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# T Cell Subsets in Normal Human Epidermis

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***Freshly isolated human lymphocytes from normal epidermis were characterized with respect to distribution of subsets. The major T cell receptor-$\alpha\beta^+$ compartment was enriched for $CD4^+$, for $CD8\alpha\alpha^+$, and for $CD4^-CD8^-$ T lymphocytes compared with peripheral blood lymphocytes. Furthermore, the majority of epidermal T lymphocytes expressed a $CD45RA^-$ $CD45RO^{high}$ $Fas^+$ memory/effector phenotype; many also expressed early-intermediate activation markers, suggesting antigenic exposure in vivo. The cutaneous lymphocyte-associated antigen was expressed by almost all epidermal T lymphocytes. A large portion also expressed the mucosal-associated $\alpha^e\beta_7$-integrin, which may mediate retention to epithelium. These data show that T lymphocytes present in normal human epidermis constitute a distinct T cell compartment with characteristics similar to that of other epithelial-associated T cell compartments. (Am J Pathol 1996, 149:665–674)***

The human skin-associated lymphoid tissue[1,2] appears structurally and functionally well equipped for continuous environmental, both infectious and physical, challenges. Keratinocytes, the principal epidermal cells, probably exert immunoregulatory functions, as they are capable of secreting a variety of cytokines and of phagocytosis and may also have antigen-presenting capacities (reviewed in Refs. 3 and 4).[5] Bone-marrow-derived dendritic cells within epidermis, dermis, and skin-draining lymph nodes serve as antigen-presenting cells in primary and secondary immune responses.[6–10] Human skin also harbors a substantial number of T lymphocytes,[11,12] and skin endothelial cells express the vascular addressin necessary to attract and direct skin-homing T lymphocytes from the circulation into the skin.[13–15]

The T lymphocytes of the skin-associated lymphoid tissue populate all three of its anatomical compartments, the epidermis, the dermal perivascular units, and the skin-draining lymph nodes. In the latter two compartments, T lymphocytes have been shown to interact with dendritic cells in antigen-elicited immune responses.[7,8,10] Similarly, epidermal T lymphocytes are often closely apposed to Langerhans cells, the epidermal subset of the skin dendritic cell population.[12] However, their functional capacities are yet unassessed.

Evidence that T lymphocyte populations at epithelial sites may be specialized to recognize not only classical major histocompatibility complex (MHC)-peptide complexes but also particular sets of nonclassical antigens and/or antigen-presenting molecules possibly synthesized by epithelial cells is provided by the unique phenotypic characteristics and T cell receptor usage/diversity of human intestinal intraepithelial lymphocytes and decidual mononuclear cells.[16–18] Thus, by inference, human epidermal T lymphocytes may also have specialized phenotypic and functional properties, perhaps comparable to intestinal intraepithelial lymphocytes.

This study was aimed at comprehensively defining the phenotypic properties of human epidermal T lymphocytes, in particular with regard to the differential expression of the accessory molecules CD4, CD8$\alpha$, and CD8$\beta$ by epidermal $\alpha\beta$ and $\gamma\delta$ T lymphocytes as well as of molecules relevant to certain defined T lymphocyte functions, using multi-parameter flow cytometry. Although results of such phenotypic analyses will only indirectly point to given biological role(s) and/or the derivation of a particular T lymphocyte

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**Table 1.** *Antibodies and Reagents Used in this Study*

| Specificity | Antibody | Clone | Isotype | Conjugate | Source* | Epitope trypsin sensitive |
|---|---|---|---|---|---|---|
| CD1a | Anti-CD1a | OKT6 | IgG1 | FITC | Ortho | No |
| CD3 | IOT3b | UCHT1 | IgG1 | FITC | Immunotech | No |
| | Anti-CD3 | UCHT1 | IgG1 | PE, biotin | Dako | No |
| CD4 | Anti-CD4 | MT310 | IgG1 | PE | Dako | Yes |
| | Leu-3a | SK3 | IgG1 | Biotin | Becton Dickinson | Yes |
| CD8α | IOT8a | B9.11 | IgG1 | Biotin | Immunotech | Yes |
| | Anti-CD8 | DK25 | IgG1 | PE | Dako | Yes |
| | Leu-2a | SK1 | IgG1 | FITC | Becton Dickinson | Yes |
| CD8β | Anti-CD8β | 2ST8–5H7 | IgG1 | FITC† | Ref. 19 | Yes |
| CD16 | Anti-Leu-11c | B73.1 | IgG1 | PE | Becton Dickinson | No |
| CD19 | Anti-Leu-12 | 4G7 | IgG1 | | Becton Dickinson | No |
| CD25 | Anti-IL-2Rp55 | 2A3 | IgG1 | PE | Becton Dickinson | Yes |
| CD122 | Anti-IL-2Rp75 | TU27 | IgG1 | PE | Becton Dickinson | No |
| CD45RA | Anti-CD45RA | 2H4 | IgG1 | RD1 | Coulter | Yes |
| CD45RO | Anti-Leu-45RO | UCHL-1 | IgG2a | | Becton Dickinson | No |
| | Anti-CD45RO | UCHL-1 | IgG2a | PE | Dako | No |
| CD57 | Anti-CD57 | NC1 | IgM | Biotin | Immunotech | No |
| CD95 | Anti-FAS | CH-11 | IgM | | UBI | No |
| L-Selectin | Anti-Leu-8 | SK11 | IgG2a | PE | Becton Dickinson | Yes |
| HLA-DR | Anti-HLA-DR | L243 | IgG2a | PE | Becton Dickinson | No |
| αeβ7 | Anti-αeβ7 | BER-ACT-8 | IgG1 | | Ref. 20 | No |
| CLA | Anti-CLA | HECA-452 | IgM | FITC | Ref. 21 | No |
| TcRαβ | Pan-TcRαβ | BMA031 | IgG2b | FITC | T Cell Diagnostics | No |
| | | BMA031 | IgG2b | Biotin | Ref. 22 | No |
| TcRγδ | Anti-TcRγδ-1 | 11F2 | IgG1 | FITC, biotin | Becton Dickinson | No |
| Mouse Ig | Goat-anti-mouse | $F(ab')_2$ | | FITC, PE | Tago | |
| Mouse IgG | Goat-anti-mouse | $F(ab')_2$ | | FITC | Cappel | |
| Mouse IgM | Goat-anti-mouse | $F(ab')_2$ | | FITC | Cappel | |
| Streptavidin | | | | Red670 | GIBCO/BRL | |

* Becton Dickinson, San Jose, CA; Cappel, Organon Teknika, Durham, NC; Coulter Corp., Hialeah, FL; Dako Corp., Carpinteria, CA; GIBCO/BRL, Bethesda, MD; Immunotech, Westbrook, ME; Ortho Diagnostic Systems, Raritan, NJ; Tago, Camarillo, CA; T Cell Diagnostics, Cambridge, MA; UBI, Lake Placid, NY.

† MAb was used as unconjugated reagent.

population, they will facilitate further more direct functional and/or developmental studies.

## Materials and Methods

### Preparation of Cell Suspensions

Clinically normal split-thickness (~3 mm) skin samples (n = 8) 10 to 15 $cm^2$ in size were obtained from randomly chosen healthy adult women undergoing reductive plastic surgery of the breast at the Brigham and Women's Hospital, Boston, MA. Separation of epidermis and dermis was accomplished by floating small skin stripes on phosphate-buffered saline (PBS) without calcium and magnesium (Dulbecco's PBS) with 0.1% trypsin (ICN Biochemicals, Cleveland, OH) and 1 mmol/L EDTA for 2 hours at 37°C. Epidermal sheets were peeled off the dermis and vigorously agitated for a few minutes in PBS, 10% fetal calf serum (FCS) (Hyclone, Logan, UT), 0.025% DNAse I (Sigma Chemical Co., St.Louis, MO) to obtain single-cell suspensions. Cell suspensions were filtered through a fine steel mesh to remove large clumps of cells. Cell viability was routinely 80 to 90%, as determined by trypan blue exclusion. Depending on the sensitivity of monoclonal antibody (MAb)-reactive epitopes to trypsin treatment (see also next paragraph and Table 1), immunostaining of epidermal cell suspensions was performed either directly after isolation or after overnight 37°C incubation in RPMI, 10% FCS, allowing re-expression of trypsin-sensitive epitopes.

As the customary approach to isolating epidermal cells involves protease (trypsin) digestion, control experiments on peripheral blood mononuclear cells tested the trypsin sensitivity of all cell surface molecules analyzed in this study before analyzing their expression by epidermal T lymphocytes. Buffy coat leukocytes were obtained from randomly selected healthy adult blood donors at the Dana-Farber Cancer Institute Blood Bank. Peripheral blood mononuclear cells were isolated by density gradient centrifugation over Ficoll-Hypaque (Pharmacia, Piscataway, NJ). One aliquot of peripheral blood mononuclear cells was stained directly after isolation, and the remainder of the cells were subjected to trypsin treatment identical to that

used to isolate epidermal cells. One aliquot of treated cells was stained directly after trypsin exposure, and the remaining cells were incubated at 37°C overnight and stained thereafter. Several cell surface molecules were sensitive to trypsin treatment, but MAb binding was recovered after overnight incubation at 37°C (Table 1).

### *Antibodies/Reagents*

Primary unconjugated and fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, or biotin-conjugated MAbs and secondary antibodies/reagents used in this study are listed in Table 1. Irrelevant MAbs of the appropriate immunoglobulin isotype were used as negative controls. MAbs were titrated and diluted in staining buffer (PBS, 0.02% sodium azide, 1% FCS, 10% human AB serum).

### *Flow Cytometry*

Staining of cells for one- and multi-color flow cytometry was performed as described.[23] Cells were washed in buffer (PBS, 0.02% sodium azide, 1% FCS) followed by a 15-minute room temperature incubation in staining buffer. Then, $2 \times 10^6$ cells/sample were reacted with various combinations of conjugated and/or unconjugated primary MAbs, washed, exposed to appropriate secondary reagents, and washed again. Blocking of unbound anti-mouse immunoglobulin binding sites was accomplished by a 10-minute room temperature incubation in PBS with 10% (v/v) normal mouse serum. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson, Santa Cruz, CA). Acquisition gates were based 1) on viability ($17 \times 10^3$ living cells/sample) and 2) on size (forward scatter) and granularity (side scatter). In some experiments, analysis gates defined by CD3 or T cell receptor expression were also used. Results were expressed as the log of fluorescence.

## *Results and Discussion*

### *Flow Cytometry of Epidermal T Lymphocytes*

Immunohistological analyses of sections and sheets of normal human epidermis have demonstrated the presence of significant numbers of T lymphocytes within the most basal keratinocyte layer, but very rarely were T lymphocytes detected in suprabasal locations.[12] Thus, although quite frequent within basal epidermis, the proportion of epidermal T lymphocytes within the total number of epidermal cells (as analyzed in this study) was expected to be low, due to the multilayered architecture of human epidermis. Multi-color flow cytometry of small cell subsets (in this study, T lymphocytes) within an overwhelming population of irrelevant cells (in this study, strongly autofluorescent keratinocytes; Figure 1a) reaches the thresholds of detectability. One approach to enriching epidermal T lymphocytes involves density gradient centrifugation-based keratinocyte depletion protocols before immunostaining, which carry the risk of susbstantial nonspecific T lymphocyte loss (unpublished results). Alternatively, specific cell subsets can be selected using elec-

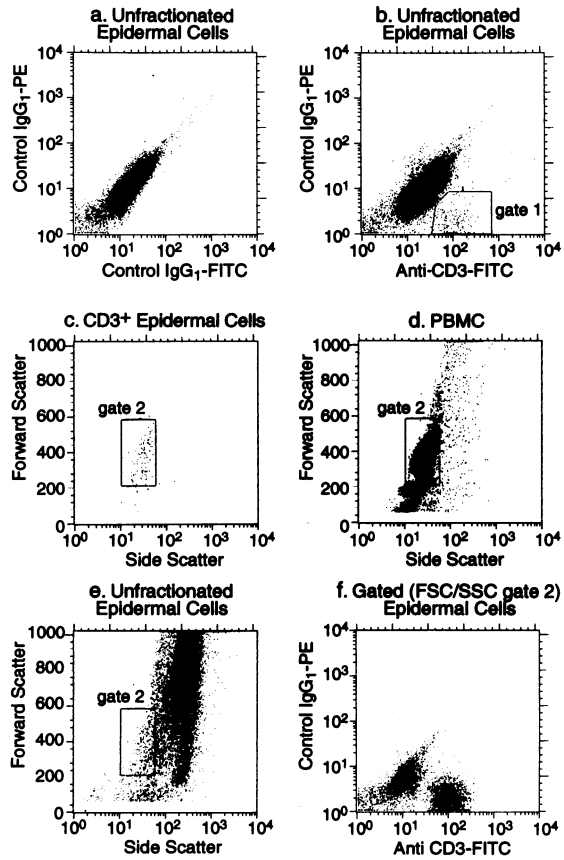


Figure 1. *Flow cytometry of freshly isolated epidermal cells, designed to optimize parameter settings on FACScan for analysis of intraepidermal lymphocytes. This was necessary as unfractionated epidermal cells (ie, mostly keratinocytes) were strongly autofluorescent with high background staining in all fluorescence parameters* (a: *PE and FITC and data not shown for Red 670*). *Epidermal cells from eight different donors were analyzed. Representative data are shown. The size of epidermal T lymphocytes was determined by analyzing the FSC/SSC distribution* (c) *of CD3+ epidermal cells (gate 1 in* b), *which compared well with the FSC/SSC profile of peripheral blood lymphocytes* (d). *Throughout this study, multi-parameter flow cytometry acquisition was performed, applying gates designed to encompass the entire epidermal T lymphocyte population (such as gate 2 in* e) *to unfractionated epidermal cells.* f: *Reduced (keratinocyte-born) autofluorescent background when gate 2 was applied during acquisition.*

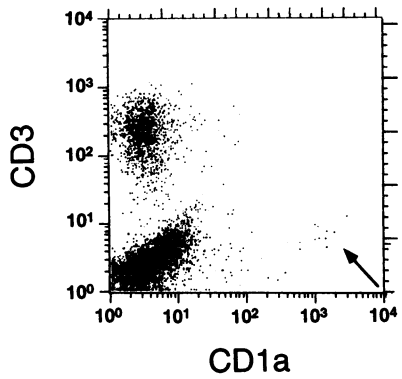


Figure 2. *CD1a/CD3 expression by epidermal cells visualized by two-color flow cytometry. MAbs were anti-CD1a-FITC and anti-CD3-PE. Epidermal cells were gated as depicted in gate 2 in Figure 1e.* Arrow *indicates strongly CD1a⁺ Langerhans cells.*

tronic gates based on cell size and granularity during flow cytometry. To determine the size of human epidermal T lymphocytes, unfractionated epidermal cells were stained with anti-CD3 MAb. CD3⁺ epidermal cells were electronically gated (gate 1 in Figure 1b) and their forward scatter (FSC) and side scatter (SSC) distribution displayed (Figure 1c). The FSC/SSC distribution of peripheral blood lymphocytes is shown for comparison (Figure 1d). In addition, immunofluorescence microscopy of keratinocyte-depleted epidermal cell suspensions revealed occasional CD1⁺CD3⁺ signals, possibly representing epidermal T lymphocyte/Langerhans cell conjugates.[24] The frequency of such conjugates, however, was below the limits of detectability by flow cytometry. Accordingly, flow cytometry of anti-CD1 and anti-CD3 double-stained epidermal cells revealed virtually non-overlapping populations (Figure 2). Throughout this study, a FSC/SSC gate designed to encompass all epidermal CD3⁺CD1⁻ T lymphocytes (such as gate 2 in Figure 1c) was applied to unfractionated epidermal cells during acquisition (Figure 1e), resulting in greatly reduced (keratinocyte-born) background staining (Figure 1f; for comparison, Figure 1b). In addition, MAbs directed against CD3 and/or T cell receptor-$\alpha\beta$ were always included in each multi-color staining to assure analysis of epidermal T cells.

The proportion of CD3⁺ cells within the total number of epidermal cells ranged between 0.3 and 1.3% (mean, 0.7 ± 0.4%) in six independent experiments (Table 2). Thus, as expected, epidermal T lymphocytes were rare compared with the total number of epidermal cells. As most epidermal T lymphocytes reside between basal keratinocytes,[12] evaluating the proportion of epidermal T lymphocytes per linear or two-dimensional unit of basal epidermis may appear biologically more significant than expressing the size of the epidermal T lymphocyte population in relation to the total number of epidermal cells present in full-thickness epidermis. However, the primary goal of this study was not so much to quantitate but rather

Table 2. *Epidermal and Peripheral Blood T Lymphocyte Subsets as Defined by T Cell Receptor (TcR) Usage and Accessory Molecule Expression*

| Experiment | | % of total ECs | % of CD3⁺ cells | | % of TcR$\alpha\beta^+$ cells | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CD3⁺ | TcR$\alpha\beta^+$ | TcR$\gamma\delta^+$ | CD4⁺ CD8$\alpha^-$ | CD4⁻ CD8$\alpha^+$ | CD4⁻ CD8$\alpha\beta^+$ | CD4⁻ CD8$\alpha\alpha^+$ | CD4⁻ CD8⁻ | CD4⁺:CD8$\alpha\beta^+$ ratio |
| 1 | ECs | 0.4 | 97.6 | 2.4 | 77.8 | 17.3 | 9.9 | 8.2 | 5.2 | 8:1 |
| | PBLs | | | | 63.3 | 35.8 | 32.7 | 2.5 | 0.9 | 2:1 |
| 2 | ECs | 0.4 | 98.7 | 1.3 | 47.3 | 43.8 | ND | ND | 8.9 | ND |
| 3 | ECs | ND | 93.4 | 6.6 | 62.2 | 21.7 | ND | ND | 15.1 | ND |
| 4 | ECs | 0.3 | 96.2 | 3.8 | 49.8 | 46.7 | 24.7 | 13.4 | 3.5 | 2:1 |
| 5 | ECs | 1.0 | 98.6 | 1.4 | 58.8 | 34.1 | 11.0 | 21.8 | 7.1 | 6:1 |
| 6 | ECs | 0.8 | 99.3 | 0.7 | 73.1 | 13.4 | 6.2 | 7.2 | 13.5 | 12:1 |
| | PBLs | | | | 63.1 | 36.5 | 33.2 | 2.4 | 0.4 | 2:1 |
| 7 | ECs | 1.3 | 96.9 | 3.1 | 60.7 | 36.8 | 23.3 | 13.5 | 2.5 | 3:1 |
| 8 | PBLs | | | | 65.7 | 31.3 | 26.3 | 2.7 | 3.0 | 2:1 |

The percentage of positive cells was calculated as follows: numbers of cells expressing the phenotypes listed in the second line divided by numbers of cells (total cells, CD3⁺ cells, TcR$\alpha\beta^+$ cells, respectively) listed in the top line multiplied by 100. The frequency of CD3⁺ cells was determined in single (anti-CD3-FITC)-stained epidermal cell samples electronically gated to acquire 17,000 living cells. The frequencies of cells expressing the TcR/CD4/CD8-defined phenotypes were determined in triple-stained epidermal cell/peripheral blood mononuclear cell samples electronically gated to acquire cells within a FSC/SSC-defined gate (see also gate 2 in Figure 1e). Similar to what has been described for the CD4 and CD8$\alpha$ epitopes recognized by MAbs anti-Leu3a, -Leu3b[43] and anti-Leu2b (to a lesser degree also anti-Leu2a),[44] respectively, the CD4 and CD8$\alpha$ epitopes recognized by the MAbs used in this study were sensitive to trypsin treatment but restored to control levels after overnight incubation at 37°C (see also Materials and Methods). Anti-CD8$\beta$ MAb (2ST8-5H7) reactivity of trypsin-treated peripheral blood lymphocytes was quantitatively recovered to control levels after 37°C incubation. The staining intensity of trypsin-treated cells, however, remained lower than that of untreated peripheral blood lymphocytes. Thus, although this staining behavior was carefully taken into consideration when calculating the proportions of CD8$\alpha\beta^+$ and CD8$\alpha\alpha^+$ epidermal T cells, respectively, a discrete over-representation of CD8$\alpha\alpha$-homodimer-expressing cells cannot be excluded. ECs, epidermal cells; PBLs, peripheral blood lymphocytes; ND, not determined.

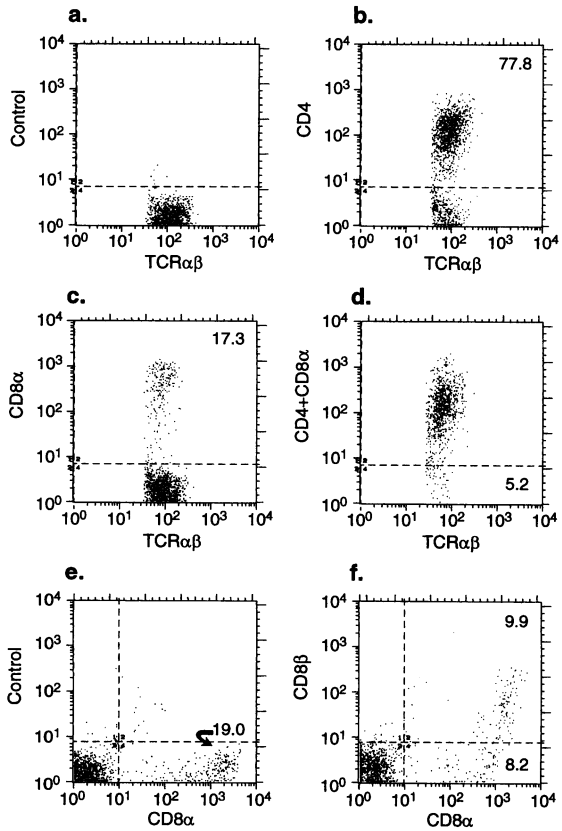


Figure 3. *CD4/CD8-defined epidermal αβ T lymphocyte subsets in sample 1 visualized by two-color* (a *to* d) *or three-color* (e *and* f) *flow cytometry. Analysis gates based on T cell receptor expression were applied throughout. MAbs were anti-TcRαβ-FITC (BMA031), anti-CD4-PE (MT310), anti-CD8α-PE (DK25), anti-CD8α-biotin (B9.11), and anti-CD8β (2ST8–5H7). Secondary reagents were streptavidin-Red 670 and goat F(ab')$_2$ anti-mouse Ig-PE.*

to phenotypically define the human epidermal T lymphocyte population, for which purpose multi-parameter flow cytometry of full-thickness epidermis appeared most appropriate.

## *Epidermal T Lymphocyte Subsets as Defined by T Cell Receptor Usage and Accessory Molecule Expression*

In agreement with previous immunohistological results,[12,25–27] multi-parameter (expression of CD3, T cell receptor-αβ, and T cell receptor-γδ, respectively) immunostaining of thorax-derived epidermal cell suspensions revealed that the majority of human epidermal T lymphocytes expressed the αβ T cell receptor whereas γδ T lymphocytes constituted between 0.7 and 6.6% of total epidermal T lymphocytes (Table 2). Epidermal αβ T lymphocytes were mostly single positive with a predominance of $CD4^+CD8\alpha^-$ over $CD4^-CD8\alpha^+$ cells (representative example shown in Figure 3, b and c) in five of seven samples and close to equal numbers of $CD4^+CD8\alpha^-$ and $CD4^-CD8\alpha^+$ cells, respectively, in samples 2 and 4 (Table 2). In samples 1 (Figure 3, e and f) and 4 to 7, $CD4^-CD8\alpha^+$ αβ T lymphocytes were further analyzed for co-expression of CD8β chains. In all of these samples, CD8αα homodimer (ie, $CD8\beta^-$)-expressing αβ T lymphocytes were detected at frequencies considerably higher and, vice versa, CD8αβ heterodimer$^+$ αβ T lymphocytes at frequencies considerably lower than those previously described for and confirmed in paired samples of peripheral blood (Table 2).[28,29] $CD8\alpha\alpha^+$ T lymphocytes constituted between one-third (samples 4 and 7) and one-half (samples 1 and 6) of the total of $CD8\alpha^+$ cells in four samples and were twice as frequent as $CD8\alpha\beta^+$ cells in sample 5 (Table 2). The so-called CD4:CD8 ratio (defined as the ratio of $CD4^+$ to $CD8\alpha\beta^+$ αβ T cells) in epidermal αβ T lymphocytes in all but one sample by far exceeded the ratio generally found in peripheral blood (Table 2).[28] The proportions of epidermal αβ T lymphocytes expressing a $CD4^-CD8^-$ (double-negative) phenotype,[30] 2.5 to 15.1%, were also larger than those generally found in adult peripheral blood (Table 2 and Figure 3d).[31,32] Epidermal γδ T lymphocytes were mostly double negative ($CD4^-CD8^-$), and cells expressing CD4 or CD8 were rare. Accurate quantification of CD4/CD8-defined γδ T lymphocyte subsets, however, was not possible due to the low frequencies of γδ T lymphocytes in all samples, 0.7 to 6.6% (Table 2). Thus, human epidermal T cells are distinguished from peripheral blood T lymphocytes within their αβ T lymphocyte subset with relatively larger proportions of cells expressing either CD4 or CD8α homodimers or a double-negative phenotype. In contrast, murine epidermal T lymphocytes are unique in their preferential T cell receptor-γδ usage.[33]

Immunohistological phenotyping of epidermal T lymphocytes from various body regions has demonstrated regional variability in the ratio of $CD4^+$ to $CD8\alpha^+$ epidermal T lymphocytes, with a predominance of $CD8\alpha^+$ cells at most sites and, consistent with this study, mostly $CD4^+$ αβ T lymphocytes in thorax epidermis.[12] Despite the lack of information regarding the relative proportions of $CD8\alpha\beta^+$ *versus* $CD8\alpha\alpha^+$ T lymphocytes at the various anatomical locations, these previous observations together with the results of this study suggest topographic differences in the functional capacities of this T lymphocyte population.

The biological role(s) and derivation of human $CD4^-CD8\alpha\alpha^+$ and $CD4^-CD8^-$ αβ T lymphocytes are unknown. However, both phenotypes have been associated with the recognition of nonclassical MHC

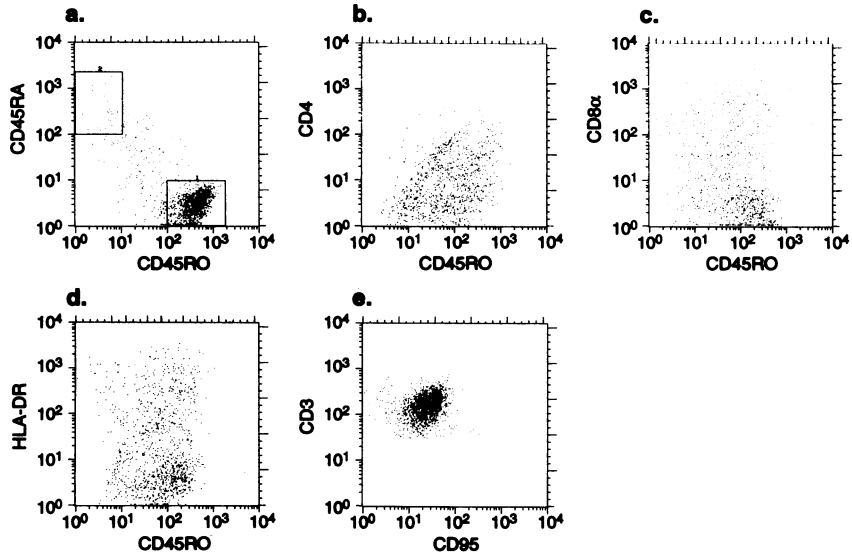


Figure 4. *CD45RO/RA and early-intermediate activation marker expression by epidermal T lymphocytes visualized by three-color* (a *to* d) *and two-color* (e) *flow cytometry. Analysis gates based on CD3 expression were applied throughout.* a *to* d: *MAbs were anti-CD3-biotin (UCHT1), anti-CD45RO (UCHL-1), anti-CD45RA-RD1PE (2H4), anti-CD4-PE (MT310), anti-CD8α-PE (DK25), and anti-HLA-DR-PE (L243). Gates in* a *define CD45RO$^{high}$ and CD45RA$^{high}$ T lymphocyte populations.* e: *MAbs were anti-CD3-PE (UCHT1) and anti-CD95, Fas (CH-11). Secondary reagents were streptavidin-Red 670, goat F(ab')$_2$ anti-mouse IgG-FITC, and goat F(ab')$_2$ anti-mouse IgM-FITC.*

ligands. Peripheral blood-derived double-negative $\alpha\beta$ T cell clones were shown to recognize monomorphic CD1 molecules as such or in association with bacterial products,[34–38] and the ligand(s) for CD8$\alpha\alpha^+$ $\alpha\beta$ T lymphocytes may also be unique.[29,39] Thus, the relatively high frequency of $\alpha\beta$ T lymphocytes of these phenotypes in human skin may reflect a relatively high incidence of nonclassical immune responses at these sites. Another line of evidence for a unique functional specialization of double-negative and possibly also CD8$\alpha\alpha^+$ $\alpha\beta$ T lymphocytes stems from analyses of the T cell receptors used by these cells in peripheral blood. Monoclonal or oligoclonal expansion was found in both T lymphocyte subsets[31,32,37,40–42] (J. Ritz, personal communication), suggesting that they respond to chronic stimulation of a small number of pathogens or autoantigens. Studies underway to characterize the T cell receptors used by the different human epidermal $\alpha\beta$ T lymphocyte subsets should provide insight into these hypotheses, in particular as it has been proposed that, although detectable in peripheral blood, double-negative and CD8$\alpha\alpha$ single-positive $\alpha\beta$ T lymphocytes are functionally active only at epithelial sites, such as skin.[17,18]

## *Most Epidermal T Lymphocytes Express a Memory Phenotype and Markers of Early-Intermediate Activation*

In all samples, the majority of epidermal T lymphocytes expressed a memory/effector phenotype as defined by the reciprocal expression of CD45RO and RA[45] (Figure 4a); 86.9 ± 5.9% (n = 8) of the T lymphocytes were CD45RO$^{high}$ and CD45RA$^-$ whereas naive/virgin epidermal T lymphocytes (defined as CD45RO$^-$/RA$^{high}$) constituted between 5 and 19% (Table 3). T lymphocytes expressing CD45RA/RO profiles indicative of a virgin-to-memory conversion were rare or absent (Figure 4a). Memory *versus* naive T lymphocytes were distributed among both CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ $\alpha\beta$ T lymphocytes (Figure 4, b and c). Only less than 2% of epidermal T lymphocytes expressed CD57, a marker of late immune responses (data not shown).[28,46] The shift from CD45RA$^{hi}$ to CD45RO$^{hi}$/RA$^-$ phenotype is, however, reversible, and consequently, all epidermal T cells expressing the CD45RA$^{hi}$ phenotype may not belong to the naive/virgin subset.[47,48] The expression of CD45RA$^{hi}$ in epidermis may reflect the presence of memory cells with different physiological characteristics. As suggested previously[12] and similar to what has been described for intraepithelial T lymphocytes in gut[49] and lung,[50] the predominance of memory over naive T lymphocytes in human epidermis must reflect the chronicity of antigenic exposures challenging this tissue. That a large proportion of epidermal T cells also showed signs of recent activation, such as expression of interleukin-2 receptor-$\alpha$ (p55, CD25), HLA-DR, and Fas CD95,[51,52] is consistent with such a concept (Figure 4, d and e; Table 3).

## *Human Epidermal T Lymphocytes Express Homing/Adhesion Molecules Related to Epithelial Sites*

Tissue-selective homing of lymphocytes is regulated in large part at the level of differential homing receptor expression by a given lymphocyte. The T lympho-

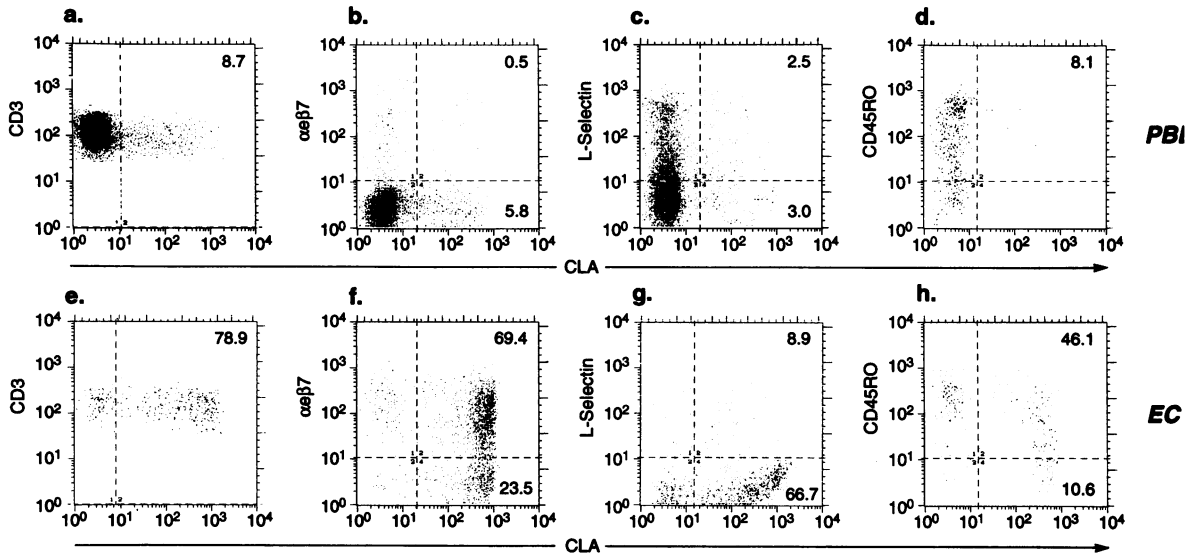


Figure 5. *Homing/adhesion molecule expression by peripheral blood T lymphocytes (PBL) and epidermal CD3$^+$ T cells (EC) visualized by three-color flow cytometry. mAb were anti-CD-PE or -biotin (UCHT1) developed with streptavidin-Red670™, anti-cutaneous lymphocyte antigen (CLA)-FITC (HECA-452), anti-integrin α$^e$β7 (BER-ACT-8) followed by goat F(ab)$^2$ anti-mouse Ig-PE, anti-L-selectin-PE (SK11) and anti-CD45RO-PE (UCHL-1). Two-color dot-blots were generated by gating on CD3$^+$ cells.*

cyte molecule responsible for skin-specific homing of T lymphocytes via interactions with endothelial E-selectin is the cutaneous lymphocyte-associated antigen (CLA).[15] Accordingly, most intraepidermal T lymphocytes in clinically normal-appearing skin (77.6 ± 9.9, n = 7) expressed CLA at high levels (Figure 5 and Table 3).[45,53] Only few epidermal T cells expressed the lymph node-homing receptor L-selectin (Figure 5g and Table 3). It should be noted, however, that the L-selectin epitope is trypsin sensitive (Table 1). Cells were therefore incubated overnight to allow re-expression of L-selectin after trypsin treatment. Re-expression of L-selectin occurred on peripheral blood T lymphocytes analyzed in parallel. It is not possible, however, to completely rule out the possibility that the procedure to prepare epidermal cells resulted in loss of L-selectin.

A candidate molecule to mediate retention of T lymphocytes within the epidermis is the $\alpha^e\beta_7$ integrin, which mediates adhesion of cultured intestinal intraepithelial T lymphocytes to epithelial cells through binding to E-cadherin.[54,55] The $\alpha^e\beta_7$ integrin is expressed by >95% of intestinal intraepithelial T lymphocytes and a large portion of pulmonary T lymphocytes but only a few percent of peripheral blood lymphocytes[56,57] (Figure 5b). Approximately 50% of epidermal T lymphocytes expressed $\alpha^e\beta_7$, and most $\alpha^e\beta_7{}^+$ T lymphocytes also expressed CLA (Figure 5f and Table 3). T lymphocytes expressing both CLA and $\alpha^e\beta_7$, however, were hardly detectable in peripheral blood (Figure 5b). Transforming growth factor-$\beta_1$ induces cell surface expression of $\alpha^e\beta_7$ on T lymphocytes *in vitro*.[58,59] Thus, it is conceivable that, once T lymphocytes have reached epidermis, keratinocyte-derived transforming growth factor-$\beta_1$ induces expression of $\alpha^e\beta_7$ and consequently causes retention of T lymphocytes via binding to E-cadherin within epidermis.[60]

The majority of T lymphocytes that migrate to the skin during experimental delayed-type hypersensitivity (influx 18 to 48 hours after antigenic challenge) express the memory/effector cell marker CD45RO as well as CLA.[45] CLA$^+$ T lymphocytes in peripheral blood are similarly CD45RO$^+$ (Figure 5d). The majority of CLA$^+$ T lymphocytes isolated from normal epidermis were also CD45RO$^+$ but CLA$^+$ CD45RO$^-$ cells were detected (Figure 5h). In contrast to T lymphocytes in normal epidermis, the majority of T lymphocytes in inflammatory skin blisters, furthermore, express L-selectin but lack $\alpha^e\beta_7$.[57] These differences might reflect tissue-selective up- and down-regulation of adhesion molecules locally in epidermis and/or that inflammation allows influx of T lymphocytes that normally do not reside within epidermis.

## Conclusion

Taken together, these data suggest that epidermal T lymphocytes share characteristics with other T lymphocytes in close proximity to epithelium. 1) The composition of αβ T lymphocyte subsets, based on CD4, CD8α, and CD8β expression, is different than

Table 3. *Activation and Homing/Adhesion Markers Expressed by Epidermal T Cells*

| | % of $CD3^+$ cells | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $CD45RO^{high}$ | $CD45RA^{high}$ | $CD25^+$ | $HLA\text{-}DR^+$ | $FAS^+$ | $CLA^+$ | $\alpha e\beta 7^+$ | L-$selectin^+$ |
| ECs | 86.9 ± 5.9<br>n = 8 | 14.2 ± 6.7<br>n = 4 | 26.1 ± 10.9<br>n = 6 | 50.6 ± 10.5<br>n = 8 | 84.0 ± 10.6<br>n = 4 | 77.6 ± 9.9<br>n = 7 | 51.4 ± 17.6<br>n = 4 | 11.5 ± 4.5<br>n = 4 |

a) EC; epidermal cells; b) number of samples tested; The percentage of positive cells was calculated as follows: numbers of cells expressing the antigens described in the second lines divided by the numbers of $CD3^+$ cells multiplied by 100. The numbers of cells were determined in double-stained (anti-CD3; MAbs directed against the antigens described in the second lines) epidermal cell samples electronically gated to acquire cells within a FSC/SSC-defined gate (see also gate 2 in Figure 1e). Values are expressed as mean ± SD. ECs, epidermal cells; n, number of samples tested.

has been found in peripheral blood. 2) The majority of epidermal T lymphocytes express a memory/effector phenotype. 3) Epidermal T lymphocytes express homing/adhesion molecules related to epithelial sites, eg, $\alpha^e\beta_7$ integrin.

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