts to determine LC density were performed at ×625 under water immersion using an ocular frame of known area.

Frozen Cross-sections. Frozen sections of normal, grafted, or cultured ear skin were prepared and stored at −30°C until use. These were fixed in acetone, rehydrated, and incubated with primary antibodies for 45–60 min and FITC mouse anti-rat IgG (at 1:100) for 45–60 min. For immunoperoxidase, peroxidase goat anti-rat IgG was used as a secondary antibody, and 3-amino-9-ethyl-carbazole was used as a chromagen to allow better differentiation of the peroxidase reaction product from the pigment in the epidermis and dermis.

Cytocentrifuge Preparations. Cell populations were suspended at 10^5/ml in RPMI plus 10% FCS and sedimented onto glass slides, air dried, and stored at −30°C until use. They were immunoperoxidase stained as above.

Two-color FACS Analysis

Cell populations were labeled first with HB38 (mouse anti-Ia^b) or with TIB 93 (mouse anti-Ia^k, as a negative control) followed by FITC-conjugated goat anti-mouse IgM (Sigma Chemical Co.). Aliquots were then labeled with one of the various rat mAbs (all IgG, see above) against the markers of interest, followed by PE-conjugated goat anti-rat IgG (γ-chain specific; Seralab) and fixed in formol-saline. Fluorescence profiles were generated using a FACScan flowcytometer and Consort 30 software (Becton Dickinson & Co.), with the valuable assistance of P. Fairchild. For this, the subset of Ia^b-positive, FITC-labeled LC was defined (relative to the negative control), and the PE fluorescence profile of this subset was acquired and displayed as a histogram. The profiles were then electronically overlaid. Note that the noncompeting rat anti-Ia^b mAb B21-2 was included in the PE analysis to confirm that all the

FITC-gated cells expressed MHC class II and were a homogeneous population.

Cell Populations

Epidermal Cell (EC) Suspensions. Epidermal sheets were prepared by floating ear halves (fresh or cultured), essentially as described (13), except that 0.2 mM EDTA was included during the trypsin digestion step. EC were either used directly or layered onto 4-ml BSA columns (p = 1.083) and centrifuged at 2,000 g for 20 min at 4°C. The interface cells were termed low density EC.

Migratory LC. Migratory LC were obtained from cultured ear halves that were floated directly on 2–2.5 ml of complete media in 24-well plates for 1–7 d. At specified times, the nonadherent migratory cells were gently resuspended, filtered through a 35-μm Nitex filter (Tekto Inc.) to remove hair and debris, pelleted, and counted in trypan blue before use. Cell populations obtained using this system between 1 and 7 d of culture were 71% Ia^+ LC (± 13% SD in 17 experiments, as described in the text).

Spleen Cell Suspensions. Single cell suspensions were prepared from spleens by gentle teasing with forceps. Erythrocytes were lysed using tris-buffered ammonium chloride.

Nylon Wool–passed T Cells (~90% pure). Unfractionated spleen cell suspensions or the high density fraction of a BSA gradient of spleen cells were passed through nylon wool columns and treated with tris-buffered ammonium chloride to lyse residual erythrocytes (5).

Immunostimulation Assays

Oxidative Mitogenesis. Graded doses of irradiated (2,000 rad, ^{137}Cs) stimulator cells were added to syngeneic sodium periodate–treated T cells (2.5 × 10^5/well) in a final volume of 0.2 ml in 96-well flat-bottomed microtest plates (23). Proliferation in the wells was measured by adding 1 μCi of [^{3}H]thymidine/well after 22–26 h. The cells were harvested 16–18 h later and counted on a β plate counter (LKB Instruments, Inc., Gaithersburg, MD). Results are the means of triplicate cultures.

Allogeneic MLR. MLR were set up as for oxidative mitogenesis assays, except that allogeneic nylon wool–passed T cells were used as responders (2.5 × 10^5/well) and proliferation was measured during a 16–18-h thymidine pulse on day 3–4.

Results

Changes in Epidermal LC after Skin Grafting. The distribution of Langerhans cells in epidermal sheets was initially visualized by immunofluorescent staining using a donor-specific anti-Ia mAb. Epidermal sheets from allografted skin were prepared between 1 and 7 d after transplantation; beyond 7 d, the grafts were too friable to permit preparation of sheets. Similar results were obtained in several strain combinations (CD2 F1 → CBA/J; C57BL/6 → B6D2 F1; C57BL/6 → C3H/HeJ; C57BL/10 → CBAB6 F1; C57BL/10 → CBA/Ca), although results are presented only for C57BL/10 → CBA/Ca.

Within the first 24 h after allografting, the epidermal LC increased markedly in size and upregulated their expression of Ia (compare Fig. 1, *a* and *b*). These changes appear to be the in vivo counterpart of those described for isolated LC cultured in the presence of TNF-α and/or GM-CSF. In addition, there was a dramatic decrease in the density of LC within epidermal sheets with time (Fig. 1, *a–d*). The efflux began promptly after grafting, particularly over the first day, with the numbers of LC falling from 921/mm² in normal C57BL/10 skin to 392/mm² after grafting on to CBA/Ca recipients (Table 1). The density was reduced to 128/mm² by 7 d, and most of the LC that persisted remained large and strongly Ia^+ (not shown). As reported by others, Ia was induced on keratinocytes of the allografts by day 7 (not shown), but the LC expressed higher levels so they could still be enumerated.

Table 1. *Changes in Epidermal LC Density after Skin Grafting*

Time	Epidermal LC density	
	Allografts	Isografts
d	*mm*$^{-2}$	
0	921 ± 204	
1	370 ± 128	392 ± 114
3	293 ± 110	317 ± 102
5	277 ± 114	469 ± 170
7	128 ± 105	390 ± 258

C57BL/10 skin grafts on CBA/Ca mice were removed at specified times, stained with anti-Ia, and the LC density was determined. Values are the mean ± SD of ≥24 random fields from three grafts at each time point, and 67 random fields for normal skin.

A similar series of CD2F1 and C57BL/10 isografts was evaluated for comparison, and results are presented for the latter. Over the first 3 d after grafting, the changes noted in epidermal LC were identical to those seen in allografts: the LC enlarged, increased their expression of Ia, and decreased in density (Table 1). Thus, the early changes noted for allografts appear to have been due to a nonspecific inflammatory response rather than the specific allograft reaction. At later times (days 5–7), the density of LC within the isografts appeared to stabilize and the LC that were present were similar in size and Ia expression to those in normal skin (not shown). This could indicate that the inflammatory response to grafting had subsided, whereas in allografts, the injury continued as rejection proceeded, or it might reflect an influx of LC from the recipient because these cells would also be detected by the anti-Ia antibody used.

Skin Organ Culture Reproduces the Changes in Epidermal LC Associated with Skin Grafting. To dissociate the effects of locally produced signals for phenotypic changes and migration of LC from those of the host, we adapted the fetal thymus organ culture system described by Jenkinson et al. (17) to skin explants. Dorsal ear skin was prepared as for grafting, and cultured on spongostan rafts in complete medium (see Materials and Methods). The cultured skin resembles a skin graft before it is vascularized in that it survives by diffusion of nutrients, but unlike a skin graft, it is influenced solely by endogenously produced mediators or by mediators added to the system.

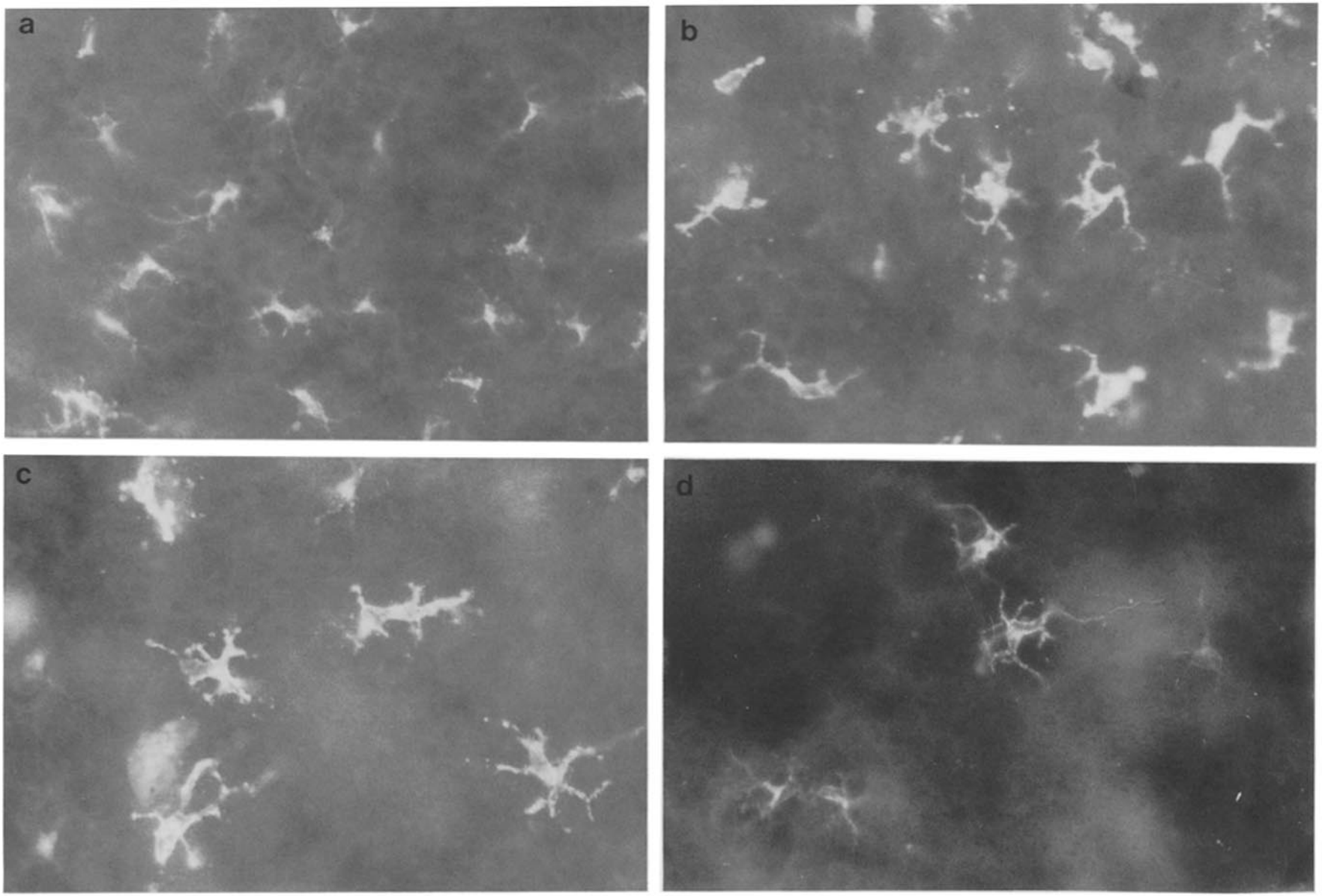


Figure 1. Changes in epidermal LC after skin grafting. Epidermal sheets of normal and grafted skin were stained with anti-Ia mAb, B21-2 (×400). (*a*) Normal skin has an extensive network of LC. (*b*) 1 d after allografting, LC increase in size and express higher levels of Ia. (*c*) 3 d and (*d*) 5 d after grafting, the number of epidermal LC is markedly reduced.

Table 2. *Changes in Epidermal LC Density during Organ Culture*

Time	Epidermal LC density
h	mm^{-2}
0	921 ± 204
4	824 ± 106
8	850 ± 144
16	810 ± 190
24	761 ± 215
48	226 ± 96
72	83 ± 59

C57BL/10 ear skin was cultured on spongostan rafts and analyzed as in Table 1. Values are the means ± SD of ≥20 random fields.

Epidermal sheets from cultured skin showed similar changes to those seen in grafted skin. Even within 4 h, there was a dramatic increase in the size of the LC and the intensity of Ia expression (not shown). There was also a steady exodus of LC from the epidermis, albeit with slightly slower kinetics compared with grafted skin (Table 2). Similar observations were noted with the DC-restricted antibody NLDC-145 on cultured skin, but this marker was considerably weaker than Ia, making it less useful for counting and photography. The similarities between allografts, isografts, and cultured skin suggest that injury alone is sufficient to stimulate dramatic phenotypic changes and the migration of epidermal LC. The fact these can occur in cultured skin indicates that the mediator(s) is locally produced.

Epidermal LC Migrate into the Dermis of Transplants and Explants. Reduced numbers of epidermal LC might have been seen in skin grafts and cultured skin because these cells died in situ, were sloughed from the surface of the epidermis (24), or migrated into the dermis. To examine the fate of epidermal LC, we applied the techniques used for evaluating epidermal sheets to the dermis of cultured and grafted skin. The normal dermis from mouse ear has relatively few Ia^+ (Fig. 2 *a*) or NLDC-145^+ cells. In contrast, strongly Ia^+ cells appeared in significant numbers within the dermis of cultured or grafted skin at the same time the number of LC decreased within the epidermis. After 24–48 h in organ culture, or 1 d after skin grafting, LC were scattered in a high focal plane in the dermal sheet, just below what had been the epidermal-dermal junction (Fig. 2 *b*). 3 d after organ culture or skin grafting, the LC were found in "cords" or strands deeper within the sheets, suggesting that the cells had entered lymphatic or vascular channels (Fig. 2 *c*). Eventually, the LC left the dermis of both the transplants and explants. These cells also stained with NLDC-145 (not shown), making it unlikely these results were due to induction of Ia on other elements of the dermis.

The distribution of LC was also examined in cross-sections of cultured skin and skin grafts. These studies confirmed that the LC initially became larger and more Ia^+ within the

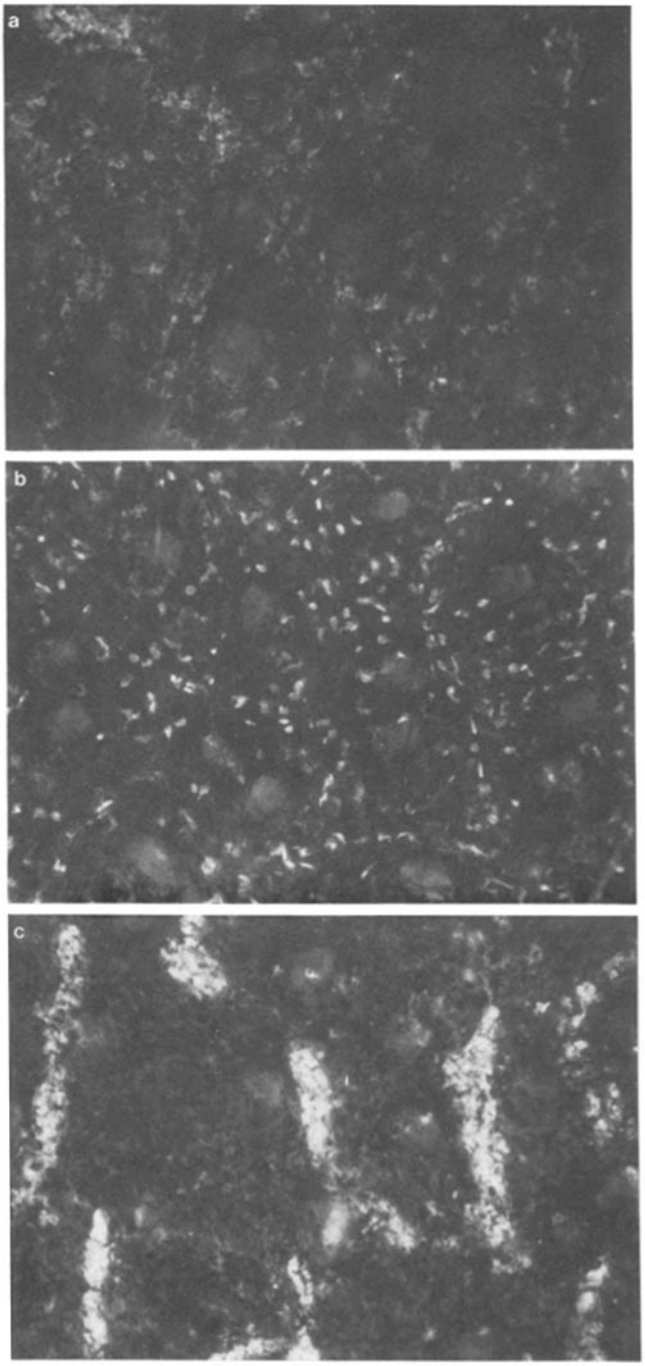


Figure 2. Epidermal LC redistribute to the dermis. Dermal sheets from cultured skin were stained with anti-Ia mAb, B21-2 (×50). (*a*) Normal dermis contains relatively few Ia^+ cells (white). (*b*) After 48 h in organ culture, numerous scattered Ia^+ cells have appeared in the dermis. (*c*) After 72 h, most Ia^+ cells in the dermis are found in groups or in cords; although not clearly visible in this photograph, discrete cells could be readily seen by microscopically focusing up and down within the plane of the tissue in the original specimen. Similar observations were made in allografts and isografts.

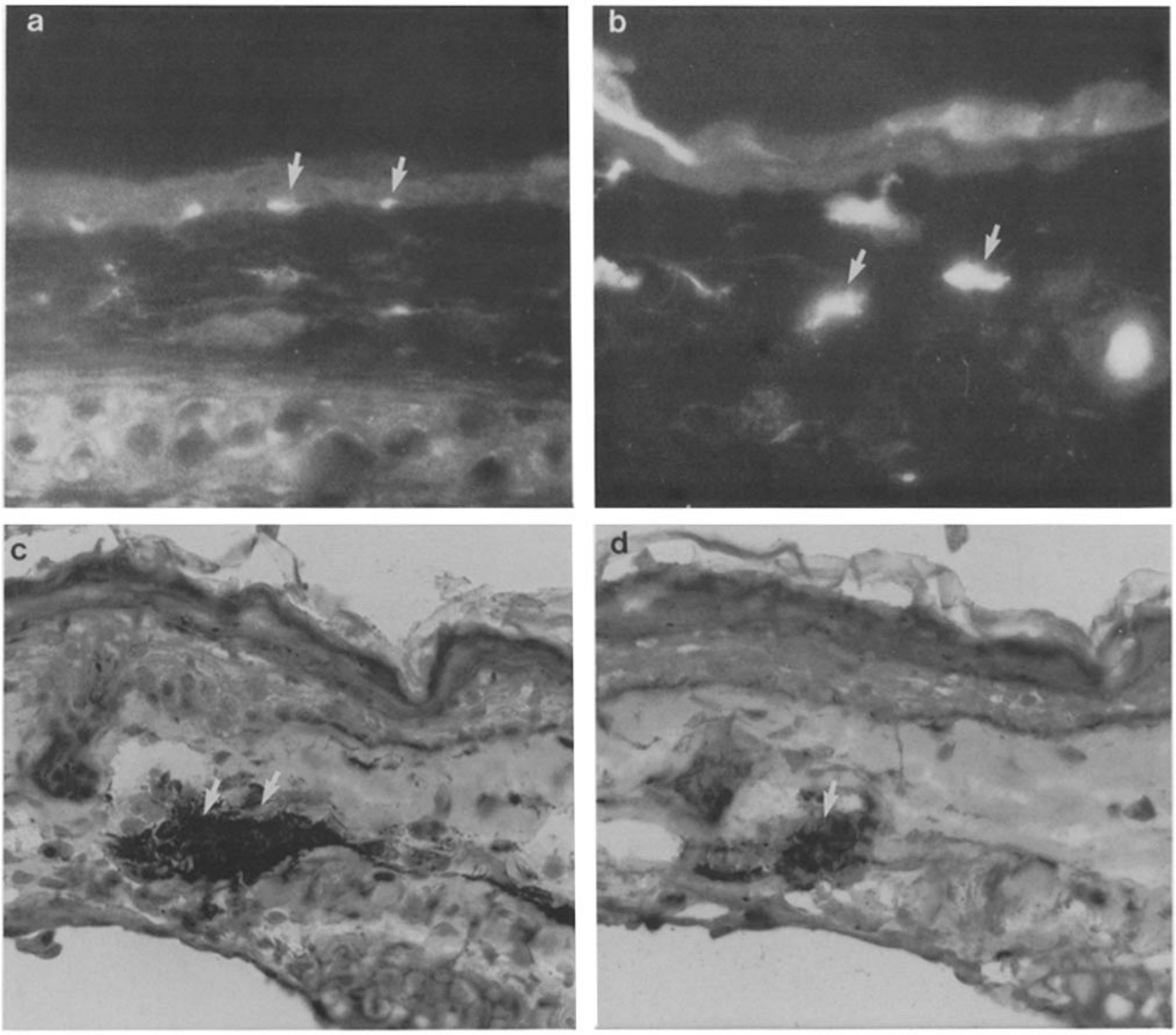
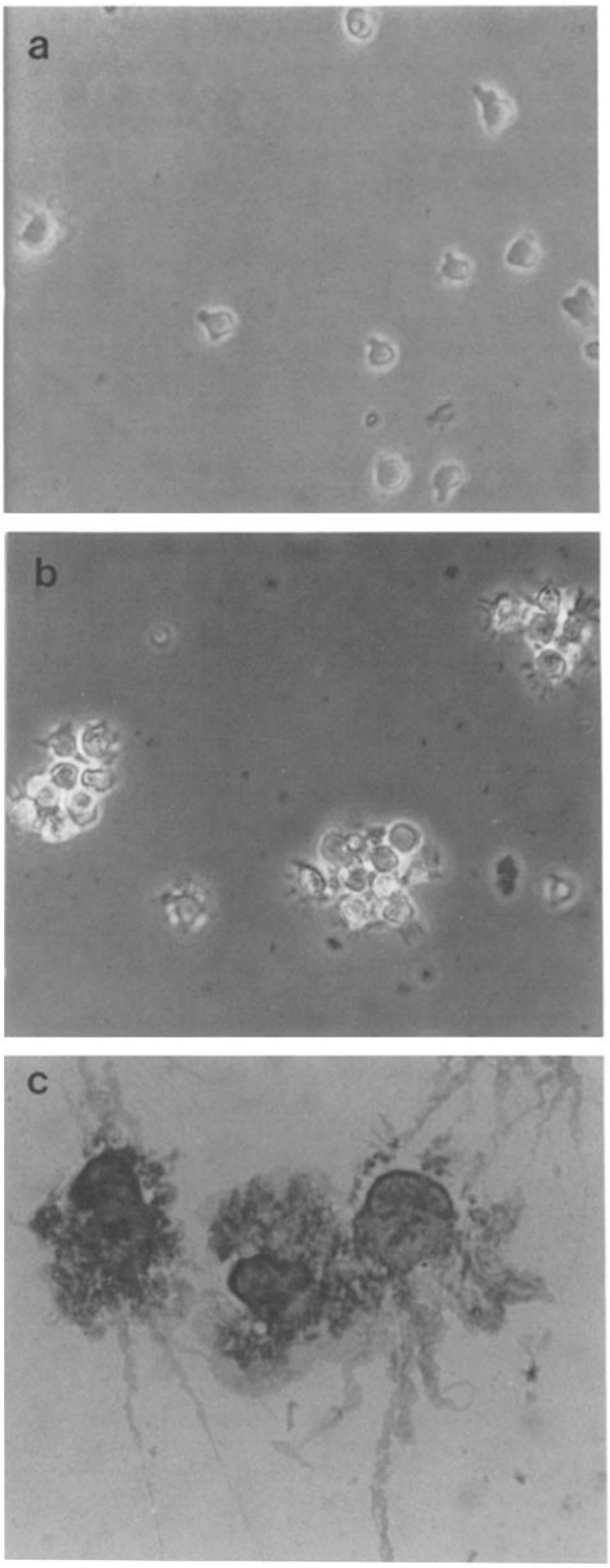


Figure 3. Redistribution of epidermal LC (*arrows*) to the dermis in cross-section. Frozen sections of (*a*) normal, (*b*) allografted, and (*c* and *d*) cultured skin stained with (*a–c*) anti-Ia mAb, B21-2, and (*d*) NLDC-145 (×400). (*a*) In normal skin, LC (white) are small and lie just above the dermal-epidermal junction. (*b*) 24 h after allografting, LC are at or straddling the epidermal-dermal junction. (*c* and *d*) After 3 d in organ culture, LC are found within the dermal lymphatics.

epidermis (not shown). Later, the cells left their predominantly suprabasal position (Fig. 3 *a*), and some cells were seen apparently straddling, or just under, the epidermal-dermal junction (Fig. 3 *b*). At later times, LC were found deeper within the dermis in groups or cords (Fig. 3 *c* and *d*) before they disappeared (not shown). Unlike the situation reported for DTH responses (24), LC were not seen in the upper portion of the epidermis, suggesting they were not being sloughed from the skin, and no obvious epidermal thickening was seen in either the transplants or explants. Thus, epidermal LC migrate into the dermis after transplantation and organ culture.

Epidermal LC Migrate Out of Cultured Skin. To determine whether LC continued to migrate out of explants into the culture medium, ear skin was floated directly on medium in 24-well plates. Initially, few cells were seen at the bottom of the wells, but after 24 h, cells with short processes were evident (Fig. 4 *a*). The number of cells increased over the next few days in culture at the rate of $\sim 8–10 \times 10^3$/ear/d (S. Ewing, unpublished observations). They became larger with more extensive cytoplasmic veils, and soon formed clusters within the wells (Fig. 4 *b*).

Cytocentrifuge preparations revealed that virtually all the nonadherent cells (95% ± 5% SD, $n = 8$) were leukocytes (TIB 122^+). In 17 experiments, 71% (±13% SD) were large Ia^+ LC with extensive cytoplasmic veils (Fig. 4 *c*). The remainder were smaller Thy-1^+, $CD3^+$, $CD4^-$, $CD8^-$



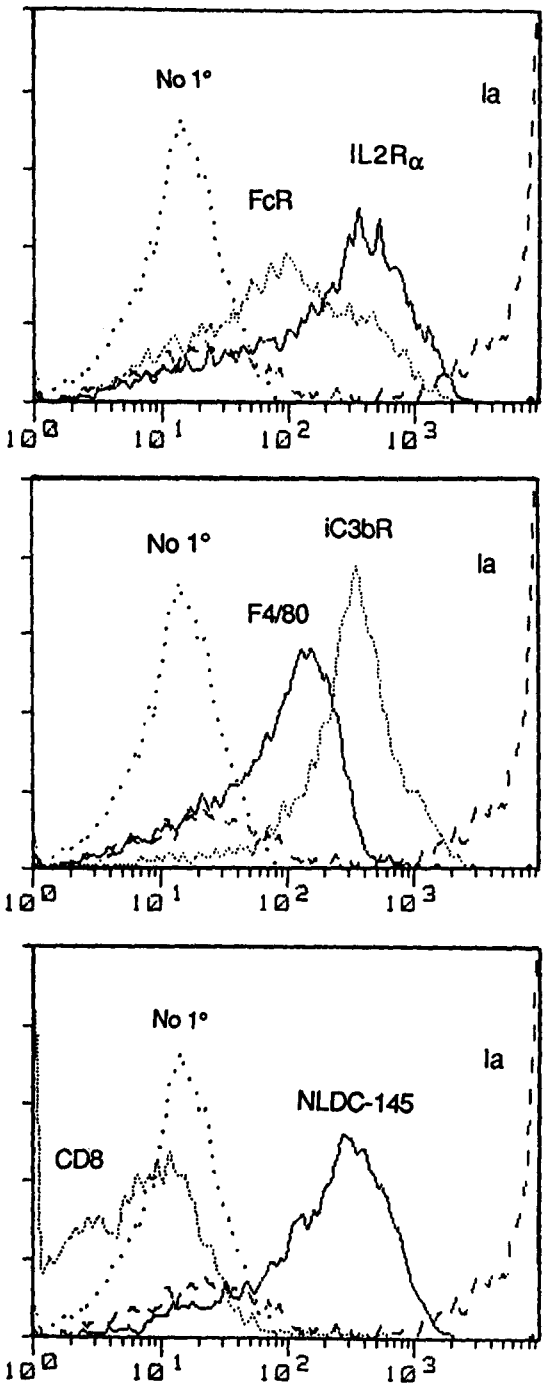


Figure 5. The phenotype of day 3 migratory LC by two-color FACS. Migratory LC were analyzed for expression of various markers (see Materials and Methods). Note that the data are displayed on a high-sensitivity scale for clear resolution of the profiles: expression of MHC class II is at least two orders magnitude greater than that of F4/80 and FcRII, for example.

Figure 4. LC migrate out of cultured skin. (*a*) A phase contrast photomicrograph of migratory cells from ears after 24 h of culture; note cells have short processes (×200). (*b*) After 5 d of culture, the cells form clusters and have extensive cytoplasmic veils (×200). (*c*) Cytocentrifuge preparation of migratory cells with the characteristic morphology of cultured LC, stained with anti-Ia (B21-2) (×1,000).

lymphoid cells, which we presume are Thy-1$^+$ TCR-γ/δ^+ dendritic epidermal cells (DEC) that also migrated from the epidermis (results not shown). Closer examination revealed that at later times, the LC were clustered with DEC, the significance of which is being investigated (C.P. Larsen, J.A.

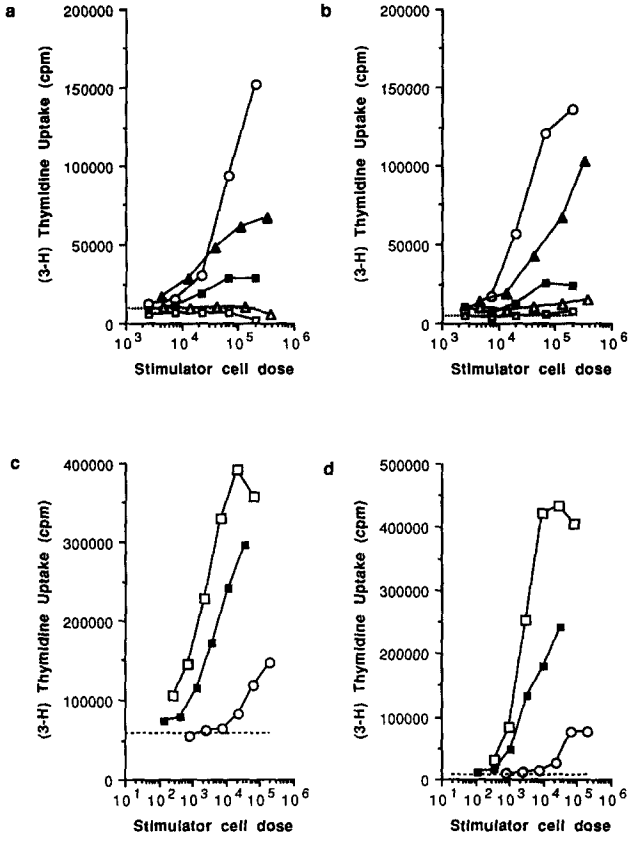


Figure 6. Functional maturation of LC accompanies migration. (*a* and *b*) C57BL/10 EC were tested for their ability to stimulate (*a*) oxidative mitogenesis of periodate-treated syngeneic T cells or (*b*) an allogeneic MLR using untreated C3H/He T cells. Unfractionated EC from fresh skin (*open squares*) were unable to stimulate either response, while EC from cultured skin (*filled squares*) showed modest immunostimulatory activity compared with whole splenocytes (*open circles*). LC-enriched low density EC from cultured skin (*filled triangles*) showed even greater immunostimulatory activity, whereas low density EC from fresh skin (*open triangles*) were unable to induce significant T cell proliferation (*a* and *b*). Dashed lines indicate T cells alone. Similar results were obtained in three experiments. (*c* and *d*) Migratory LC were tested for their ability to stimulate (*c*) oxidative mitogenesis or (*d*) an allogeneic MLR, as above. LC harvested after 3–4 d of culture (*open squares*) were more immunostimulatory than LC harvested after 1 d of culture (*filled squares*); whole splenocytes (*open circles*) were tested for comparison. Similar results were obtained in five experiments.

Roake, and J.M. Austyn, unpublished observations). There were no cells in the wells with detectable staining for CD4, CD8, or Ig. In addition to the nonadherent cells, the wells also contained an adherent population with the phenotype FcRII$^+$, CD11b/18$^+$ (iC3b receptor), F4/80$^+$, Ia$^-$, presumably macrophages from the dermis. These cells were not harvested but stained in the wells or on glass coverslips (results not shown). Thus, the migratory population was apparently comprised of LC, Thy-1$^+$ DEC, and macrophages, although the nonadherent fraction used in subsequent studies (below) contained only the former two cell types. The accumulation of LC in the culture medium provided direct evidence for migration of epidermal LC out of the skin via the dermis.

Migratory LC Begin to Acquire the Phenotype of DC. Having observed a rapid upregulation of Ia on epidermal LC, we next examined whether other phenotypic changes occurred. As reported by others (11, 25), resident epidermal LC were found to express Ia, CD45 (leukocyte common antigen), CD11b/CD18 (iC3b receptor), NLDC-145, FcRII, and F4/80, but IL-2Rα was not detectable (not shown). The latter three markers are particularly informative, FcRII and F4/80 being downregulated during maturation of cultured LC, while IL-2Rα is acquired (11, 26). Negative controls were CD4 (TIB 207) and CD8 (TIB 105).

At least the majority of LC remaining in epidermal sheets after 24 h of culture expressed high levels of Ia and also stained for FcRII and F4/80, but not IL-2Rα (not shown). The phenotype of migratory LC that had left the skin and accumulated in the culture medium after 1, 3, and 5 d of culture was then examined by single- and two-color immunoperoxidase staining of cytospins, revealing marked heterogeneity in the expression of these markers (not shown). These findings were confirmed by two-color FACS analysis of day 3 migratory LC, which demonstrated variable expression of the various markers, with undetectable to modest levels of FcRII and F4/80, modest to high levels of the IL-2Rα, NLDC-145, and CD11b/CD18 (iC3bR, which changes little during maturation), and very high levels of Ia (Fig. 5). Thus, migratory LC begin to acquire markers associated with mature DC, but do not need to be fully mature in phenotype before they migrate from the epidermis.

Functional Maturation Accompanies Migration. To determine whether functional maturation of epidermal LC occurred during culture, we first prepared epidermal cell suspensions from fresh or 1-d explants and assessed their ability to stimulate oxidative mitogenesis (a rapid, polyclonal, proliferative response of periodate-treated T cells) and the primary allogeneic MLR. Bulk and low-density EC suspensions, containing ~1% and ~2–7% Ia$^+$ LC, respectively, were found to be more effective stimulators when prepared from cultured skin than fresh skin (Fig. 6, *a* and *b*), although they were considerably less potent than spleen cells. This indicates that functional maturation begins in situ within the epidermis.

The stimulatory activity of migratory LC was then tested at various times after culture. In both assays, day 1 migratory cells (containing 60% ± 14 SD Ia$^+$ LC) were ~25–50 times more potent than spleen cells on a per cell basis, while day 3 migratory cells (containing 65% ± 9 SD Ia$^+$ LC) were ~75–100 times more potent (Fig. 6, *c* and *d*). In five independent experiments, the day 1 migratory cells were less stimulatory than day 3, 4, or 5 migratory populations; this could not be accounted for simply by the number of LC present. These results indicate that LC do not need to be fully mature in a functional sense before they migrate from the skin.

Discussion

DC within nonlymphoid tissues are thought to be important "passenger leukocytes" that sensitize host T cells after transplantation (reviewed in reference 3), but there is little

information on the behavior of these cells in grafts. While it has been assumed that epidermal LC from skin allografts migrate into the draining nodes and initiate rejection, the evidence for this is largely circumstantial. For example, the importance of a lymphatic drainage for rejection of skin allografts was demonstrated in classical studies (14, 15), and although DC can be isolated from afferent lymph as veiled cells, an epidermal origin for these cells has not been proven. It is possible to isolate antigen-laden DC from draining lymph nodes after the application of contact sensitizers (10), implying that these cells migrate from the epidermis. However, these contact allergens might have been acquired en route within the lymph, or within the node itself.

In the current study, the use of epidermal and dermal sheets prepared from transplanted skin allowed us to demonstrate directly the migration of LC out of the epidermis into the dermis, together with a number of other changes in the cells. Within 4 h, the cells increased both in size and Ia expression, and significant migration from the epidermis was evident within 24 h. This was concomitant with the appearance of strongly Ia^+ cells within the dermis, which were later arranged in distinct cords or strands, presumably within lymphatics. These events were similar in allografts and isografts, certainly over the first few days after grafting. They were also seen in an organ culture model where the cells continued to migrate into the culture medium, providing a source of cells that spontaneously migrated from the skin for in vitro analysis. This revealed heterogeneity in phenotype and increased immunostimulatory function. The fact that some migratory cells more closely resembled LC in situ, at least with respect to the markers examined, indicates that LC do not need to become phenotypically fully mature before they can migrate. The hierarchy of function (day 5 migratory cells > day 1 migratory cells > spleen > day 1 EC in situ > day 0 EC in situ) points to a similar conclusion. Thus, after grafting (and in culture), LC begin to mature into cells more closely resembling lymphoid DC and migrate out of the tissue.

It is notable that after grafting LC did not appear to move upwards in the epidermis, and keratinocyte hyperplasia was not evident. This is in contrast to a report on the behavior of LC during a human delayed-type hypersensitivity (DTH) response, where marked epidermal thickening was observed and LC appeared to be carried upwards, ultimately, it was suggested, to be sloughed from the surface (24). Possibly, there are differences in the behavior of epidermal LC in sites containing rapidly generated secondary immune reactions (e.g., DTH) compared with nonspecific inflammatory or nascent primary immune responses; it would be of interest to examine the behavior of epidermal LC during second-set rejection of skin allografts. It is also striking that the migration of LC from epidermis to dermis, and thence out of the skin, could occur in the absence of any lymph or capillary flow. Similar directed migration has been reported in vivo, in that DC home to T-dependent areas of lymph nodes or spleen, respectively (4, 7). Directed migration has also been observed in other organ culture systems: DC migrate spontaneously out of murine islets of Langerhans (Larsen, C., K. Leow, and J. Austyn, unpublished observations), but migrate into the medulla of embryonic thymus rudiments (27).

We feel that the changes noted in the LC of skin grafts do not merely reflect the normal turnover of these cells. First, the changes in morphology, size, and Ia expression were seen for the majority of the resident population of LC. In contrast, normal ear skin has only a small percentage of large and strongly Ia^+ LC (25), which may represent cells turning over at the basal rate. Second, the efflux of LC in both transplants and explants was rapid (half-life, ~1–2 d). Two previous studies that focused on the origin and turnover of LC using bone marrow chimeras found the half-life of LC in situ to be >5 wk (28, 29). Interestingly, in both studies, the turnover of LC in nonrejecting skin grafts (parent → F_1) was found to be faster (half-life, ~2 wk), although time points earlier than 11 d were not examined.

In injured or inflamed tissues, signals may be produced that promote the maturation of LC and their efflux from the tissues. The stimulus(i) for maturation and migration of LC has not been defined, but it would appear to be a consequence of a relatively "nonspecific" response (e.g., inflammation) rather than part of the adaptive immune response (allograft reaction), since similar results were obtained for allografts and isografts, as well as in organ culture. Moreover, the response appears to be a "local" one and there seems to be no requirement for mediators produced by infiltrating host cells or from the graft bed, because only endogenously produced mediators are present in organ culture. Thus, local inflammation appears to be a sufficient stimulus to induce LC maturation and migration.

In light of the similar movement of LC within cultured and grafted skin, the finding that LC migrate out of skin into the culture medium in vitro strongly suggests they exit similarly in vivo. Almost certainly, they can then migrate as veiled cells in lymphatics to draining nodes and home to T-dependent areas. The rapid departure of LC from injured or inflamed tissues would provide an efficient means of transporting whatever antigens are present (if any) to the lymphoid tissues where antigen-specific T cells can be encountered. In addition, the upregulation of membrane Ia, and presumably specific MHC-peptide complexes, is likely to increase the cell's ability to present the relevant peptides sampled in the periphery. The fact that some migrating LC in vitro are relatively immature also raises the possibility that, once they are within the node, they may be able to acquire and process soluble antigens that were carried in the lymph, this ability seeming more typical of immature rather than mature cells (12). Although LC migrate similarly out of both allografts and isografts, in the case of an allogeneic skin graft, the delivery of allogeneic LC into the recipient's nodes would provide a powerful stimulus for initiation of rejection.

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