# In Vitro Migration of L3T4$^+$ Cloned T Cells to Epidermis: Possible Role for Keratinocyte-Derived Factors

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Three types of L3T4$^+$ cloned T cells with different antigen specificities, auto-, allo-, and antigen-reactive, were characterized with respect to their migratory potential using an in vitro migration assay under agar gel. Autoreactive T cells, BB5, and alloreactive T cells, SK 1, both of which have been proved to be epidermotropic in vivo, showed specific directional migration to the epidermis, whereas no directional migration was seen with non-epidermotropic cloned T cells and freshly isolated lymph node T cells. Both BB5 and SK 1 cells were equally attracted to all the epidermal fragments tested regardless of their I-A antigens. The directional migration of BB5 cells to the epidermis was significantly inhibited by the co-cultivation with the epidermis, but not the dermis. Studies with cell lines, the conditioned media (CM), and recombinant interleukin (IL) 1, 2, and 3 revealed that BB5 cells were chemotactically attracted to a transformed keratinocyte cell line PAM212 and, to a lesser extent, to the CM from PAM212 and IL-2, but not to IL-1 and IL 3. These results suggest that epidermotropic T cells may be preferentially trapped in an area with a high concentration of keratinocyte-derived growth factors as well as IL-2. *J Invest Dermatol 92:360–365, 1989*

In various lymphocyte-mediated skin diseases, T lymphocytes show a specific affinity for the epidermis, which is known as epidermotropism [1]. These T cells, when localized within the dermis, have the tendency to migrate across the dermal-epidermal junction and infiltrate the overlying epidermis [1]. Unfortunately, the mechanism(s) governing such epidermotropic behavior of these T cells is still largely unknown. This is probably due to the paucity of the relevant in vitro assay to accurately reflect epidermotropism and the difficulty of obtaining T-cell clones with epidermotropic nature both in vivo and in vitro.

Our previous reports demonstrated that class II antigen-restricted, Lyt-1$^+$23$^-$, L3T4$^+$ cloned T cells specific for self- or allo-I-A can migrate into the epidermis following their intradermal inoculation into the footpads of syngeneic or allogeneic mice with the appropriate I-A antigens and cause the destruction of the epidermis via the release of cytotoxic lymphokines [2–5]. These studies raised the question of whether the observed migration of the T-cell clone into the epidermis would be dependent on the interaction between the injected T-cell clone and the immunocompetent cells of the host, or whether the T-cell clone is capable of migrating into the epidermis without involving other cells in the host. Therefore, the in vitro migration of the T cells was studied using a migration assay under agar gel, as described by Laroche et al, for measurement of skin-directed migration of peripheral blood lymphocytes from Sézary syndrome patients [6]. In the present report, we first demonstrate that certain autoreactive or allo-I-A reactive cloned T cells that have been proven to be epidermotropic in vivo, but not other T cells, show directional migration toward the epidermis in vitro. Furthermore, studies with cell lines indicate that keratinocyte-derived factors are responsible for the epidermotropic migration of the T cells.

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Abbreviations:
B6: C57BL/6J
CGG: chicken γ-globulin
CM: conditioned media
ETAF: epidermal cell-derived thymocyte activation factor
GM-CSF: granulocyte-macrophage stimulating factor
IL: interleukin
LCF: lymphocyte chemoattractant factor
LFA-1: lymphocyte function-associated antigen 1
MoAb: monoclonal antibody

## MATERIALS AND METHODS

**Mice** Female C57BL/6 (B6), B10.BR, and B10.D2 mice were obtained from the Charles River Japan, Inc. and The Jackson Laboratory (Bar Harbor, ME). They were maintained in our animal facility and were used predominantly at 8 to 12 weeks of age.

**Cloned T-Cell Lines** General characteristics of the cloned T-cell lines used are shown in Table I. Two different kinds of cloned autoreactive T cells, exclusively specific for I-A$^b$, designated clones BB5 and C10, were established in previous experiments [2–5]. Both clones are derived from B6 mice, are of Thy 1$^+$, Lyt-1$^+$23$^-$, and L3T4$^+$ phenotype, and produce lymphotoxin and interferon-γ in response to I-A$^b$. BB5 cells, when injected into the footpads of syngeneic B6 mice, migrate into and cause the destruction of the epidermis [2,3]. C10 cells, in contrast, are completely incapable of migrating into the epidermis [3]. In some experiments, a cloned L3T4$^+$ B6 cell line, termed 82F12, specific for chicken γ-globulin (CGG) associated with the syngeneic I-A$^b$ molecule [4] and a cloned L3T4$^+$ A.TH cell line, termed SK.1, specific for I-A$^{k,b,f,r}$ molecules [2], were also used. Clone SK 1 is also capable of migrating into the epidermis upon its intradermal inoculation into the footpads of the appropriate allogeneic recipients with I-A$^{k,b,f,r}$ molecules and causes the destruction of the epidermis [2], whereas clone 82F12, like C10,

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**Table I.** Description of Cloned T-Cell Lines Used in This Study

| Clone designation | Origin | Antigen specificity | Function (in vitro) | Epidermotropism (in vivo) |
|---|---|---|---|---|
| BB5 | C57BL/6J | I-$A^b$ | Helper/cytotoxic | + |
| C10 | C57BL/6J | I-$A^b$ | Helper/cytotoxic | − |
| 82F12 | C57BL/6J | CGG ± I-$A^b$ | Helper | − |
| SK 1 | A.TH | I-$A^{k,b,f,r}$ | Helper/cytotoxic | + |

is not epidermotropic in vivo [3]. These clones (BB5, C10 and SK 1) were maintained by weekly feedings with mitomycin C-treated syngeneic (BB5, C10) or appropriate allogeneic (SK 1) spleen cells and rat interleukin-2 (IL-2) [2,3]. 82F12 was maintained in the same manner but with 250 μg/ml CGG [4].

**In Vitro Migration Assay** The technique for the in vitro migration of murine T cells was adapted from the migration assay described previously by Laroche [6]. Briefly, a gel was prepared in a tissue culture dish (Falcon 3002) using 1% agar (Agar Noble, Difco Laboratories, Detroit, MI) in RPMI 1640 supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, 1% L-glutamine, and 10% heat-inactivated fetal bovine serum (FBS; M.A. Bioproducts, Walkersville, MA). A row of three wells spaced 1 mm apart was cut in each agar plate using a stainless steel punch. The center well of each row received 4 μl of T-cell suspension ($6 \times 10^4$ cells/well). A 0.25-$mm^2$ fragment of tissue was added to the outer well as a test attractant. Epidermal fragments from footpad skin were obtained by mechanically separating them from the dermis following incubation in 0.02% EDTA for 2 h at 37°C. The inner well contained medium alone as a control. Each plate was composed of a maximum of four rows to eliminate overlap of adjacent gradients. The plates were incubated at 37°C in a fully humidified atmosphere containing 5% $CO_2$ in air for 48 h and was examined with an inverted microscope. The number of the cells that migrated under the gel and was observed in the space immediately between the wells, was enumerated on both sides of the center wells. The results were expressed as a migration index (MI):

$$\text{MI} = \frac{\text{Number of cells migrating to the test well}}{\text{Number of cells migrating to the control well}}.$$

This evaluation was performed in a blind fashion by an investigator who was uninformed of the treatment. Each Table is representative of three to four independent experiments. Each value represents the mean ± S.D. derived from six determinations.

**Cell Lines** PAM212 cells, a spontaneously transformed BALB/c-derived keratinocyte cell line, was kindly provided by Dr. Kiyoshi Nishioka (Kitasato University, Japan) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. The murine fibroblast line L929, provided by Dr. Nancy Ruddle (Yale University), was maintained in the same manner.

**IL-1, IL-2, IL-3, and Conditioned Media from PAM212 and L929** Recombinant human IL-1, murine IL-2, and murine IL-3 were purchased from Genzyme Corporation (Boston, MA). Conditioned media (CM) were prepared by incubating PAM212 cells or L929 cells at a density of $1 \times 10^6$ cells/ml in the complete media for 48 h.

**Statistical Analysis** The statistical significance of differences was evaluated by means of the two-tailed Student's $t$ test; $P < 0.05$ (2-tailed test) was considered significant.

## RESULTS

**Ability of Autoreactive T cells to Migrate to the Skin in Vitro** Previous studies have demonstrated that the migratory ability of lymphocytes in vitro depends on stage of the activation: lymphocytes become most motile after peak thymidine incorporation has occurred [7]. Therefore, in order to determine at what stage of activation the random or directional migration of the autoreactive T cells becomes maximal, BB5 cells were harvested from culture on days 3, 5, 6, and 8 after antigenic stimulation and were examined for their ability to migrate to the skin using a migration assay under agar gel. Inverted phase-contrast microscopic observations showed that at days 5 and 6 after antigenic stimulation, BB5 cells stopped growing and changed their shape and size gradually from large and spindle-shaped to small and round. The maximal level of $^3$H-thymidine incorporation was observed on day 2, as previously described [4]. As shown in Table II, BB5 cells, when harvested 3 d after initiation of stimulation with antigen, were most motile without any attracting tissue, whereas the skin-directed migration that was expressed as an MI reached a maximal levels with those harvested on day 5. With respect to the random migration, other cloned T cells tested were most motile, regardless of their antigen specificity, when harvested on day 3 of culture (data not shown), although the absolute number of migrated cells varied considerably from clone to clone. SK 1 cells showed greatest random migration, i.e., 5 times more than BB5 cells, and 82F12 cells demonstrated the least random migration. We then decided to use cloned T cells harvested from culture 5 d after antigenic stimulation for all further assessment of the directional migration.

**Comparison of the In Vitro Migration of Autoreactive, Alloreactive and Antigen-Reactive T Cells** Three types of class II antigen-restricted, Lyt-$1^+$ L3T$4^+$ cloned T cells with different antigen specificities were characterized with respect to their directed migratory potential in vitro: autoreactive T cells, BB5, and C10; allo-I A-specific T cells, SK 1; and CGG-specific T cells, 82F12. As shown in Table III, the tissue fragments from various organs were used as attracting tissues. BB5 and SK 1, both of which had been shown to be epidermotropic in vivo [2,3], showed skin-directed migration, whereas no directed cell movement was seen with non-epidermotropic clones, C10 and 82F12. Furthermore, epider-

**Table II.** In Vitro Migration of BB5 Cells Harvested on Various Days After Antigenic Stimulation

| | Test Attractant | | | | | |
|---|---|---|---|---|---|---|
| | Medium | | | Skin | | |
| Days of Culture | T[a] | C[b] | MI[c] | T[a] | C[b] | MI[c] |
| 3 d | 367 ± 72 | 373 ± 53 | 0.98 ± 0.08 | 931 ± 139 | 490 ± 154 | 2.01 ± 0.40 |
| 5 d | 55 ± 19 | 51 ± 12 | 1.06 ± 0.29 | 510 ± 107 | 152 ± 29 | 3.41 ± 0.56 |
| 6 d | 25 ± 8 | 23 ± 6 | 1.10 ± 0.26 | 280 ± 71 | 95 ± 18 | 2.95 ± 0.47 |
| 8 d | 4 ± 2 | 5 ± 2 | 1.08 ± 0.46 | 45 ± 13 | 27 ± 5 | 1.67 ± 0.32 |

[a] Number of cells migrating to the test well.
[b] Number of cells migrating to the control well.
[c] Migration Index.

**Table III.** Migration Pattern of Class II Antigen-restricted Cloned T-Cell Lines

| Attractant | Cloned T-Cell Line BB5 | C10 | 82F12 | SK 1 |
|---|---|---|---|---|
| Medium | 1.02 ± 0.23 | 1.06 ± 0.25 | 0.97 ± 0.15 | 1.06 ± 0.18 |
| Skin | 3.37 ± 0.65[a] | 1.29 ± 0.29 | 1.21 ± 0.24 | 1.75 ± 0.34[b] |
| Muscle | 1.07 ± 0.22 | 0.98 ± 0.25 | 0.96 ± 0.15 | 1.11 ± 0.12 |
| Kidney | 0.94 ± 0.17 | 1.04 ± 0.16 | N.D.[c] | 0.99 ± 0.17 |
| Liver | 1.24 ± 0.27 | 1.16 ± 0.11 | N.D.[c] | N.D.[c] |
| Epidermis | 4.13 ± 0.65[a] | 1.34 ± 0.20 | 1.10 ± 0.18 | 1.86 ± 0.29[a] |
| Dermis | 1.31 ± 0.48 | 1.05 ± 0.17 | 0.96 ± 0.16 | 1.23 ± 0.17 |

[a] $P < 0.001$ vs. medium control.
[b] $P < 0.005$ vs. medium control.
[c] Not done.

mal fragments were found to be as effective as skin fragments in attracting clones BB5 and SK 1 (Table III, Fig 1). Neither C10 nor 82F12 showed such a directed migration toward the epidermis. Freshly isolated lymph node T cells, like C10 and 82F12, did not show any directed migration (data not shown). These results indicate that the epidermotropic migration by the cloned T cells demonstrated in vivo is accurately reflected in our in vitro migration assay under agar gel and that the in vitro migration to the epidermis is not restricted to autoreactive T cells.

**Specificity of In Vitro Migration** Clones BB5 and C10 show reactivity exclusively directed against self-I-$A^b$ molecule [4], while clone SK 1 is alloreactive to either I-$A^k$ or I-$A^b$ and to a lesser extent to I-$A^f$ and I-$A^r$ [2]. Therefore, it was of interest to examine whether these cloned T cells could migrate preferentially to the epidermis with the relevant I-A molecules. The data depicted in Table IV demonstrate that the epidermotropic cloned T cells, BB5 and SK 1, were equally attracted to all the epidermal fragments tested, regardless of whether the epidermal fragment had the relevant or irrelevant I-A molecules. This suggests that the in vitro epidermotropic migration of either BB5 or SK 1 is independent of their reactivity to I-A molecules.

To further test the specificity of the migration, competition experiments in which fragments of epidermis or dermis were added to the center wells containing BB5 cells were performed. The effects of the addition on the directional migration of BB5 cells were assessed by the inhibition of migration toward the outer well containing the test attractant. As shown in Table V, the directional movement of BB5 cells toward the epidermis was significantly inhibited by the co-cultivation with the fragment of epidermis in the center well, although the random migration, which was assessed by the migration toward the control well, was significantly increased. The co-cultivation with the fragment of dermis showed neither inhibition of the directional movement nor increase of random migration. The viability of BB5 cells was not affected by the addition of the fragment of epidermis or dermis. These results indicate that epidermotropic clone BB5 is capable of migrating specifically towards the epidermis without requiring the participation of second-set immunocompetent cells such as macrophages.

**In Vitro Migration of the Epidermotropic T Cells to the Keratinocyte Cell Line** Because the murine footpad epidermis is predominantly composed of three cell populations, a numerically large population of keratinocytes and a small population of Langerhans cells and Thy $1^+$ dendritic cells, we next investigated whether a transformed murine keratinocyte cell line PAM212, which had been shown to be devoid of such non-keratinocyte cell types, could attract BB5 cells in vitro as well as the epidermal fragment. The reason we chose PAM212 cells as a source of murine keratinocytes was based on the preliminary observation that purified keratinocyte population without contamination by non-keratinocyte cell types was not obtained from primary culture of epidermal cells even after repetitive treatment with MoAb such as anti-I-A MoAb and anti-Thy-1.2 MoAb. The murine fibroblast line L929 was employed as a control. As shown in Table VI, PAM212 cells appeared to be more effective than the epidermal fragment in attracting BB5 cells, whereas L929 induced no directed migration in BB5 cells, just as the dermal fragment.

Keratinocytes have been shown to produce IL-1 [8], IL-3 [9], IL-6 [10], and granulocyte-macrophage colony-stimulating factor (GM-CSF) [11]. Therefore, to determine whether soluble factors released from the epidermis or PAM212 cells were responsible for the in vitro epidermotropic migration demonstrated here, CM collected from PAM212 cells was tested for its ability to attract BB5 cells in the migration assay. The results (Table VII) show that the CM from PAM212 was not as effective an attractant as PAM212 cells themselves even when the undiluted CM was used; although the mean MI value obtained with the CM was significantly higher than that either with medium or with CM from L929. Recombinant IL-2 at a

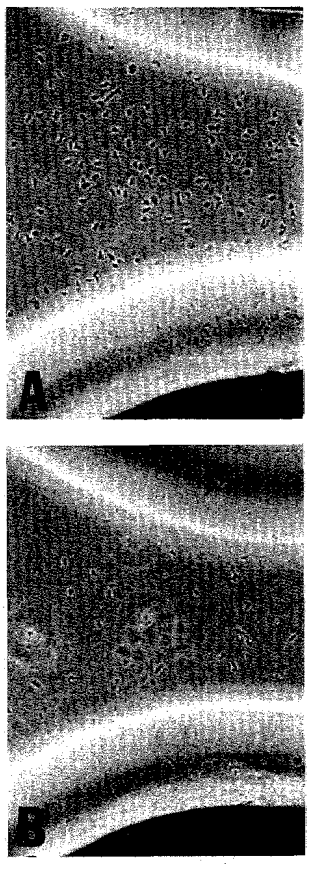


**Figure 1.** *A:* BB5 cells migrating from a center well *(bottom)* to an outer well *(top)* containing epidermal fragment. *B:* BB5 cells migrating from a center well to an outer well containing dermal fragment.

**Table IV.** Specificity of In Vitro Migration of Class II Antigen-restricted Cloned T-Cell Lines

| Attractant | I-A | Cloned T-Cell Line BB5 | C10 | SK 1 |
|---|---|---|---|---|
| Medium | – | 1.08 ± 0.16 | 1.09 ± 0.13 | 1.00 ± 0.21 |
| B6 epidermis | b | 4.06 ± 0.88[a] | 1.32 ± 0.20 | 1.85 ± 0.28[a] |
| B10.D2 epidermis | d | 3.89 ± 0.70[a] | 1.23 ± 0.17 | 1.76 ± 0.29[a] |
| B10.BR epidermis | k | 3.66 ± 0.49[a] | 1.26 ± 0.19 | 1.92 ± 0.43[b] |

[a] $P < 0.001$ vs. medium control.
[b] $P < 0.005$ vs. medium control.

concentration of 100 U/ml showed a marginal but significant chemotactic activity for BB5 cells, whereas neither rIL-1 nor rIL-3 appeared to be chemotactic in this system. Similar negative results were reproducibly obtained in experiments using a range of concentrations of rIL-1 and rIL-3 from1 to 50 U/ml (data not shown).

## DISCUSSION

Much attention has recently been directed to the mechanism(s) of lymphocyte migration from the bloodstream into lymphoid tissues, and the receptor-ligand type of interaction between lymphocyte and postcapillary high endothelial venules (HEV) has been proved to be essential for migration [12]. On the other hand, studies addressing the factors that govern the epidermotropic migration of certain T cells have been few in number, because no attempt has been successful in demonstrating the in vitro migration of T cells to the epidermis. There are some possible reasons for the failure of previous investigators. First, directional migration (chemotaxis) of lymphocytes, unlike random migration (chemokinesis), has been difficult to demonstrate using the standard filter assays applied to studies of neutrophil motility [7]. Second, the heterogeneity of lymphocyte populations and the possible variation in the rate of migration, as demonstrated by Hoffman [7], may have made previous attempts to demonstrate the in vitro migration difficult. Even if the very small number of epidermotropic T cells that are probably present in the bulk lymphocyte preparations were attracted to the epidermis, they are not likely to be detected in the in vitro migration assay. This view is supported by our finding that the freshly isolated bulk lymphocyte population did not show directional migration to the epidermis. Moreover, the capacity of T cells to migrate to the epidermis may depend on the stage of activation or position in the cell cycle of the T cells. In fact, the skin-directed migration of BB5 cells began to increase after the DNA synthesis peaked and became maximal on day 5, at which time the T cells were in plateau growth phase (Table II). This observation held true for other cloned T cells (data not shown). However, and in contrast to previous studies [7], with respect to the random migration, our T cells appeared to be far less motile in the plateau growth phase as compared to those in the exponential growth phase. For these reasons, our in vitro migration assay was performed using epidermotropic or non-epidermotropic cloned T cells in the plateau growth phase. Thus, the availability of cloned T cells with epidermotropic nature and the technique of the in vitro migration assay has made the demonstration of epidermotropism of certain T cells possible.

Although BB5 cells and SK 1 cells were comparable with regard to their ability to migrate to the epidermis in vivo, the MI of BB5 cells was much higher than that of SK 1 cells. Differences in the random migration may offer a partial explanation for this apparent discrepancy, because SK 1 cells showed epidermis-directed migration comparable to that of BB5 cells with respect to the absolute number of migrated cells. In considering that the co-cultivation with the fragment of epidermis to the center well increased random migration, it seems likely that keratinocyte-derived factors are not only chemotactic but are also to some extent chemokinetic. To differentiate between these two effects, "checkerboard analysis," in which varying concentrations of the factors are added to the test and control wells, will be needed.

Specifically sensitized lymphocytes are shown to preferentially accumulate at sites of antigenic deposition [13]. However, whether these specifically sensitized T cells are attracted to the site of specific antigen deposition by the chemotactic gradient that accounts for neutrophil accumulation, or whether their random movement is arrested only when the antigenic structure they are specific for is presented on the surface of another cell of appropriate type [14], is still speculative. The demonstration by Tyler et al [15] that cloned cytolytic T cells specific for non-H-2 alloantigen of epidermal cells, designated Epa-1, could cause necrosis of epidermal structures in vivo, appears to indicate that antigen might be chemotactic for specifically sensitized T cells. Our data, however, that self-I-$A^b$ specific BB5 cells were attracted not only to the epidermis with the appropriate antigen (I-$A^b$), but also to the epidermis with the wrong antigen (I-$A^d$), are consistent with our previous observation that the addition of anti-I-A MoAb to the epidermis did not affect the migration pattern of BB5 cells [16]. These data also provide evidence against the notion that the T cells are specifically attracted to the site of antigen deposition.

What then is the mechanism by which epidermotropic T cells are attracted to the epidermis? Evidence is accumulating that growth factors for lymphocytes induce a chemotactic response [17,18]. These growth factors include IL 1 [19,20], IL 2 [18], and lymphocyte chemoattractant factor (LCF) [17,18]. Either IL 1 or LCF appears to induce migration in resting and activated T cells [18–20], whereas IL 2 acts solely on activated T cells [18]. In considering that freshly isolated lymph node T cells showed no directed migration to the epidermis and that BB5 cells harvested on day 8 were marginally attracted to the epidermis, epidermal fragments appeared to be inert in resting T cells. In this regard, factors released from the epidermis, especially keratinocytes, may induce a chemoattractant response in a manner analogous to IL 2. How do these growth factors such as IL 2 give directionality to T-cell migration? T cells activated with antigens or mitogens have been shown to be extremely motile and adhesive as compared to resting T cells. Therefore, activated T cells moving randomly could be preferentially trapped in an area with a

**Table V.** Competition Experiments of Directional Migration of BB5 Cells to Epidermis

| Competition | Attractant Epidermis T | Epidermis C | Epidermis MI | Dermis T | Dermis C | Dermis MI | Medium T | Medium C | Medium MI |
|---|---|---|---|---|---|---|---|---|---|
| None | 401 ± 60 | 109 ± 30 | 3.80 ± 0.53 | 74 ± 6 | 61 ± 11 | 1.24 ± 0.17 | 50 ± 9 | 50 ± 7 | 1.02 ± 0.23 |
| Epidermis | 353 ± 97 | 285 ± 42 | 1.27 ± 0.35[a] | 187 ± 42 | 188 ± 57 | 1.03 ± 0.10 | 159 ± 56 | 162 ± 37 | 0.98 ± 0.20 |
| Dermis | 320 ± 55 | 94 ± 14 | 3.49 ± 0.73 | 44 ± 9 | 40 ± 5 | 1.12 ± 0.16 | 41 ± 12 | 37 ± 3 | 1.07 ± 0.32 |

[a] $P < 0.01$ vs "none".

**Table VI.** Preferential Migration of BB5 Cells to Keratinocyte Cell Line

| Attractant | BB5 cells | | |
|---|---|---|---|
| | T | C | MI |
| Medium | 31 ± 9 | 33 ± 9 | 1.00 ± 0.28 |
| Epidermis | 328 ± 40 | 107 ± 16 | 3.12 ± 0.52[b] |
| Dermis | 42 ± 12 | 38 ± 8 | 1.10 ± 0.20 |
| PAM212[a] | 404 ± 42 | 104 ± 22 | 4.01 ± 0.51[b] |
| L929 | 95 ± 12 | 77 ± 16 | 1.21 ± 0.16 |

[a] $4 \times 10^3$ viable cells were added to the outer well as a test attractant.
[b] $P < 0.001$ vs. medium control.

high concentration of growth factors, when they proliferate, thereby accumulating.

Until recently, epidermal cell-derived thymocyte activating factor (ETAF), which has been shown to be identical to IL 1, has been regarded as the most probable T-cell chemoattractant [19] that offers a good explanation for epidermotropism of T cells. However, the effect of ETAF on T-cell chemotaxis has been exclusively demonstrated using a bulk population of unsensitized human T cells [18]. In this study, we have failed to demonstrate such a chemoattractant activity of IL 1 with a bulk population of unsensitized T cells (data not shown) and cloned T cells. These differences may result from differences in the method used for the in vitro migration assay or may reflect a possible low sensitivity of murine lymph node T cells to rIL 1 used in this study. Alternatively, ETAF/IL 1 may not play a critical role in epidermotropism of T cells, as suggested by our previous study in which increased production of ETAF was not accompanied by an increase in epidermotropic migration of BB5 cells [21].

GM-CSF [11] is another possible chemotactic factor for T cells. The fact that GM-CSF supports the growth of certain helper T cell lines [22] suggests a potential role for GM-CSF in epidermotropic migration of T cells. This potential role for GM-CSF is particularly interesting in the context of the documented capacity for GM-CSF and PAM212 CM devoid of IL 2 to sustain the growth of IL-2-dependent, epidermotropic cloned T cells, BB5 and SK 1 (Shiohara, unpublished observations). In this respect, the possibility that differential expression of receptors specific for GM-CSF and/or as-yet unidentified, keratinocyte-derived growth factors could account for the difference in the ability of T cells to migrate to the epidermis may be worth exploring. However, in our preliminary experiments, recombinant GM-CSF did not show significant chemotactic activity for BB5 cells when used at a range of concentrations from 10 to 500 U/ml. When we compared the proliferation of BB5 cells induced by IL 2 and GM-CSF, IL 2 was much better than GM-CSF (Shiohara, unpublished observations), and the failure to observe significant chemotactic activity with GM-CSF reflects that a much higher concentration of GM-CSF than we could obtain in this preliminary experiment may be required. Thus, it remains to be determined whether GM-CSF would be chemotactic for BB5 cells. Alternatively, in addition to GM-CSF, keratinocytes may produce other growth factors that can support the growth of epidermotropic T cells, thereby rendering them capable of migrating to the epidermis. It was unfortunate that IL2 and the CM from PAM212 were not as effective an attractant as PAM212 cells themselves (Table VII), thus making the chemotactic reaction of the T cells to cytokines such as GM-CSF more difficult to interpret. Because one requirement for chemotaxis of cells has been shown to be that the cells should be able to detect concentration differences across their own length [23], our migration assay system employed here may have been sensitive enough to detect the concentration differences produced by cytokine-producing cells themselves, but not that produced by the cytokines.

**Table VII.** Preferential Migration of BB5 Cells to PAM212 CM and rIL 2

| Attractant | BB5 cells | | |
|---|---|---|---|
| | T | C | MI |
| Medium | 43 ± 7 | 42 ± 4 | 1.01 ± 0.14 |
| PAM212 | 719 ± 69 | 172 ± 12 | 4.17 ± 0.25[a] |
| PAM212 CM[c] | 154 ± 17 | 104 ± 11 | 1.49 ± 0.23[b] |
| L929 CM[c] | 63 ± 14 | 58 ± 7 | 1.09 ± 0.21 |
| rIL 1[c] | 83 ± 10 | 79 ± 8 | 1.11 ± 0.09 |
| rIL 2[c] | 256 ± 37 | 177 ± 18 | 1.45 ± 0.16[b] |
| rIL 3[c] | 64 ± 6 | 61 ± 7 | 1.05 ± 0.10 |

[a] $P < 0.001$ vs. medium control.
[b] $P < 0.05$ vs. medium control.
[c] Four microliters of rIL 1, 2, or 3 (100U/ml) or CM from PAM212 or L929 were added to the outer well as a test attractant.

The ability of MoAb to lymphocyte function-associated antigen 1 (LFA-1) to inhibit the in vitro epidermotropic migration of BB5 cells [24] suggests that LFA-1 on the T cells is interacting with its ligand, a soluble factor released from keratinocytes. This view is further supported by our recent observation that locally administered MoAb to LFA-1 prevents cutaneous graft-versus-host disease by epidermal invasion of BB5 cells [25]. Thus it appears likely that the interaction of LFA-1 with its ligand may synergize with the activation signal delivered by keratinocyte-derived growth factors.

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