Mouse Dendritic Epidermal T Cells Exhibit Chemotactic Migration Toward PAM 212 Keratinocyte Culture Supernatants

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Dendritic epidermal T cells (DETCs) are Thy-1$^+$, CD45$^+$, CD3$^+$, CD4$^-$, CD8$^-$, and T-cell receptor-Vγ3/Vδ1$^+$ leukocytes that reside normally in adult mouse skin. We have demonstrated previously that keratinocytes serve as adhesion substrates for DETCs, and that interleukin 7 (IL-7), which is produced by keratinocytes, serves as a growth factor for DETCs. The present study was conducted to address the mechanisms by which DETCs migrate into the epidermis, reasoning that keratinocytes may also be a source of chemotactic activity. Short-term DETC lines were ^{35}S-labeled and tested for migration toward Pam 212 keratinocyte culture supernatants using a modified Boyden chamber method; cell movement from upper chambers toward test samples in lower chambers was traced by counting radioactivity. DETC displayed rapid (within 60 min) and marked (>50%) migration toward keratinocyte supernatants. The majority of cells that had migrated into keratinocyte supernatants expressed the Vγ3 T-cell receptor, thus verifying that the migrating cells were DETCs. Addition of keratinocyte supernatants to the upper chambers completely blocked migration, suggesting its chemotactic nature. By contrast, no DETC migration was observed toward 3T3 fibroblast supernatants. Chemotactic activities were 1) produced by Pam 212 cells even in the absence of serum; 2) greater than 12 kD in size; 3) heat and pH labile; 4) trypsin sensitive; and 5) precipitated by 60–100% ammonium sulfate. Several cytokines (e.g., IL-1α and IL-8) failed to mediate DETC migration when added to the lower chambers. Likewise, the same cytokines, when added to the upper chambers, failed to inhibit DETC migration toward Pam 212 supernatants. These results support our hypothesis that keratinocytes facilitate the residence of DETC in epidermis by secreting unique chemotactic factors, by providing adhesion substrates, and by elaborating specific growth factors. Key words: $\gamma\delta$ T cell/locomotion/homing.
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A population of dendritic Thy-1$^+$/CD45$^+$/CD3$^+$/CD4$^-$ /CD8$^-$/T-cell receptor (TCR)-Vγ3/Vδ1$^+$ leukocytes resides normally in adult mouse epidermis; these cells have been designated dendritic epidermal T cells (DETC) (reviewed in [1]). DETCs are now known to display several properties of immunologic relevance: 1) proliferation in response to mitogenic stimuli, such as Con A, immobilized anti-CD3, or phorbol ester and Ca ionophore [2–4]; 2) secretion of relatively large amounts of interleukin 2 (IL-2), IL-3, and γ-interferon [3,4]; 3) cytotoxicity that is directed against tumor targets, such as the YAC-1 lymphoma, fibrosarcomas, melanomas, and transformed keratinocytes [5,6];* and 4) recognition via a $\gamma\delta$ TCR of one or more molecules expressed by transformed keratinocytes [7]. Based on these properties and the extremely limited diversity seen with TCR gene rearrangement, DETCs have been postulated to play an important role in maintaining the integrity of cells within the epidermis [8–10]. This postulate has led to the concept that DETCs are positioned to recognize neighboring cells that are damaged, transformed, infected, or otherwise "stressed" *via* their $\gamma\delta$ TCR, and then to eliminate them by a cytotoxic mechanism.

Several lines of evidence suggest that DETCs are derived from precursors in the fetal thymus: 1) intravenous injection of fetal thymocytes into athymic mice populates the epidermis with typical DETC [11]; 2) transplantation of fetal thymic lobes into athymic mice produces a similar effect [12]; and 3) both thymocytes from fetal mice (sixteenth day of gestation) and DETCs utilize the same unusual combination of rearranged TCR gene segments (Vγ3/Vδ1) [12–14]. Recognizing their thymic origin and the obvious requirement to travel to skin, a major interest in our laboratory has been to identify their mechanisms of adhesion and locomotion. We first observed that DETCs isolated from adult mouse skin will migrate preferentially back to the skin following intravenous injection, suggesting that they retain their homing properties [15].

To achieve an epidermal location, DETCs must have the potential to interact with several different adhesion ligands along their migratory pathway, including those expressed on endothelium, dermal connective tissues, basement membrane, and, ultimately, keratinocytes. In fact, DETCs have been shown to bind to endothelial cells, type I collagen, fibronectin, laminin, type IV collagen, and keratinocytes, satisfying at least some of these requirements

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[16,17].†‡ On the other hand, two critical questions remain with respect to the final steps in reaching the epidermis: 1) do DETCs have the capacity to exhibit directed movement, and 2) do keratinocytes produce relevant chemotactic molecules?

Previous experience suggests that keratinocytes are a logical source of molecules that are chemotactic for DETC. In fact, keratinocytes are known to secrete a variety of molecules that are chemotactic for other leukocytes, including IL-1 for conventional T cells and B cells, IL-8 for T cells and neutrophils, anionic neutrophil-activating peptides, which are specific for neutrophils, and lipid attractants such as leukotriene B_4 and 12-hydroxyeicosatetraenoic acid (reviewed in [18]). Thus, our hypothesis was that DETCs have the capacity to move in response to chemotactic gradients and that migration into epidermis is facilitated by one or more related keratinocyte-derived soluble factors.

MATERIALS AND METHODS

Cells Epidermal cells were isolated from truncal skin in 6–8-week-old female CBA mice (Harlan, Indianapolis, IN) by two sequential trypsin treatments, as described previously [2,4]. The recovered cells, including about 1% DETCs, were then enriched for DETC by centrifugation over Histopaque (1.083; Sigma, St. Louis, MO); cells recovered from the medium interface ("interface" epidermal cells) typically contained 10–20% DETCs, as judged by FACS analyses [4,19]. Cells were cultured in 24-well plates (Corning, Corning, NY) in complete RPMI (RPMI 1640 supplemented with 10% fetal bovine serum [FBS], 10 mM HEPES, 1% nonessential amino acid mixture, 20 μM L-glutamine, 10 μM sodium pyruvate, 1% penicillin/streptomycin, 50 μg/ml gentamicin, 10 μM 2-mercaptoethanol, and 1 μg/ml indomethacin) containing 2 μg/ml Con A (Pharmacia, Piscataway, NY) and 5 U/ml human rIL-2 (R&D Systems, Minneapolis, MN). DETCs were expanded by restimulation with Con A (1 μg/ml) on day 7 and by repeated feeding with IL-2 (5 U/ml) every 3–4 d. Ordinarily, cells harvested from 2–4-week-old cultures, containing 60–80% DETC, were used for cell migration assays.

Splenic T cells isolated from CBA mice using nylon wool columns were cultured for 24 h in complete RPMI with 5 U/ml IL-2 alone or for 3 d in complete RPMI with both Con A (2 μg/ml) and IL-2 (5 U/ml), and tested for migration. The BALB/c mouse keratinocyte line, Pam 212 [20], was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 10 Mm HEPES, 1% nonessential amino acids, 20 μM L-glutamine, 10 μM sodium pyruvate, and 1% penicillin/streptomycin (complete DMEM), as reported previously [21]. For supernatant collection, Pam 212 cells were cultured for 24 h at a cell density of $1-2 \times 10^6$ cells in complete DMEM or DMEM supplemented with all the above additives except for FBS (FBS-free DMEM).

Cell Migration Assays Cell migration was measured using 6.5-mm Transwell units (Costar #3422; Cambridge, MA), in which upper chambers (100-μl volume) and lower chambers (600-μl volume) are separated by a 10-μm-thick, multi-pore polycarbonate membrane, with a pore size of 8 μm. Short-term cultured DETCs or splenic T cells were radiolabeled for 16 h with 10 μCi/ml of ^{35}S-methionine (New England Nuclear, Boston, MA) in methionine-free DMEM (Gibco) supplemented with 10% FBS and 5 U/ml IL-2. Cells were washed extensively with phosphate-buffered saline to remove free radioactivity and suspended in complete DMEM or FBS-free DMEM at a cell density of $5-10 \times 10^5$ cells/ml. Cell suspensions (100-μl/chamber) were placed in the upper chambers, and test samples (i.e., Pam 212 keratinocyte supernatants, 600-μl/chamber) were added to the lower chambers. After incubation at 37°C in an atmosphere of 5% CO_2 and 100% relative humidity, cells were recovered from upper and lower chambers by extensive pipetting and then counted for radioactivity. Cells remaining at the bottoms of lower chambers or on the membranes were extracted in 2% Triton X-100 and counted for radioactivity as well. Based on these counts, percent migration was calculated by dividing the cpm collected from lower chambers by total cpm recovered.

Flow-Cytometric Analyses Anti-Thy-1.2 (Becton Dickinson, Mountainview, CA), biotinylated anti-Vγ3 TCR (Pharmingen, San Diego, CA), FITC-conjugated anti-rat IgG (Jackson, West Grove, PA), and FITC-streptoavidin (Boehringer Mannheim, Indianapolis, IN) were used to identify DETCs. Anti-Thy-1.1 (Becton Dickinson) and biotinylated anti-Vβ3 (Pharmingen) were used as irrelevant antibody controls. Immunofluorescence staining was performed as described previously [4], and samples were analyzed with a FACSCAN (Becton Dickinson).

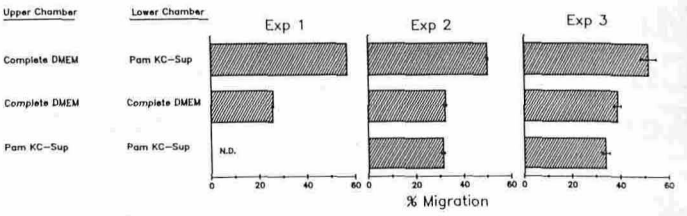

Figure 1. Migration of DETCs toward Pam 212 keratinocyte culture supernatants. Short-term DETC lines were ^{35}S-labeled, suspended in medium containing 10% FBS (complete DMEM), and placed into the upper chambers. Pam 212 keratinocytes were cultured for 24 h in complete DMEM, and culture supernatants (Pam KC-Sup) were added to the lower chambers. Fresh medium (complete DMEM) was added to lower chambers as negative controls, and Pam 212 supernatants were added to the upper chambers to block chemotactic migration. Samples were incubated for 3 h at 37°C, and percent migration was calculated by the radioactivity recovered from the lower chambers divided by total recovery radioactivity. Data shown are the mean ± SEM of percent migration from duplicate samples.

Other Reagents Mouse recombinant IL-1α, IL-4, tumor necrosis factor-α, granulocyte/macrophage-colony stimulating factor, and human rIL-8 were purchased from Genzyme (Cambridge, MA); mouse rIL-2 and rIL-7 were from R&D System. Trypsin immobilized on agarose beads was purchased from Sigma and used at a specific activity of 10 U/ml.

RESULTS

Pam 212 Keratinocyte Culture Supernatants Contain Chemotactic Activity for DETCs In the first experiments, we examined whether DETCs have the capacity to migrate toward Pam 212 keratinocyte culture supernatants. Short-term (2–4-week-old) DETC lines, which had been stimulated initially with Con A and expanded thereafter with IL-2, were radiolabeled by ^{35}S-methionine and placed into upper chambers of the Transwell units. Pam 212 cells were cultured for 24 h in DMEM supplemented with 10% FBS, and these culture supernatants were added to lower chambers. Samples were incubated for 3 h and migration was assessed by counting the radioactivity in each chamber. Considerable migration was observed even toward unconditioned DMEM, which presumably reflected random migration. In three independent experiments, addition of Pam 212 supernatants to the lower chambers resulted consistently in a significant augmentation of migration (Fig 1). This migration appeared to represent cell chemotaxis, rather than chemokinesis, because the number of migrating cells returned to baseline levels when supernatants were added to both upper and lower chambers (Fig 1, experiments 2 and 3).

To rule out the possibility that keratinocytes had simply activated one or more chemotactic molecules already present in FBS (e.g., complement) rather than producing a new molecule, Pam 212 cells were cultured in FBS-free DMEM in the next set of experiments. Even under serum-free conditions, we observed specific migration of these short-term DETC lines toward Pam 212 supernatants (Fig 2). This migration was again blocked by adding the same supernatants to the upper chambers (Fig 2, Experiment 2). We concluded that FBS is not required for the generation of this chemotactic activity by Pam 212 cells, and subsequent experiments were all performed in the FBS-free system.

Flow-Cytometric Analysis of Epidermal Cells That Have Migrated into Keratinocyte Supernatants Although the short-term DETC lines used for migration assays contained 60–80% Thy-1$^+$ and Vγ3 TCR$^+$ leukocytes (i.e., DETCs), it remained to be confirmed whether the migration assessed by counting radioactivity indeed represented the movement of DETCs. To address

† Edelbaum D, Kaminski MJ, Cruz PD Jr., Bergstresser PR, Takashima A: Binding of dendritic epidermal T cells to endothelial cell monolayers and extracellular matrix proteins (manuscript submitted).

‡ Takashima A, Edelbaum D, Cruz PD, Jr., Bergstresser PR: Selective adhesion of mouse dendritic epidermal T cells to keratinocyte monolayers (manuscript submitted).

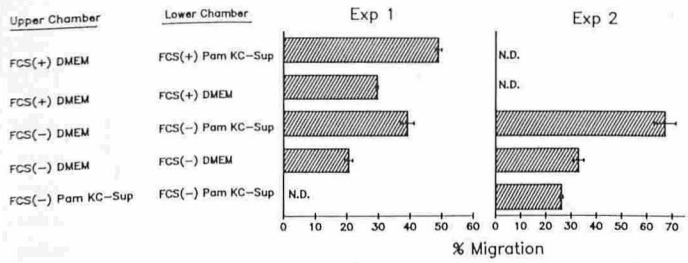

Figure 2. Secretion of DETC chemotactic activities by Pam 212 keratinocytes in the absence of FBS. Pam 212 cells were cultured for 24 h in DMEM in the presence or absence of 10% FBS, and these culture supernatants (Pam KC-Sup) were added to the lower chambers. DETCs suspended in the indicated media were placed into the upper chambers. Data shown are the mean ± SEM of percent migration at 3 h from duplicate samples.

this question, cells that had migrated into Pam 212 supernatants were harvested from the lower chamber and then analyzed for their expression of Thy-1 antigen and Vγ3 TCR. Not only were the majority of cells originally plated in the upper chambers Thy-1$^+$ and Vγ3 TCR$^+$ (Table I), even higher proportions of the cells recovered from lower chambers expressed the same markers (Table I, Fig 3). Based on these data, we calculated that 68% of all cells, 96% of Thy-1$^+$ cells, and 78% of Vγ3 TCR$^+$ cells were recovered from the lower chambers (Table I), justifying our use of short-term DETC lines to study DETC migration.

Biologic Characterization of Keratinocyte-Derived DETC Chemotactic Activities The next series of experiments was conducted to characterize the migration of DETCs. Time-course studies showed that specific migration (i.e., percent migration to Pam 212 supernatant minus percent migration to fresh media) was seen as early as 1 h, reaching a plateau by 2 h (Fig 4). Dose-response studies showed that Pam 212 supernatants contained relatively high amounts of chemotactic activity; they were active at concentrations as low as 12% (v/v) (Fig 5). Addition of the same supernatants to upper chambers inhibited migration in a dose-dependent fashion, again indicating the chemotactic nature of this migration (Fig 5). To test cell specificity for this phenomenon, Pam 212 keratinocyte and 3T3 fibroblast lines were cultured in parallel, and culture supernatants were compared for their chemotactic activities. In contrast to Pam 212 supernatants, 3T3 supernatants mediated minimal, if any, specific cell migration (Fig 6). When short-term cultured splenic T cell lines were tested, they also exhibited significant migration toward Pam 212 keratinocyte supernatants (Fig 6). We thus concluded that relevant chemotactic activity is produced preferentially by keratinocytes and that this activity is capable of attracting not only DETCs, but other T cells as well.

Biochemical Properties of Pam 212 Keratinocyte-Derived DETC Chemotactic Activities Pam 212 supernatants were subjected to a series of pretreatment protocols to examine several biochemical properties of the chemotactic activity (Fig 7). Specific activity was preserved after dialysis of supernatants for 3 d, using a 12-Kd cutoff membrane, suggesting that the relevant factor(s) has a

Table I. Migration of Thy-1$^+$, Vγ3 TCR$^+$ Cells Toward Pam 212 Keratinocyte Culture Supernatants

	Total Cells	Thy-1$^+$ Cells	Vγ3$^+$ Cells
Added in upper chamber	6.0×10^4 cells/well	3.8×10^4 (63.0%)[a]	3.8×10^4 (62.6%)
Recovered in lower chamber	4.1×10^4	3.6×10^4 (88.8%)	2.9×10^4 (72.0%)
Percent Migration	68.0%	95.8%	78.1%

[a] Number in parentheses represent the percent positive cells.

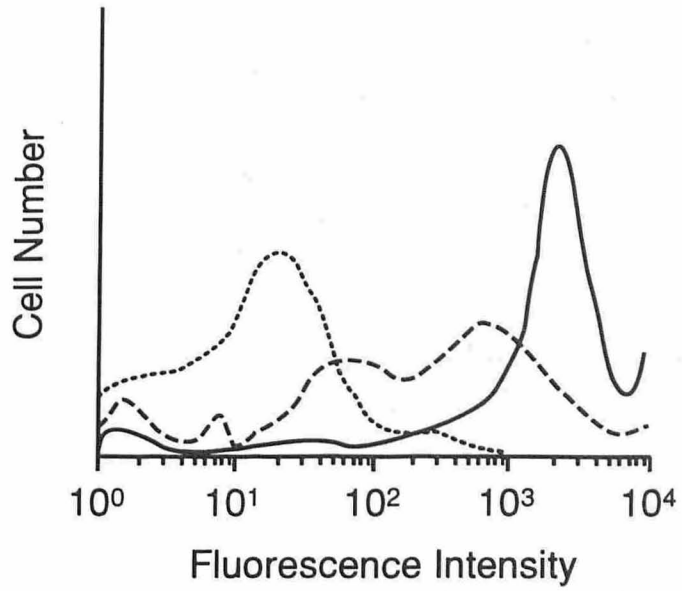

Figure 3. Expression of Thy-1 and Vγ3 TCR by migrating DETCs. A DETC line was suspended in FBS-free DMEM and placed into upper chambers and Pam 212 supernatants were added to the lower chambers. Cells were harvested from the lower chambers after 2-h incubation, and stained for Thy-1 *(solid line)* or Vγ3 TCR *(broken line)*. Control staining with irrelevant MoAb is shown by *dotted lines*.

relatively large molecular size. Boiling as well as pretreatment with strong acid or base resulted in significantly reduced chemotactic activity, suggesting that the factor(s) is heat and pH labile. Trypsin pretreatment abolished the biologic activity completely, indicating that the factor(s) contains trypsin-sensitive peptides. The chemotactic activity was precipitated with 60–100% ammonium sulfate, suggesting that the factor(s) is a macromolecule. Taken together, these features indicate that the relevant factor(s) in the Pam 212 supernatant is polypeptides, rather than lipid metabolites or another small molecule.

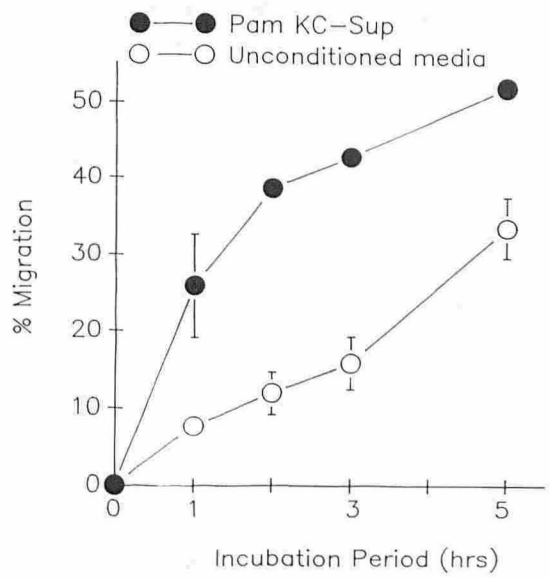

Figure 4. Kinetics of DETC chemotaxis. DETC suspended in FBS-free DMEM were placed into upper chambers, and Pam 212 culture supernatants prepared in FBS-free DMEM (●) or unconditioned FBS-free DMEM (○) were added to lower chambers. Data shown are the mean ± SEM of percent migration after 1–5 h from duplicate samples.

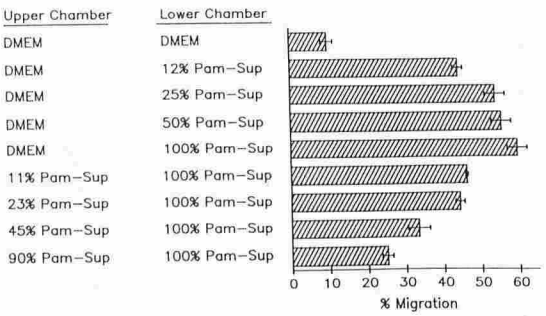

Figure 5. Dose response of DETC migration toward Pam 212 keratinocyte culture supernatants. DETCs suspended in fresh FBS-free DMEM were placed into upper chambers, and Pam 212 supernatants (Pam-Sup) (0-100% in FBS-free DMEM) were added to lower chambers. Graded doses of Pam 212 supernatants (0–90% v/v) were also added to upper chambers to block chemotactic migration. Data shown are the mean ± SEM of percent migration at 2 h from duplicate samples.

Recombinant Cytokines Do Not Mediate or Block DETC Chemotaxis Because Pam 212 keratinocytes and mouse epidermal cells produce a wide range of cytokines (reviewed in [22,23]), we asked whether DETC chemotaxis is mediated by one that was obvious. As noted in Fig 8*A*, the addition of mouse rIL-1α, IL-2, IL-4, IL-7, tumor necrosis factor-α, granulocyte/macrophage-colony stimulating factor, or human rIL-8 to the lower chambers failed to mediate specific migration of DETC. We then tried to reverse the chemotactic gradient by adding the same panel of cytokines to the upper chambers. None of the cytokines tested, however, was able to inhibit DETC migration toward Pam 212 supernatants (Fig 8*B*). Although IL-1 and IL-8 have been shown to attract T cells [24–26], we also failed to detect significant activity for DETC in our system even at different doses (0.13 or 1.25 ng/ml IL-1α; or 0.2, 2, or 20 ng/ml IL-8) or after different incubation periods (1, 3, or 5 h; data not shown).

DISCUSSION

The reported experiments have demonstrated that Pam 212 keratinocytes secrete one or more factors that cause directed DETC migration. In sum, these migratory responses were: 1) specific for Pam 212 supernatants (3T3 fibroblast culture supernatants failed to attract DETC); 2) chemotactic rather than chemokinetic in nature (addition of Pam 212 supernatants to the upper chambers prevented migration); 3) time dependent (half maximal migration was observed within 60 min); 4) dose dependent (half maximal activity was attained even at an eightfold dilution of supernatants); and 5) mediated by a heat- and pH-labile, trypsin-sensitive molecule having a MW higher than 12 Kd.

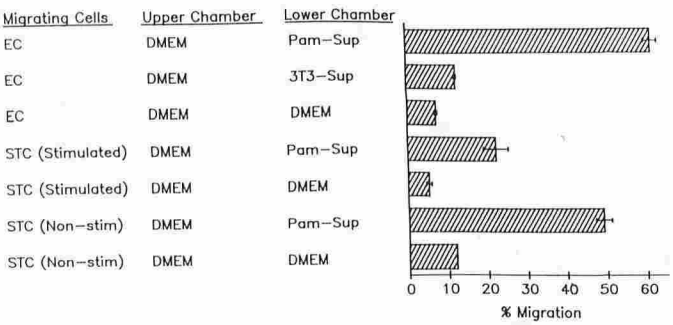

Figure 6. Specificity of DETC migration. A short-term DETC line (EC) was prepared by culturing CBA mouse epidermal cells for 14 d in the presence of 2 μg/ml Con A and 5 U/ml IL-2. Short-term splenic T-cell lines (STC) were prepared by culturing CBA mouse splenic T cells for 24 h with IL-2 alone (non-stimulated) or for 3 d with both Con A and IL-2 (stimulated). These lines were ^{35}S-labeled and tested for migration toward the FBS-free DMEM that had been conditioned by Pam 212 cells (Pam-Sup) or 3T3 fibroblasts (3T3-Sup). Data shown are the mean ± SEM of percent migration after 2 h from duplicate samples.

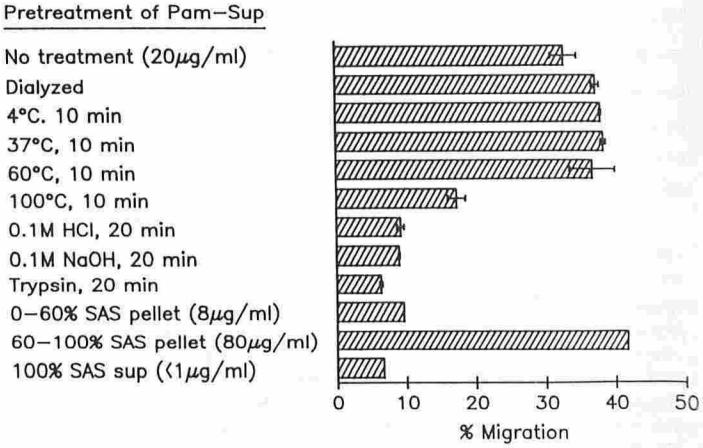

Figure 7. Biochemical properties of Pam 212 keratinocyte-derived chemotactic activities. Pam 212 keratinocyte supernatants (Pam-Sup) in FBS-free DMEM were subjected to several different pretreatments: dialysis (3 d) at 4°C using membranes with a 12-kD cutoff point, incubation (10 min) at different temperatures, incubation (20 min) at acidic or basic pH followed by neutralization, incubation (20 min) with immobilized trypsin at 37°C, or ammonium sulfate precipitation followed by extensive dialysis and lyophilization. Chemotactic activities were tested using a ^{35}S-labeled DETC line. Data shown are the mean ± SEM of percent migration from duplicate samples. SAS, saturated ammonium sulfate.

With respect to the source of chemotactic activity, a growing literature now documents the capacity of keratinocytes to secrete several different molecules that are chemotactic for cells of leukocyte lineage (reviewed in [18,22,23]). From this list, relevant candidate molecules include IL-1, IL-8, anionic neutrophil-activating peptides, and monocyte chemotaxis and activating factor (reviewed above). In fact, each of these four cytokines possesses some of the features characteristic of the DETC chemotactic activity found in Pam 212 supernatants. On the other hand, our experiments have suggested that neither IL-1 nor IL-8 is responsible. rIL-1α or rIL-8, when added to the lower chambers, failed to attract DETC and, when added to the upper chambers, failed to prevent migration. Although compelling, these negative results are not yet definitive, because these molecules may synergize with others and because all concentrations have not been tested. Further studies, not in the scope of this communication, will be required, and blocking experiments using antibodies against known chemoattractants and biochemical purification of chemotactic molecules are currently being conducted.

With respect to DETCs, the target of chemotactic activity, a major interest in our laboratory has been to elucidate mechanisms by which they (or their thymic precursors) home to the epidermis where they then take up residence. We and others have observed, using cell-adhesion assays, that DETCs are capable of binding to endothelial cell monolayers as well as to several different extracellular matrix proteins present in dermal connective tissue and at the basement membrane [16,17].† This work has led to the assumption that DETCs migrate from the blood vessels to the dermo-epidermal junction by interacting in sequence with these adhesion substrates. Our present study provides an additional clue in this physiologic migration; DETC movement from dermis into epidermis may be facilitated by factors secreted by keratinocytes. Once the epidermis is reached, it should be noted that keratinocytes presumably provide high-affinity adhesion substrates, because DETCs display *in vitro* a capacity to bind selectively to keratinocyte monolayers.‡ Very re-

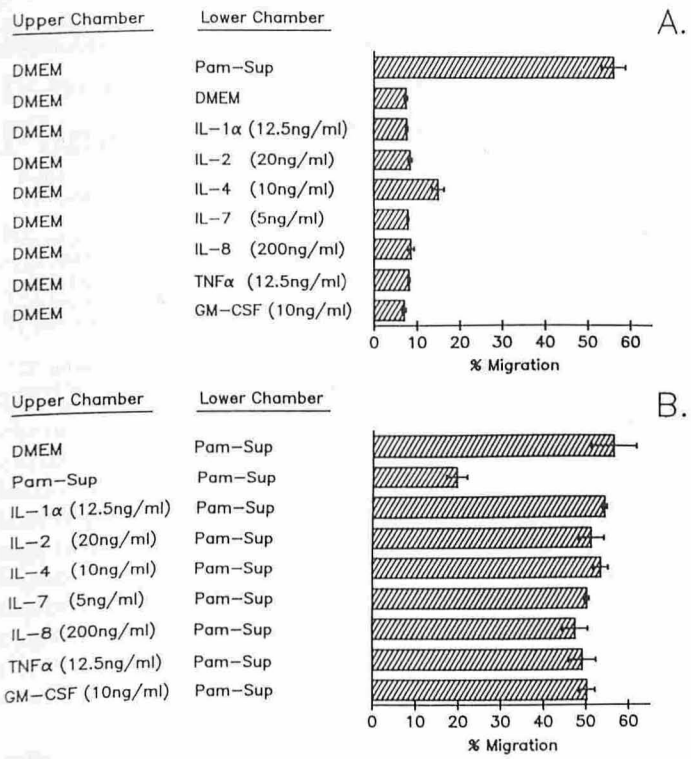

Figure 8. Failure of recombinant cytokines to mediate or to inhibit DETC migration. In one set of experiments *(A)*, several different cytokines were added to lower chambers to test their abilities to mediate DETC chemotaxis. In another set *(B)*, the same cytokines were added to upper chambers to block chemotactic migration toward Pam 212 culture supernatants. Data shown are the mean ± SEM of percent migration at 2 h from duplicate samples. Pam-Sup, Pam 212 supernatants; TNFα, tumor necrosis factor-α; GM-CSF, granulocyte/macrophage-colony stimulating factor.

cently, we have observed that keratinocytes are also capable of secreting physiologically relevant amounts of IL-7 and that rIL-7 as well as keratinocyte-conditioned media sustain the survival and promote the growth of DETC in culture.§ Thus, keratinocytes appear to play several key roles in each step of DETC movement, residence, and survival by producing gradients of chemotactic activity, by providing adhesion substrates, and by secreting growth factors.

With respect to cell specificity, we observed that chemotactic activity was not detectable in 3T3 fibroblast culture supernatants. We interpret these results to suggest that the relevant activity is present at higher concentrations in epidermis compared with the dermis, thereby producing a chemotactic gradient to promote epidermal-directed movement. However, two questions remain to be addressed to validate the physiologic relevance of this possibility. First, we do not know as yet whether normal mouse keratinocytes also produce relevant chemotactic activities. Mouse keratinocytes did grow, although relatively slowly, using DMEM supplemented with 20% FBS,‡ and DETC did migrate toward culture supernatants from these normal keratinocytes (data not shown). However, we could not block this migration by adding the supernatants to the upper chambers, suggesting that FBS at high concentrations had produced higher rates of random migration, preventing us from detecting chemotactic migration. Second, Pam 212 keratinocyte supernatants attracted not only DETC, but also splenic T cells, which do not ordinarily reside in the epidermis. On the other hand, it is likely that the residence of leukocytes within epidermis depends on adhesion molecules as well as chemotactic factors. With respect to cell adhesion, DETCs have demonstrated markedly greater affinities for keratinocyte substrates compared with splenic T cells.‡ Therefore, it is our assumption that adhesion molecules, rather than chemotactic factors, are responsible for the long-term residence of DETC within the epidermis.

From a broader perspective, interaction with cells in epithelial tissues must also be responsible for the preferential and specific localization of γδ T cells in other organs, such as the gut and reproductive tract. These γδ T cells are quite diverse, each having features unique to their tissue of residence. They differ with respect to 1) TCR gene usage (e.g., Vγ3/Vδ1 in epidermis, Vγ2/Vδ6 in lung, and Vγ5/Vδ4,5,6, and 7 in intestine) [9]; 2) ontogeny (e.g., DETCs are derived from precursors that appear in day 16 fetal thymus, whereas intestinal γδ T-cell development occurs after birth) [12]; 3) thymic dependency for their development (e.g., DETCs develop in a thymic-dependent mechanism, whereas intestinal γδ T cells are of extrathymic origin) [27]; and 4) homing specificity (e.g., γδ T cells isolated from skin preferentially migrate to the skin and thymus, but not to other epithelial tissues) [15]. In light of these differences, the important remaining question concerns the mechanisms that govern their selective homing. With the assays developed in our laboratory to examine γδ T-cell homing (adhesion to epithelial cell monolayers, migration toward epithelial cell culture supernatants, and survival and growth in the presence of epithelial cell-derived factors), it should be possible to address these questions of leukocyte/tissue specificity. Ultimately, such analyses should allow us to better understand the roles of γδ T cells in these organs, as well as those of DETCs in the protective immunity that occurs where interface with the environment occurs.

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