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Both Dendritic Cells and Memory T Lymphocytes Emigrate From Organ Cultures of Human Skin and Form Distinctive Dendritic–T-Cell Conjugates

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Prior studies of mouse skin in organ culture have shown that dendritic cells selectively emigrate from the explants over 1–3 d. This emigration may model the movements of dendritic cells that can occur *in situ*, as in transplantation and contact sensitivity. In this study, we cultured explants of normal human skin that had been removed with a dermatome. Dendritic cells with characteristic morphology and mixed leukocyte response-stimulatory activity emigrated. The dendritic cells had the expected phenotype, e.g., rich in major histocompatibility complex class II and accessory molecules such as B7-1, intercellular adhesion molecule-1, and leukocyte function–associated antigen-3. Small lymphocytes also were present in the emigrated populations and proved to be T cells exclusively, almost entirely of the TcR$\alpha\beta$ and memory type (CD45RA^{weak}, CD45RO^{+}, LFA-3/CD58^{+}), with a CD4:CD8 subset ratio of about 2:1. Some of the T cells were bound tightly to the dendritic cells. These conjugates did not dissociate after exposure to trypsin or to calcium- and magnesium-free medium, or during cytofluorography. This made it possible to sort distinct populations of single dendritic cells, single T cells, and conjugates of the two cell types. Conjugates would continue to form from mixtures of separated dendritic cells and T cells in culture. Therefore, cutaneous dendritic cells and memory T lymphocytes emigrate from human skin explants, and some of these cells form distinctive conjugates that we hypothesize contribute to immunologic recall reactions. *Keyword: Langerhans cells.*
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Among the distinctive features of dendritic cells are their migratory properties. Migration has been studied to a large extent in skin. During contact sensitivity, dendritic cells (Langerhans cells) are noted in the afferent lymph [1,2] and in the draining lymph node [3,4]. After skin transplantation, dendritic cells leave the epidermis and undergo changes that include increased expression of major histocompatibility complex (MHC) class II [5]. Because dendritic cells are known to gain access to afferent lymphatics [1,6–8], the migration of these potent antigen-presenting cells into the lymph and then to the draining lymph node may account for the need for intact, cutaneous afferent lymphatics during the primary response to transplants [9] and contact allergens [10] *in situ*. In recall or delayed-type hypersensitivity reactions, dendritic cells also are juxtaposed to the infiltrates of dermal mononuclear cells [11].

When dendritic cells are pulsed with antigens *ex vivo* and are injected into mice, CD4^{+} T cells are primed in the draining lymphoid organs [12–14]. Austyn *et al* [15] showed that dendritic cells, when placed into the bloodstream or paws of mice, migrate to the T-cell areas in the draining lymphoid tissue, i.e., spleen and lymph node, respectively. If antigens are deposited intramuscularly, the dendritic cells from the corresponding afferent lymphatics carry that antigen in a form stimulatory for T cells [16]. Therefore, the migratory properties of dendritic cells likely interface with their antigen-presenting functions to sensitize T cells *in situ*.

If explants of mouse [5] or human skin [17] are placed in organ culture, dendritic cells selectively migrate into the medium surrounding the explant. This system might be useful in the further characterization of dendritic-cell migration and should provide access to cutaneous dendritic cells in disease states. An example is human immunodeficiency virus-1 (HIV-1) infection. Dendritic cells are found in the epithelia covering all of the organs involved in the sexual transmission of HIV-1 [18], making their susceptibility to HIV-1 an important area of study.

Because of our interest in the role of dendritic cells during transplantation and during HIV-1 infection, we set up organ cultures of normal human skin. Specimens were removed with a dermatome from skin that otherwise would have been discarded after plastic surgery. When the explants were placed in culture, dendritic cells emigrated and exhibited a characteristic morphology, phenotype, and T-cell–stimulatory function. What we found, however, was that cutaneous T-cell receptor (TcR)$\alpha\beta^{+}$ T cells also emigrated, making both T cells and dendritic cells accessible in a highly enriched form for study. We describe several properties of these T cells, including their ability to form distinctive conjugates with autologous dendritic cells.

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Abbreviation: PE, phycoerythrin.

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MATERIALS AND METHODS

Culture Medium RPMI 1640 (Cellgro, Fisher Scientific, Springfield, NJ) was supplemented with 10 mM HEPES, 50 μM 2-mercaptoethanol, 100 U/ml penicillin-100 μg/ml streptomycin, 2 mM L-glutamine, and either 10% fetal bovine serum (Gibco BRL) or 10% normal human serum (obtained from laboratory donors).

Preparation of Cell Suspensions Split-thickness, normal breast or abdominal skin was removed with a dermatome after cosmetic surgery. The skin was washed twice with sterile Ca^{++}- and Mg^{++}-free phosphate-buffered saline, incubated in medium with 200 μg/ml gentamicin (Gibco BRL) for 1 h at 4°C, washed twice in sterile Ca^{++}- and Mg^{++}-free phosphate-buffered saline, and floated as 3 × 3-cm explants dermal side down, each in 15 ml of medium in 100-mm dishes (3003, Falcon, Oxnard, CA). After 2–5 d at 37°C, the skin was removed and the debris digested with 400 Mandl units/ml Collagenase D (1088 882, Boehringer Mannheim, Indianapolis, IN) for 1 h at 37°C. This was essential to be able to harvest the cells without marked losses due to trapping within collagenous debris. The cells were pooled and washed in medium, and the numbers of viable cells (greater than 95%) were assessed by Trypan blue (Gibco BRL) exclusion.

Skin cells also were prepared from epidermal sheets and dermal explants as described [19,20], except that the dendritic cells were enriched by flotation on 13.5% metrizamide [21].

Immunolabeling and Cell Sorting Greater than 2×10^4 skin cells per well were placed in a 96-well V-bottom tray (Flow/ICN, Horsham, PA), and 100 μl of the appropriate dilution of primary monoclonal antibody (**Table I**, *Results*) was added for 30 min at 4°C. The cells were washed four times in phosphate-buffered saline containing 5% fetal bovine serum and 10 mM azide, exposed to fluorescein isothiocyanate (FITC)-conjugated $F[ab']_2$ fragment goat-anti-mouse IgG (Cappel Research Products, Durham, NC) for 30 min at 4°C, washed, and incubated in 1% normal mouse serum for 5–15 min at 4°C. Cells then were exposed for 30 min to phycoerythrin (PE)-conjugated anti-human leukocyte antigen (HLA)-DR (to identify MHC class-II-rich dendritic cells; Becton Dickinson Immunocytometry Systems, Inc., San Jose, CA), washed, fixed in 10% formalin for 10 min, washed again, and analyzed on a FACScan (Becton Dickinson Immunocytometry Systems). The controls for nonspecific Ig binding (FITC channel) were nonreactive IgG_1, IgG_{2a}, or IgG_{2b} (Sigma Chemical Co.) and PE-conjugated IgG_{2a} (Becton Dickinson Immunocytometry Systems). As described in *Results*, the skin-cell suspensions could be sorted into dendritic cells, T lymphocytes, and dendritic-cell–T-cell conjugates using a FACStar^{PLUS} (Becton Dickinson Immunocytometry Systems) with laser excitation of 200 mW at 480 nM (Innova 90-5 Argon laser, Coherent, Inc., Palo Alto, CA).

Light Microscopy of Cytospin Smears Fifty microliters of skin cells (4×10^5/ml) were cytospun onto precleaned microscope slides (Baxter Diagnostic Inc., Parkway, NJ) using a Shandon Cytocentrifuge (Shandon Inc., Pittsburgh, PA). The slides were removed immediately from the holders, air-dried for 1 h, fixed in acetone (Fisher Scientific, Fair Lawn, NJ) for 10 min at room temperature, air-dried, rehydrated with Tris-buffered saline (pH 7.4), and incubated with anti-CD3 (Leu 4), anti-CD4 (Leu 3a), anti-CD5 (Leu 1), anti-CD8 (Leu 2), or anti-HLA-DR (9.3C9, HB180) for 30–60 min in a humidified atmosphere at room temperature. The cytospins were washed four times with Tris-buffered saline, exposed to horse radish peroxidase–conjugated $F[ab']_2$ donkey-anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 30–60 min, washed, exposed to the HRP substrate diaminobenzidine (prepared according to manufacturer's instructions; Polysciences Inc., Warrington, PA) for 10–30 min, and washed with distilled water. Cytospins could be stained with Giemsa (Fisher Scientific, Pittsburgh, PA) before coverslipping with a phosphate-buffered saline/glycerol mix (Sigma) and photography on a Nikon Optiphot Microscope (Morell Instrument Co. Inc., Melville, NY).

T-Cell Proliferative Responses The mixed leukocyte response-stimulating activity of skin emigrés was assessed in cultures of 1.5×10^5 allogeneic T cells in triplicate, as described [22]. To detect T-cell proliferation in the emigrés themselves, 4×10^4 cells in 96-well round-bottom microtest trays (Flow/ICN) were cultured without or with human recombinant interleukin-2 (rIL-2) (799 068, Boehringer Mannheim) or Concanavalin A (3 μg/ml, Boehringer Mannheim). Proliferation was monitored either by ^{3}H-thymidine uptake or by staining cytospins of the cultures with the monoclonal MIB-1 antibody to the Ki-67 nuclear antigen (AMAC, Inc., Westbrook, ME) that is expressed in cycling cells.

RESULTS

Preliminary experiments were performed using standard epidermal- and dermal-cell suspensions [19,20]. Typical epidermal and dermal dendritic cells and some small lymphocytes were noted. However, the large majority of keratinocytes prompted us to explore a biologic feature to isolate dendritic cells, i.e., the selective emigration of dendritic cells from organ cultures of skin [5].

Skin Emigrés Contain Potent Immunostimulatory Cells When normal skin was removed with a dermatome and cultured, many cells emigrated into the medium within a day and for several days thereafter. Large dendritic cells were abundant, but there were many small lymphocytes as well. The average yield of leukocytes was 57,000 ± 6200 cells/cm^2 (mean ± SEM from the first 13 experiments). This was comparable to that obtained when separated epidermis and dermis were dissociated by standard methods (26,200 ± 6504 epidermal and 25,900 ± 9712 dermal leukocytes/cm^2 of skin; mean ± SEM from 11 experiments). However, skin emigrés were greater than 95% viable and had few contaminating keratinocytes, whereas dissociated cells had many dead cells and an overwhelming majority of keratinocytes. When cryosections of the skin explants were stained for dendritic cells (anti-CD1a and anti–HLA-DR) before and after 4 d of culture, large numbers remained in both the epidermal and dermal regions of the explant. Nevertheless, when pieces of skin were replated into fresh medium after 4 d of culture, few additional cells emigrated, implying that an initial stimulus for the emigration had subsided (see *Discussion*).

The emigrated populations were potent stimulators of the primary mixed leukocyte response (30 to 100 times more potent than blood cells), much like skin-derived dendritic cells prepared by standard methods (**Fig 1**; compare *a* and *b* and [23,24]).

Light Microscopy of the Emigrated Cells Live emigrated cells were examined at 37°C in an inverted-phase contrast microscope. The dendritic cells extended large sheet-like processes ("veils") in several directions. These processes formed and retracted continually. Some of the small round lymphocytes were bound to the dendritic cells (one to three T cells per dendritic cell, but usually one). The conjugates were stable for hours, and we could not dissociate them with trypsin (0.25% for 5 min, 37°C) or

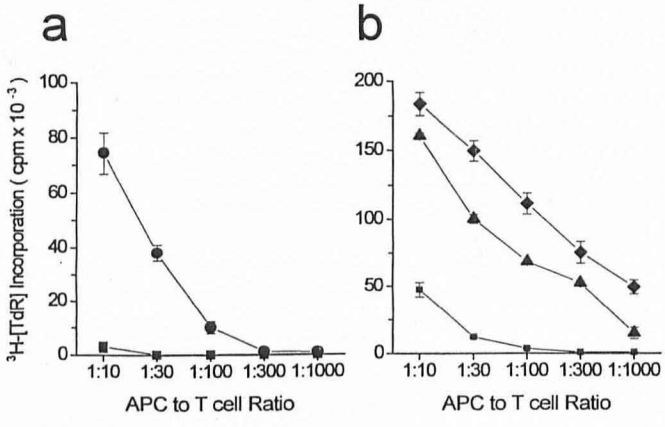

Figure 1. Strong T-cell–stimulatory activity of cells emigrating from skin explants. Activity was assessed by adding graded doses of irradiated (1500 rads ^{137}Cs) skin-derived cells to a constant number (1.5×10^5) of allogeneic T cells in flat-bottom microtest wells. After 5 d, ^{3}H-[TdR] at 1 μCi/well was added for 12 h. Potency is reflected by the activity of the skin cells relative to the standard stimulator population used in tissue typing, i.e., peripheral blood mononuclear cells (*squares*). In *a*, the stimulators were derived by emigration from skin explants (*circles*), whereas in *b* the stimulators were obtained from separated epidermis (*triangles*) or dermis (*diamonds*), as described [19,20]. Error bars represent mean ± SEM of triplicate cultures from one of four similar experiments comparing skin cells from four separate individuals. APC, antigen-presenting cells.

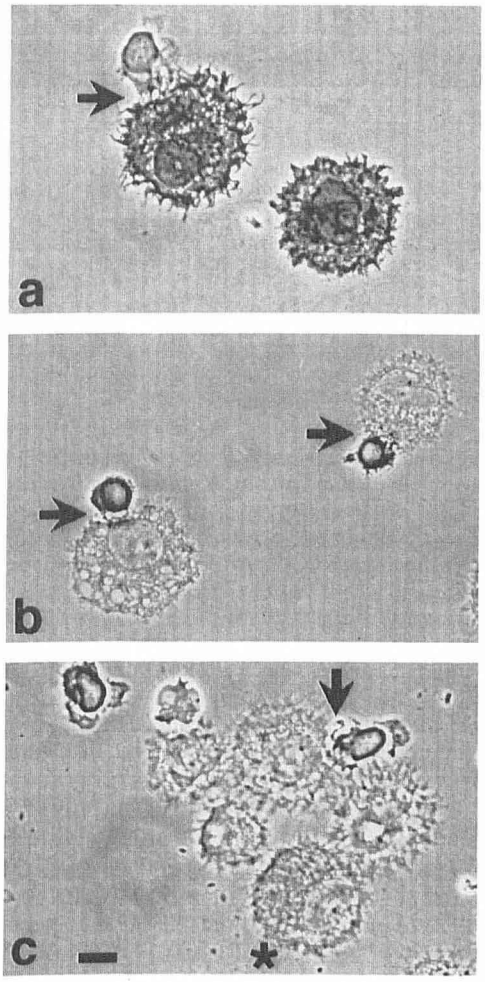

Figure 2. Features of emigrated skin cells in cytospin preparations. Skin emigrés were immunoperoxidase-stained with anti–HLA-DR (*a*), anti-CD3 (*b*), or anti-CD4 (*c*). Conjugates are shown (*arrows*). Dendritic cells are strongly HLA-DR$^+$, but the lymphocytes are DR$^-$ (*a*). In contrast, the lymphocytes are CD3$^+$ and the dendritic cells CD3$^-$ (*b*). CD4$^+$ lymphocytes, free and bound, are present (*c*). Although Langerhans cells express CD4 [26], less than 10% of emigrated dendritic cells were stained by the immunoperoxidase method (*asterisk*; this cell stained with a blush of color on the original cytospin). Representative fields from one of six identical experiments (six different individuals) are shown. *Bar*, 10 μm.

Ca^{++}- and Mg^{++}-free Hanks (1 mM ethylenediamine tetraacetic acid and 1% bovine serum albumin, 4°C).

The emigrés were cytospun onto slides and stained with monoclonal antibodies. The dendritic cells were strongly MHC class-II–positive, and the lymphocytes were CD3$^+$ **(Fig 2*a,b*)**. Both CD8$^+$ (not shown) and CD4$^+$ lymphocytes were noted, either free or attached to the dendritic cells **(Fig 2*c*)**.

Phenotype of Migrating Leukocytes by Cytofluorography

Skin emigrés were stained with a panel of antibodies and FITC-goat anti-mouse Ig, and counterstained with PE-anti–HLA-DR to identify dendritic cells. Fluorescence-activated cell sorter (FACS) profiles from one of three similar experiments representing three different individuals are shown in **Fig 3**, and **Table I** summarizes the fluorescence intensities from three experiments.

Skin emigrés were all CD45$^+$ leukocytes **(Fig 3)**. There were only rare monocytes (CD14$^+$), B cells (CD19$^+$, CD21$^+$), and natural killer cells (CD16$^+$). CD68$^+$ monocytes were seen in dermal suspensions, as described [19], but not in the migrants (not shown). Instead, the emigrés consisted almost entirely of three groups: HLA-DR$^+$ dendritic cells, HLA-DR$^-$ T cells, and their conjugates.

Most HLA-DR$^-$ profiles expressed the T-cell markers CD2, CD3, CD5, and CD7. The TcRs were of the αβ variety, with only trace γδ cells. The ratio of CD4 to CD8 subsets was typical of blood-derived cells, i.e., about 2:1. Most T cells had the memory phenotype [25], i.e., CD58/LFA-3$^+$, CD45RA^{weak}, and CD45RO$^+$ **(Fig 3**; *horizontal rows 2, 3, 5, 6*). T-cell–activation antigens were trace (CD25 IL-2 receptor and CD80/B7-1 costimulator) or absent (HLA-DR). The T cells expressed the CD11a and CD29 integrins.

The striking finding in the FACS was the presence of profiles that were both HLA-DR$^+$ and CD3$^+$. These corresponded to the conjugates observed by light microscopy **(Fig 2)**, as verified by cell sorting (below). No such conjugates have been observed in cultures of blood leukocytes that are enriched in dendritic cells (not shown). Some T-cell antigens (CD2, CD3, CD8, TcRαβ) separated the conjugated **(Fig 3**, *arrows*) from the unconjugated dendritic cells. However, anti-CD4 and anti-CD5 did not provide a separation, probably because CD4 **(Fig 2*c***, asterisk, and [22,26]) and CD5 [27] can be expressed by dendritic cells as well as T cells. The phenotype of the T cells within the conjugates seemed identical to that of the T cells that had not bound to dendritic cells. Dendritic-cell–T-cell conjugates were comparable if normal human serum or fetal bovine serum was present in the culture medium. It is likely that both epidermal and dermal dendritic cells migrated (below) and formed

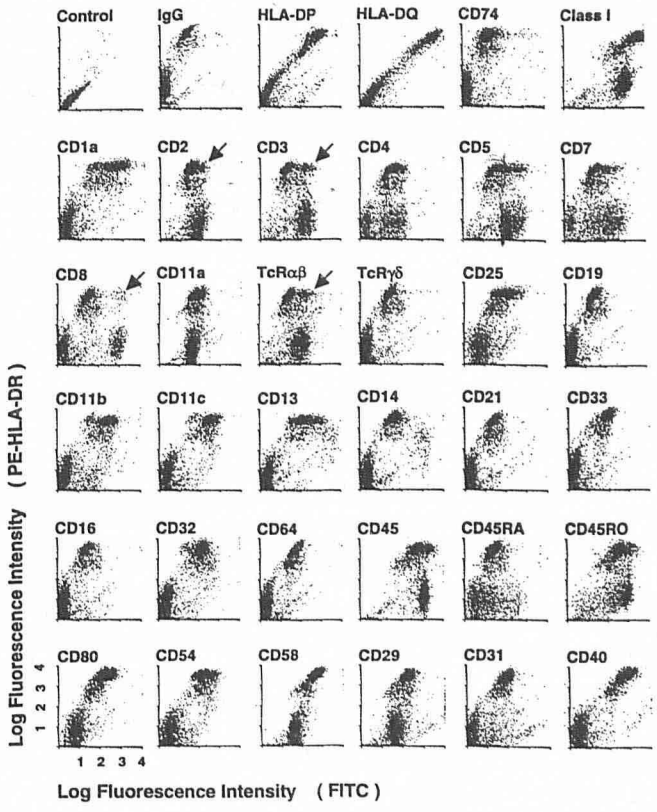

Figure 3. Two-color immunolabeling of the migrants from human skin explants. One color (PE channel, *ordinate*) is HLA-DR staining to identify dendritic cells. The second color (*abscissa*) is produced by applying a monoclonal antibody as indicated, followed by FITC anti-mouse Ig. The control panel shows cells stained with nonreactive mouse IgG, FITC anti-mouse Ig, and nonreactive PE-labeled, isotype-matched antibody. The IgG panel is the staining of cells incubated with nonreactive mouse IgG, FITC anti-mouse Ig, and PE–HLA-DR. These panels demonstrate the background fluorescence of the strongly DR$^+$ dendritic cells. The HLA-DR$^-$ cells are not stained above the nonreactive isotype-matched, PE control (compare first two boxes). Within the DR$^+$ profiles are conjugates of dendritic cells and T cells, highlighted (*arrows*) in the CD2, CD3, αβ, and CD8 boxes. The data are from one of three experiments, further summarized in **Table I**.

Table I. Cell-Surface Antigens of Skin Emigrés[a]

Monoclonal Antibody			Mean Fluorescence Intensity	
Specificity	Isotype	Source/Name[b]	Dendritic Cells	T Cells
MHC				
HLA-DR	IgG_{2a}	BDIS	3600	9
HLA-DQ	IgG_1	ATCC/HB103	2000	4
HLA-DP	IgG_{2a}	Gift-S.Y. Yang, NY/PL-15	675	3
HLA-A,B,C	IgG_{2a}	ATCC/HB95	4000	945
CD74/Ii	IgG_1	Serotec	18	2
T Cell				
CD2 (LFA-2)	IgG_1	ATCC/HB195 (TS2/18)	20	64
CD3	IgG_1	Gift-R. Evans, NY/Leu 4	21	113
CD4	IgG_1	Gift-R. Evans, NY/Leu 3a	31	45
CD5	IgG_1	Serotec	47	141
CD7	IgG_1	Serotec	22	113
CD8	IgG_1	BDIS/Leu 2	20	667
TcR$\alpha\beta$	IgG_1	BDIS	21	63
TcR$\gamma\delta$	IgG_1	BDIS	21	3
Lineage				
CD1a	IgG_1	ATCC/CRL8020 (OKT6)	875	3
CD13	IgG_1	Dako/M812	168	3
CD14	IgG_{2b}	ATCC/TIB228 (3C10)	34	3
CD33	IgG_{2a}	M195[c]	70	2
CD19	IgG_1	Amac 1283	19	3
FcγR/C3R				
CD16	IgG_1	3G8	18	2
CD32	IgG_{2b}	ATCC/HB217	120	3
CD64	IgG_1	ATCC/HB9469	50	3
CD21	IgG_{2a}	ATCC/HB135	24	2
CD45				
CD45	IgG_{2a}	ATCC/HB196	650	560
CD45RA	IgG_{2a}	R.M. Steinman/4G10	26	20
CD45RO	IgG_{2a}	UCHL-1[d]	245	600
Integrins/Ig adhesins				
CD11a	IgG_1	ATCC/HB202 (TS1/22)	93	105
CD11b	IgG_{2a}	ATCC/CRL8026 (0KM1)	275	3
CD11c	IgG_1	UO1/521	295	3
CD31 (PECAM)	IgG_{2a}	HEC-7[e]	65	3
CD54 (ICAM-1)	IgG_1	84H10[f]	110	4
CD58 (LFA-3)	IgG_1	ATCC/HB205	293	32
Activation Antigens				
CD25 (IL-2R)	IgG_1	AM47	73	7
CD40	IgG_1	Biosource CT-CD40	600	4
CD80 (B7)	IgG_1	BDIS	118	6

[a] Results of three identical experiments are tabulated as averages of the mean fluorescence intensity for large (dendritic) and small (T) cells defined by scatter analysis as in **Fig 4**, strategy II. As best as could be determined, T cells bound to dendritic cells had the same phenotype as the free T cells shown here. Background mean fluorescence intensity for staining with nonreactive Ig was 2 for T cells and 22 for dendritic cells.

[b] BDIS, Becton Dickinson Immunocytometry Systems; ATCC, American Type Culture Collection.

[c] Scheinberg DA, Tanimoto M, McKenzie S, Strife A, Old LJ, Clarkson BD. Monoclonal antibody M195: a diagnostic marker for acute myelogenous leukemia. *Leukemia* 3:440–445, 1989.

[d] Smith SH, Brown MH, Rowe D, Callard RE, and Beverley PCL. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. *Immunology* 58:63–70, 1986.

[e] Muller WA, Ratti CM, McDonnell SL, Cohn ZA. A human endothelial cell-restricted, externally disposed plasmalemmal protein enriched in intercellular junctions. *J Exp Med* 170:399–414, 1989.

[f] Makogoba MW, Sanders ME, Ginther Luce GE, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S. ICAM-1 a ligand for LFA-1-dependent adhesion of B, T, and myeloid cells. *Nature* 331:86–88, 1988.

conjugates with the T cells, because preliminary studies revealed that standard epidermal- and dermal-cell suspensions also contained HLA-DR^+, $CD3^+$ dendritic-cell–T-cell couples (unpublished observations).

The MHC class-II–rich emigrés had the phenotype of mature skin dendritic cells [19,20,28]. Actually, there were two subsets (epidermal and dermal), the more numerous having more HLA-DR and CD1a (**Fig 3**). Lenz *et al* [19] showed that epidermal dendritic cells have more DR and CD1a than those from the dermis, which would suggest that the emigrated dendritic cells come primarily from the epidermis. However, it is also possible that dermal dendritic cells up-regulate CD1a during migration. High levels of HLA-DP, HLA-DQ, and class I were expressed, but invariant chain (CD74) was absent (**Fig 3**; *top horizontal row*). The extreme intensities of dendritic-cell staining for classes I and II MHC made it difficult to compensate the FACS instrument fully such that all leukocytes were displayed simultaneously on the dot blots. Whereas CD4 and CD11a levels looked comparable on the DR^+ and DR^- populations, the staining of the DR^+ subset was much weaker when the higher autofluorescence of dendritic cells was taken into account (control and IgG panels, *row 1*). With the exception of CD14, dendritic cells expressed several myeloid markers: CD11b, CD11c, CD13, and CD33 (**Fig 3**, *row 4*). The only Fcγ receptor detected was CD32; CD16 and CD64 were weak or absent (**Fig 3**, *row 5*). Dendritic cells had high CD45RO and low CD45RA, as is typical of activated leukocytes. Dendritic cells expressed many adhesion/activation molecules: CD80/B7-1, CD54/intercellular adhesion molecule-1 (ICAM-1), CD58/leukocyte function–associated antigen-3, CD29/β1 integrin, CD31/platelet endothelial cell adhesion molecule-1, and CD40 (**Fig 3**, *row 6*).

Table II. Proliferative Activity of Skin-Cell Emigrants[a]

Stimulus	Experiment 1 (^{3}H-[TdR] cpm)		Experiment 2 (^{3}H-[TdR] cpm)	Experiment 3 (% Ki-67$^+$)
	Skin Cells	PBMC	Skin Cells	Skin Cells
R10	680 ± 72	319 ± 23	1,056 ± 163	8 ± 0.9
IL-2	3,918 ± 120	838 ± 153	4,254 ± 173	25 ± 1.0
Concanavalin A	24,416 ± 1,285	22,042 ± 1,928	28,394 ± 681	75 ± 1.6

[a] Skin cells and peripheral blood cells (PBMC) were cultured with culture medium (R10), 10% IL-2, or 3 μg/ml concanavalin A for 3 d. Proliferation was monitored by measuring the ^{3}H-[TdR] incorporated (cpm, mean ± SEM of triplicate cultures from two of four similar experiments) during the final 12 h of culture and the expression of Ki-67 nuclear antigen by immunostaining. Several fields of at least 600 profiles each were counted, and the percentages of Ki-67$^+$ (mean ± SEM) are provided from one of two similar experiments. Replicate experiments were carried out using cells from different donors.

Proliferative Capacity of Skin-Derived T Cells The presence of tight conjugates between dendritic cells and T cells suggested that the T cells would proliferate in culture. When dendritic cells are presenting nominal antigens, alloantigens, or superantigens, they bind efficiently and stimulate T cells in multicellular aggregates [29–32]. However, the skin cells exhibited low levels of ^{3}H-thymidine uptake (**Table II**). Labeling for the Ki-67 antigen (expressed in the nucleus of cycling cells) was not detected at time 0, but was detected in a fraction of the cells after 3 d of culture (**Table II**). The T cells were competent to proliferate, as the mitogen Concanavalin A induced ^{3}H-[TdR] uptake and Ki-67 staining that were as strong as those observed with blood mononuclear cells (**Table II**). Some of the skin-cell emigrants responded to IL-2 with an increased frequency of Ki-67 staining and cell size. However, Ki-67 staining did not appear to be restricted to the T cells in clusters.

Isolated Dendritic Cells and T Cells Form Additional Conjugates To determine whether emigrated dendritic cells and T cells could form additional conjugates, we sorted the populations into free dendritic cells, free T cells, and dendritic-cell–T-cell couples. This was achieved using two methods (**Fig 4**). In one (strategy I), the suspensions were stained with anti-CD3. Single T cells were small (low forward scatter) and CD3$^+$, whereas single dendritic cells were CD3$^-$ and large. Dendritic-cell–T-cell conjugates were CD3$^+$ and large. In a second method (strategy II), the single T cells were not occupied by anti-CD3 but instead were collected as small cells first. The large profiles then were stained with anti-CD3 to sort the large CD3$^-$ cells (dendritics) from large CD3$^+$ cells (dendritic–T-cell couples).

Both methods yielded 98% pure free T cells and free dendritic cells. The free T cells were small, CD3$^+$, and DR$^-$; the free dendritic cells were large, CD3$^-$, and DR$^+$; the conjugates contained large DR$^+$, CD3$^-$ dendritic cells with small DR$^-$, CD3$^+$ lymphocytes (**Fig 5**; note some free T cells and dendritic cells in comparable numbers disrupted from conjugates after sorting).

Separated free dendritic cells and T cells were returned to culture. New dendritic cell–T-cell conjugates formed after about 2 d, as assessed by direct observation and by FACS studies (**Fig 6**). Again, most conjugates consisted of a large dendritic cell coupled to a small T lymphocyte, but sometimes two or three T cells were bound.

DISCUSSION

The leukocyte emigration described here provides access to cutaneous dendritic cells and T cells in both normal skin and skin in several disease states. It is known that dendritic cells can be isolated from the epidermis and dermis of human skin [19,20,28,33], although the emigration phenomenon provides these virtually free of keratinocytes, in contrast to previous methods. Furthermore, memory TcR$\alpha\beta^+$ T lymphocytes, which are found in skin usually at the epidermal-dermal junction [34], also emigrate when skin is explanted into culture. In mouse skin explants, TcR$\gamma\delta^+$ T cells emigrate [5].

The emigration of cutaneous leukocytes has a physiologic parallel. Both dendritic cells [1,6–8] and T cells with a memory phenotype [35] can be found in afferent lymph, suggesting that these cells move from tissue spaces into lymph channels *in situ*. Mackay *et al* [36] have described dendritic–T-cell conjugates in afferent lymph; these results suggest that the interaction we describe in culture also can take place *in situ*.

The mechanism of cell emigration from skin may be characterized using the culture system described here. Perhaps explantation of skin triggers a finite release of cytokines, e.g., if contact allergens are applied to mouse skin, there is a marked up-regulation of IL-1β mRNA in dendritic cells [37].

The interaction between dendritic cells and T cells to form conjugates is strong and is not disrupted even during cell sorting.

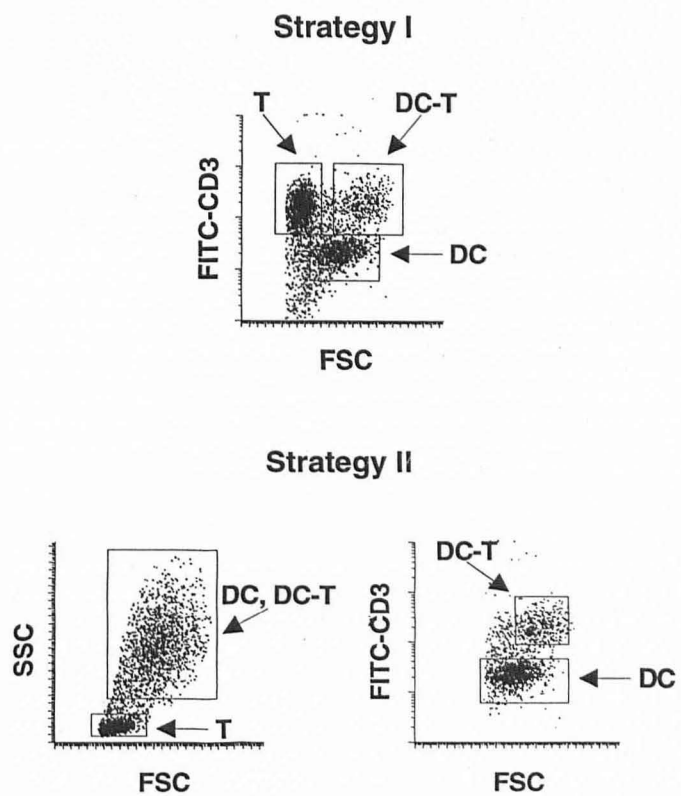

Figure 4. Two approaches for sorting single dendritic cells (DC), single T cells (T), and dendritic–T-cell conjugates (DC-T) from human skin emigrants. In one method (strategy I), the suspensions are labeled directly with anti-CD3, the CD3$^+$ and CD3$^-$ fractions are separated, and the CD3$^+$ cells are subsequently resorted to divide the large and small fractions. In strategy II, the single T cells are first isolated on the basis of low forward light scatter (FSC). The T cells in the conjugates then are labeled with anti-CD3 and isolated from the free dendritic cells. SSC, side light scatter.

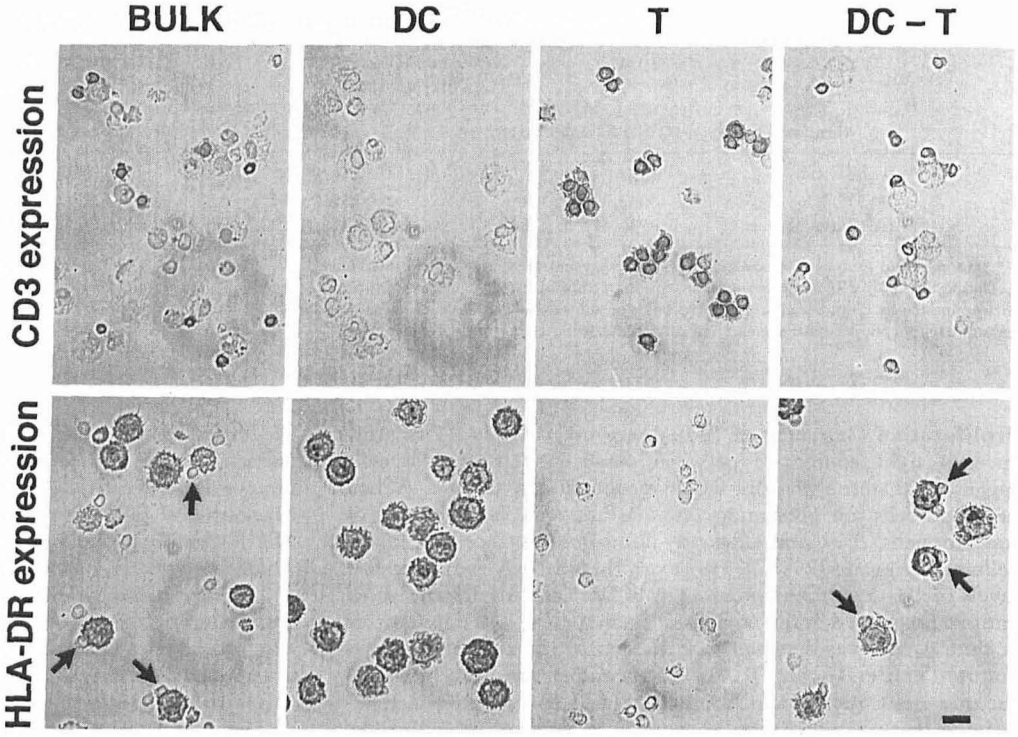

Figure 5. Efficiency of separation of skin emigrés by cell sorting. Cytospins were prepared of bulk skin cells (BULK) and the three sorted cell populations: dendritic cells (DC), T cells (T), and dendritic-cell–T-cell conjugates (DC-T). Each cytospin was immunoperoxidase-stained with either anti-CD3 (*top row*) or anti–HLA-DR (*bottom row*). Dendritic cell–T-cell conjugates are highlighted (*arrows*). These observations were made on 10 separate occasions using skin from ten different individuals. *Bar*, 20 μm.

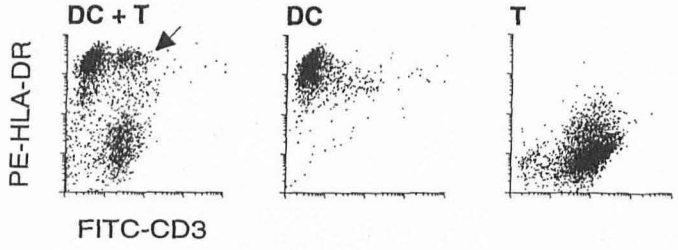

Figure 6. Formation of dendritic–T-cell conjugates after culture of sorted free dendritic cells and T cells. Dendritic–T-cell conjugates were removed from skin emigrés by sorting **(Fig 4)**. The single dendritic cells (DC) and T cells (T) then were cultured alone or mixed in equal numbers (DC + T). Aliquots were monitored in the hemocytometer to determine when conjugates had reformed in significant numbers, here at 3 d. At this time, the suspension was harvested and stained with monoclonal antibodies to reveal the newly formed conjugates (*arrows*) and to compare the dendritic cell–T-cell cocultures with the two cell types cultured separately. Similar observations were produced using specimens from different donors in at least five individual experiments.

The basis for conjugation might be studied first by sorting the free dendritic cells and T cells and then by identifying monoclonal antibodies that block conjugate formation.

Although some of the emigrated T cells expressed Ki-67 antigen after culture, we did not observe high levels of ^{3}H-[TdR] uptake or blast formation. The behavior of the dendritic–T-cell conjugates differs from the large multicellular aggregates that develop when dendritic cells are carrying known antigens or superantigens [29–32]. The latter T cells actively proliferate and emigrate from the aggregates as lymphoblasts.

We suspect that dendritic cell–T-cell conjugation could contribute to cutaneous recall responses. During recall reactions (delayed-type hypersensitivity), dendritic cells are juxtaposed to the mononuclear cells that constitute the reaction [11]. When epidermal dendritic cells are placed in culture or are induced to migrate *in vivo*, the antigen-presenting cells undergo a series of changes that are termed "maturation" [38–40]. Maturation refers to the acquisition of strong T-cell–stimulatory activity, and it is associated with the up-regulation of MHC as well as accessory molecules such as CD80/B7-1, CD86/B7-2, and CD54/ICAM-1 [5,41–43]. Memory T cells likewise are qualitatively different from naive T cells with respect to enhanced expression of adhesion molecules such as CD2, CD11a, and CD58 [44]. During explantation, skin dendritic cells likely are induced to mature. The resulting changes in surface adhesion molecules then may lead to the binding of memory T cells and facilitate the response should antigen be present, as in delayed-type hypersensitivity reactions.

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