Role of Integrin α_E(CD103)β_7 for Tissue-Specific Epidermal Localization of CD8$^+$ T Lymphocytes

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Tissue-specific T cell localization is crucial for immune surveillance of normal tissues and the pathogenesis of inflammatory disorders. In psoriatic skin, CD8$^+$ lymphocytes predominantly reside within the epidermis, whereas CD4$^+$ T cells are most abundant within the dermis. Molecular mechanisms guiding this spatial compartmentalization are not completely understood, however. Here, we demonstrate that 55% (±9.7%, n = 14) of the epidermal T cells, predominantly of the CD8$^+$ phenotype, expressed the integrin α_E(CD103)β_7. In contrast, only 5% (±2.0%) of the dermal T cells were α_E(CD103)β_7^+. Integrin α_E(CD103)β_7 was not detected in normal skin (n = 10), and less than 1% of peripheral blood lymphocytes derived from normal (n = 11) or psoriatic (n = 10) donors expressed α_E(CD103). When cultured T lymphoblasts (n = 12 donors) were stimulated with transforming growth factor β_1, expression of integrin α_E(CD103)β_7 was induced on 52.8% (±16.2%) of CD8$^+$ cells, but only on 6.1% (±2.3%) of CD4$^+$ cells, suggesting selective inducibility on CD8$^+$ lymphocytes. Whereas similar overall expression of transforming-growth-factor-β_1-specific mRNA was detected in normal and psoriatic skin by real-time quantitative polymerase chain reaction, immunohistochemistry revealed focal overexpression of transforming growth factor β_1 underneath psoriatic, but not normal, epidermis. This heterogenous transforming growth factor β_1 expression may contribute to induction of α_E(CD103) *in vivo*. Adhesion of transforming-growth-factor-β_1-stimulated CD8$^+$, but not CD4$^+$, T cells to cultured keratinocytes and psoriatic epidermis in frozen sections could be significantly inhibited by antibodies that blocked the α_E(CD103)/E–cadherin interaction. Co-culture of lymphoblasts and keratinocytes resulted in marginal enhancement of α_E(CD103)β_7 expression in some cases. Overall, integrin α_E(CD103)β_7 appears to contribute to tissue-specific epidermal localization of CD8$^+$ T lymphocytes. ***Key words: $\alpha_E\beta_7$/CD103/CD8$^+$ T cells/epidermotropism/integrin/psoriasis/TGF-β_1. J Invest Dermatol 117:569–575, 2001***

Spatial compartmentalization and tissue-specific localization of T lymphocytes are essential requirements for immune surveillance of normal skin and the pathogenesis of inflammatory diseases. Subpopulations of T cells localize to distinct cutaneous compartments, and the pattern of T cell distribution may be characteristic for certain disorders (Groves and Kupper, 1996). The tissue-specific localization of T cells is guided by differential expression of cytokines and chemokines and their receptors (Taub, 1996; Schön and Ruzicka, 2001). In addition, a complex array of adhesion molecules (Springer, 1994; Butcher and Picker, 1996) expressed by both T cells and the resident cutaneous tissue is thought to mediate T cell migration and retention (Nickoloff *et al*, 1990; Singer *et al*, 1990; Picker *et al*, 1993, 1994). Thus, the functional analysis of adhesion molecules, such as members of the integrin family (Hynes, 1992), has shed light on key pathogenic events during the development of inflammatory skin disorders (Singer *et al*, 1990). Members of the β_1, β_2, and β_7 subfamilies of integrins are expressed by T cells (Hemler, 1990; Springer, 1994). Among these, the β_7 integrins, including α_4(CD49d)β_7 and α_E(CD103)β_7, have a restricted expression pattern (Wagner *et al*, 1996). Integrin α_4(CD49d)β_7 is involved in T cell localization to organized lymphoid structures through binding to Mad-CAM (Berlin *et al*, 1993; Rott *et al*, 1996). In contrast, the α_E(CD103)β_7 integrin is expressed by more than 95% of intestinal intraepithelial T lymphocytes, more than 40% of lamina propria lymphocytes, and less than 2% of peripheral blood T lymphocytes (PBL) (Cerf-Bensussan *et al*, 1987; Kilshaw and Baker, 1988; Parker *et al*, 1992), and is thought to contribute to localization of diffusely distributed T cell subsets to the intestinal epithelium (Kilshaw, 1999), presumably through binding to E-cadherin (Cepek *et al*, 1994; Karecla *et al*, 1995, 1996; Higgins *et al*, 1998). Indeed, when integrin-α_E(CD103)-deficient mice were studied, they exhibited a reduced number of mucosal intraepithelial T cells (Schön *et al*, 1999). There is growing evidence, however, that the α_E(CD103)β_7 functions are not restricted to T cells within

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Abbreviations: AAB, adhesion assay buffer; CLA, cutaneous lymphocyte antigen; MACS, magnetic cell separation; PBL, peripheral blood lymphocytes.

the intestinal mucosa, but also affect intraepithelial T cells in other tissues whose epithelium expresses the ligand for $\alpha_E(CD103)\beta_7$, E-cadherin. In the skin, expression of $\alpha_E(CD103)\beta_7$ has been shown for epidermal T lymphocytes in some forms of inflammatory disorders (de Vries *et al*, 1997; Walton *et al*, 1997) and cutanous T cell lymphomas (Simonitsch *et al*, 1994; Dietz *et al*, 1996; Schechner *et al*, 1999). A functional role of $\alpha_E\beta_7$ for cutaneous T cell localization and/or retention, however, has not been demonstrated. In addition, mechanisms involved in regulation of $\alpha_E(CD103)\beta_7$ expression on epidermal T cells still remain unknown. *In vitro*, transforming growth factor β_1 (TGF-β_1) can maintain the $\alpha_E(CD103)\beta_7$ phenotype on intestinal intraepithelial T lymphocytes and induce expression of integrin $\alpha_E(CD103)\beta_7$ on PBL (Kilshaw and Murant, 1991; Parker *et al*, 1992; Cerwenka *et al*, 1994). It is currently not clear, however, whether TGF-β_1 contributes to the induction of $\alpha_E(CD103)\beta_7$ *in vivo*. To assess whether $\alpha_E(CD103)\beta_7$ is involved in tissue-specific localization of cutaneous T cell subpopulations, we have studied its expression in a common human skin disorder, psoriasis. In psoriatic lesions, $CD8^+$ T cells localize preferentially to the epidermal compartment, whereas $CD4^+$ T cells predominantly reside within the dermis (Hammar *et al*, 1984; Bos and de Rie, 1999). Here, we present data demonstrating a preferential expression of $\alpha_E(CD103)\beta_7$ on epidermal $CD8^+$ T cells within psoriatic lesions and show that $\alpha_E(CD103)\beta_7$ can be specifically upregulated by TGF-β_1 on $CD8^+$ T cells where it mediates adhesion to cultured keratinocytes as well as to psoriatic epidermis. In addition, our data suggest that focal upregulation of TGF-β_1 in psoriatic skin might be involved in the upregulation of $\alpha_E(CD103)\beta_7$ *in vivo*. T cell contact to cultured keratinocytes appears to play a limited, if any, role for induction of $\alpha_E(CD103)\beta_7$. Overall, our data suggest that the $\alpha_E(CD103)\beta_7$ integrin is involved in tissue-specific epidermal localization of $CD8^+$ T cells in psoriasis and that microenvironmental factors may induce its expression *in vivo*.

MATERIALS AND METHODS

Antibodies and secondary reagents Monoclonal antibodies (MoAb) against human antigens were as follows: anti-CD3ε (UCHT-1, Dako Diagnostica, Hamburg, Germany), anti-CD4 (MT310, Dako), anti-CD8α (DK25, Dako), anti-CD11a (SPV-L7, Zytomed, Berlin, Germany), anti-CD30 (Ber-H8, Pharmingen, San Diego, CA), anti-CD103 [Ber-Act8, Dako; αE7-1, αE7-2, αE7-3 (Russell *et al*, 1994), gifts from M.B. Brenner, Brigham and Womens' Hospital, Boston, MA; 2G5, Immunotech Marseille, France], anti-cutaneous lymphocyte antigen (anti-CLA) (HECA-452, Pharmingen), anti-E-cadherin [E4.6 (Cepek *et al*, 1994), gift from M.B. Brenner], anti-gp80 (BT15, Schön *et al*, 1995), anti-B-CAM (VF18, Schön *et al*, 2000), and anti-TGF-β_1 (TB21, Serotech, Oxford, U.K.). Nonbinding isotype-matched MoAb were also used as controls (mouse IgG1, MOPC 21, rat IgM, MOPC104E; Sigma, Deisenhofen, Germany). For two-color fluorescence-activated cell sorter (FACS) analysis, fluorochrome [fluorescein isothiocyanate (FITC) or R-phyroerythrin (RPE)] conjugates of the above MoAb were used. For immunohistochemistry, biotinylated goat antimouse serum (Vector, Burlingame, CA) was used as secondary reagent.

Isolation, culture, and stimulation of PBL PBL were isolated by Ficoll-Paque gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden) from buffy coats or heparinized blood of adult donors, followed by passage over nylon wool columns (Robbins Scientific, Sunnyvale, CA) for T cell enrichment. The resulting populations were generally more than 80% $CD3^+$ as determined by FACS analysis. The lymphocytes were then cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Biochrom, Berlin, Germany), 2 mM L-glutamine, 100 U per ml penicillin, 100 U per ml streptomycin, 0.25 U per ml amphotericin B, 15 mM HEPES, 10 mM nonessential amino acids (MEM), 10^{-5} M β-mercaptoethanol, 20 U per ml interleukin-2 (all from Gibco BRL, Karlsruhe, Germany), and 0.5–1 μg per ml phytohemagglutinin (PHA; Gibco BRL or Sigma). After 5–8 d of culture, the populations were generally more than 90% $CD3^+$ T cells. Cultured PHA lymphoblasts were then stimulated with 2 ng per ml of recombinant human TGF-β_1 (Gibco BRL) for 5–14 d to induce expression of the $\alpha_E(CD103)\beta_7$ integrin (Kilshaw and Murant, 1991; Parker *et al*, 1992). For isolation of $CD4^+$ and $CD8^+$ T cell subpopulations, TGF-β_1 stimulated PHA blasts were subjected to negative selection by magnetic cell separation (MACS; Miltenyi, Bergisch-Gladbach, Germany). The purity of the resulting cell populations was generally greater than 96% as determined by FACS analysis.

Keratinocyte culture Primary keratinocytes were prepared from surgical specimens of normal human skin as described previously (Schön *et al*, 1995) and propagated in serum-free keratinocyte growth medium (Gibco BRL) supplemented with 50 μg per ml bovine pituitary extract, 0.2 ng per ml epidermal growth factor (EGF), 2 mM L-glutamine, and 100 U per ml penicillin/streptomycin. The growth medium contained low concentrations of Ca^{2+} (0.09 mM) to prevent keratinocyte differentiation.

FACS analysis For FACS analysis, cells were washed twice with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin and 0.01% sodium azide (FACS buffer). Cells were then incubated with the primary MoAb at 1 μg per 10^6 cells in 200 μl FACS buffer for 15–20 min at 4°C. When unlabeled primary MoAb were used, cells were washed three times with FACS buffer, incubated with FITC- or RPE-coupled secondary reagents (DAKO) for 20 min at 4°C, and washed again three times. After the staining procedure, cells were fixed in 1% paraformaldehyde. Cell surface fluorescence was analyzed using a FACScan and the Cell Quest software (Becton Dickinson, Heidelberg, Germany).

Immunohistochemistry Immunohistochemical studies were performed on acetone-fixed serial cryostat-cut sections (5–7 μm) by the ABC immunoperoxidase method (Vector). Briefly, sections were incubated with 10% goat serum in PBS for 10 min followed by the primary MoAb at 10 μg per ml for 30 min. After three washes in PBS, endogenous peroxidase was blocked by 0.3% H_2O_2, and slides were washed three times again. Sections were then incubated with the secondary biotin-conjugated antibody (Vector) at 20 μg per ml for 30 min, washed three times again, and incubated with streptavidin-horseradish-peroxidase complex (Dako). Bound MoAb was visualized using 3-amino-9-ethyl-carbazole (Sigma) as chromagen. Sections were counterstained with Gill's hematoxylin (Sigma) and $LiCO_3$. For two-color immunohistochemistry, the sections were incubated with the unlabeled MoAb αE7-1 for 30 min at 4°C followed by FITC-conjugated goat antimouse Ig (Vector). Thereafter, the slides were washed three times and incubated with RPE-conjugated anti-CD8 or anti-CD4 MoAb.

Real-time quantitative polymerase chain reaction (PCR) (TaqMan) Biopsies from normal (n = 7 healthy volunteers; three female, four male; median age 38.6 y) and lesional psoriatic skin (n = 24 patients with chronic plaque psoriasis untreated for at least 2 wk; 10 female, 14 male; median age 36.2 y) were homogenized in liquid nitrogen using a Mikro-Dismembrator U (Braun Biotech, San Diego, CA), and RNA was extracted with RNAzol according to the manufacturer's protocol (Tel-Test, Friedensburg, TX). Four micrograms of RNA were treated with DNAseI (Boehringer Mannheim, Mannheim, Germany), and reverse transcribed using oligo-$dT14_{-18}$ (Gibco BRL) and random hexamer primers (Promega, Madison, WI). Fifty nanograms of the resulting cDNA were amplified in the presence of 12.5 μl of TaqMan Universal Master Mix (Perkin Elmer, Foster City, CA), 0.625 μl of hTGF-β_1-gene-specific TaqMan probe (Perkin Elmer), 0.5 μl of gene-specific forward and reverse primers (Perkin Elmer), and 0.5 μl of water. As an internal positive control, 0.125 μl of 18S ribosomal RNA specific TaqMan probe (Perkin Elmer) and 0.125 μl of 18S ribosomal RNA specific forward and reverse primer (Perkin Elmer) were added to each reaction. Gene-specific probes used FAM as reporter whereas probes for the internal positive control were associated with the VIC reporter. Samples underwent the following stages: (1) 50°C for 2 min; (2) 95°C for 10 min; and (3)–(43) 95°C for 15 s followed by 60°C for 1 min. Gene-specific PCR products were measured by means of an ABI Prism 7700 Sequence Detection System (Perkin Elmer) continuously during 40 cycles. Target gene expression was normalized between different samples based on the expression of the internal positive control.

Radiolabeling of activated T lymphoblasts and static adhesion assays Cultures of mixed lymphoblasts (>92% $CD3^+$ cells), MACS-enriched $CD4^+$ T cells, or MACS-enriched $CD8^+$ T cells (>96% pure) were metabolically labeled with ^{35}[S]-methionine (0.3 mCi per 10^7 cells) for 8 h. After three washes in adhesion assay buffer (AAB; PBS supplemented with 5% IgG-free FBS, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 15 mM HEPES), lymphoblasts were incubated with an anti-α_E(CD103)

MoAb (Ber-Act8), the nonbinding isotype-matched control MoAb, or the surface-binding anti-CD30 MoAb (Ber-H8) at 2 μg per 10^6 cells for 15 min. Normal human keratinocytes in first or second passage were grown to a confluency of approximately 70% in 96-well microtiter plates (Nunc, Roskilde, Denmark), rinsed twice with AAB, and incubated with the anti-E-cadherin MoAb E4.6, the isotype-matched nonbinding control MoAb, or the surface-binding anti-gp80 or anti-B-CAM MoAb at 3 μg per well for 15 min. After two washes in AAB, T cells were allowed to adhere to keratinocyte monolayers at 37°C for 45 min in a humidified incubator. Unbound cells were washed off in a standardized fashion, and remaining cells were lyzed using a 2% sodium dodecyl sulfate buffer. Bound radioactivity was quantitated with a liquid scintillation counter (Packard, Groningen, The Netherlands). Experiments were performed in sextuplicate.

Modified Stamper–Woodruff assays Air-dried, cryostat-cut frozen tissue sections of psoriatic human skin (n = 8 patients; three female, five male; median age 34.6 y; with chronic plaque psoriasis untreated for at least 2 wk) were equilibrated with AAB. Prior to adhesion, the tissue sections were incubated with the anti-E-cadherin MoAb (E4.6), the anti-gp80 MoAb (BT15), or the nonbinding isotype-matched control MoAb (MOPC 21), whereas mixed lymphoblasts, $CD4^+$ T cells, and $CD8^+$ T cells were incubated with an anti-α_E(CD103) MoAb (Ber-Act8), the anti-CD30 MoAb (Ber-H8), or the nonbinding isotype-matched control MoAb for 15 min, respectively. Sections were then overlayered with lymphocytes at a density of 5×10^5 cells per section and incubated at 37°C and 5% CO_2 for 45 min. Unbound cells were removed by gently dipping the slides into PBS in a standardized fashion. Thereafter, sections were fixed in 4% paraformaldehyde for 10 min and stained with hematoxylin. Lymphoblasts were quantitated microscopically as bound cells per 0.5 mm epidermis in tissues from three different donors in each experiment.

Co-culture experiments To assess the influence of keratinocytes on T cell expression of α_E(CD103), human keratinocytes in first or second passage were cultured in 12-well culture dishes to an estimated confluency of 70%. The culture supernatants were saved as conditioned medium. Co-culture experiments with T lymphocytes were performed as follows. (i) Keratinocyte monolayers were fixed by –20°C methanol for 15 min, washed extensively with PBS, and equilibrated with culture medium. Cultured or freshly isolated lymphoblasts were added at a density of 2×10^6 cells per ml in either RPMI 1640 lymphoblast growth medium supplemented with 3% FBS, conditioned medium supplemented with 3% FBS, 15 mM HEPES buffer, 10 mM MEM, 20 U per ml interleukin-2, and 10^{-5} M β-mercaptoethanol, or a 1:1 mixture of both media. (ii) Cultured or freshly isolated lymphoblasts were added to viable keratinocytes using the medium conditions described above. For comparison, parallel cultures were established without keratinocytes, in which the lymphoblasts were cultured in the presence of PHA, EGF, Mg^{2+} and Ca^{2+}, or TGF-β_1 under otherwise identical medium conditions. The proportion of $\alpha_E(CD103)\beta_7^+/CD3^+$, $\alpha_E(CD103)\beta_7^+/CD4^+$, $\alpha_E(CD103)\beta_7^+/CD8^+$ and $CLA^+/CD103^+$ lymphocytes was assessed by two-color FACS analysis after 0 d, 3–5 d and 7–8 d.

Statistical analysis Data were presented as means ± SD. Statistical significance was assessed by a two-tailed Student's t test, and $p < 0.05$ was considered significant.

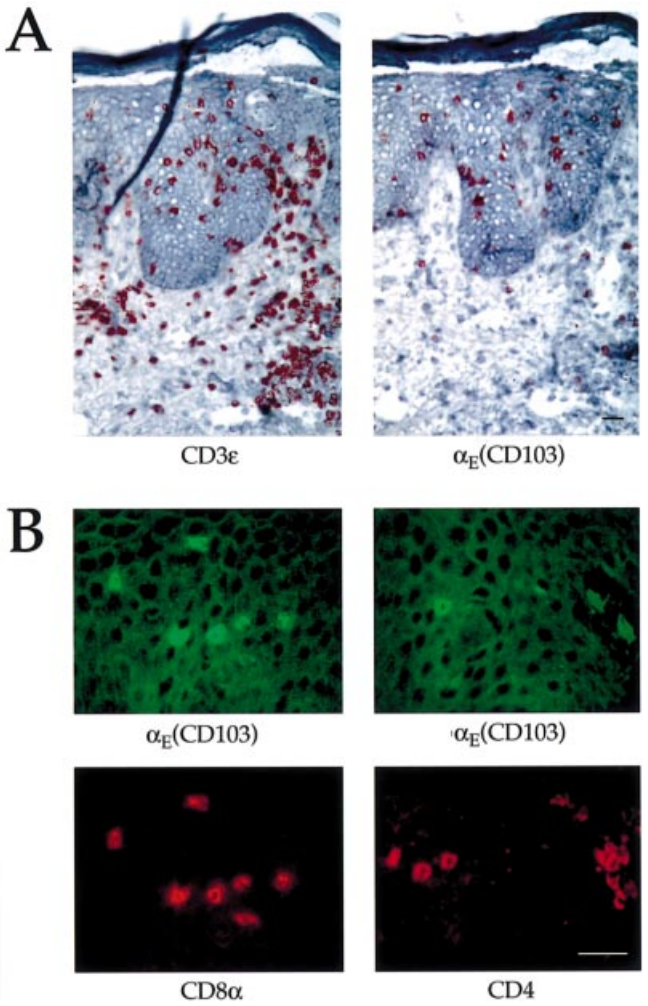

Figure 1. Integrin $\alpha_E(CD103)\beta_7$ is preferentially expressed by $CD8^+$ epidermal T cells in psoriatic skin. (*A*) Sequential cryostat-cut sections of psoriatic skin were stained by the ABC immunoperoxidase method using anti-CD3 (*left panel*) and anti-α_E(CD103) MoAb. The panels depicted are representative for tissues from 14 psoriasis patients. *Scale bar*: 20 μm. (*B*) Sequential frozen sections of psoriatic skin were subjected to two-color immunofluorescence staining using an FITC-conjugated anti-α_E(CD103) MoAb (green fluorescent signals), followed by PE-conjugated anti-CD4 (red signals) or PE-conjugated anti-CD8α (red signals) as indicated. Pictures were taken from the same microscopic fields using an FITC and a PE filter. The photomicrographs shown here are representative of tissue specimens from five different patients. *Scale bar*: 20 μm.

RESULTS

Integrin α_E(CD103) is preferentially expressed on epidermal $CD8^+$ T cells in psoriatic skin When the phenotype of T lymphocytes was assessed by immunohistochemistry in psoriatic lesions (n = 14), prominent expression of integrin $\alpha_E(CD103)\beta_7$ was found on T cells within all viable layers of the epidermis (**Fig 1*A***). Based upon quantitation of T cells in sequential cryostat-cut sections, 55.0% (±9.7%) of epidermal $CD3^+$ T cells but only 5.7% (±2.0%) of dermal T cells expressed α_E(CD103). In contrast, T cells were very rarely seen in the epidermis of normal skin (three out of 10 biopsies with one or two epidermal T cells).

To further characterize the T cell subset expressing the $\alpha_E(CD103)\beta_7$ integrin, two-color immunofluorescent stainings were performed on cryostat-cut sections of psoriatic skin (n = 5). It was found that $\alpha_E(CD103)\beta_7$ was primarily expressed by $CD8^+$ T cells whereas only few cells were of the $CD4^+/CD103^+$ phenotype (**Fig 1*B***). This observation is consistent with the preferential epidermal localization of $CD8^+$ T cells in psoriatic lesions (Hammar *et al*, 1984; Bos and de Rie, 1999) and the proposed association of α_E(CD103) with T cells of the CD8 lineage (Kilshaw and Murant, 1990; Lefrancois *et al*, 1994). As the ligand for $\alpha_E(CD103)\beta_7$, E-cadherin, is expressed throughout the viable epidermal cell layers (Furukawa *et al*, 1994), the expression of $\alpha_E(CD103)\beta_7$ by epidermal $CD8^+$ T cells suggested a role of this adhesion receptor for tissue-specific epidermal localization or retention of $CD8^+$ T cells. Few dermal $CD8^+$ T cells also expressed $\alpha_E(CD103)\beta_7$ (estimated 5%–10% based upon two-color immunofluorescence).

Given the prominent expression of $\alpha_E(CD103)\beta_7$ in psoriatic but not normal skin, we assessed its expression on freshly isolated T cells of the peripheral blood (PBL) from healthy volunteers (n = 12) and psoriasis patients (n = 9). As expected (Rott *et al*, 1996), FACS analysis revealed expression of α_E(CD103) on 0.86% (±0.26%) of $CD3^+$ PBL from normal donors. Interestingly, there was no increase in $\alpha_E(CD103)\beta_7$ expression on PBL isolated from

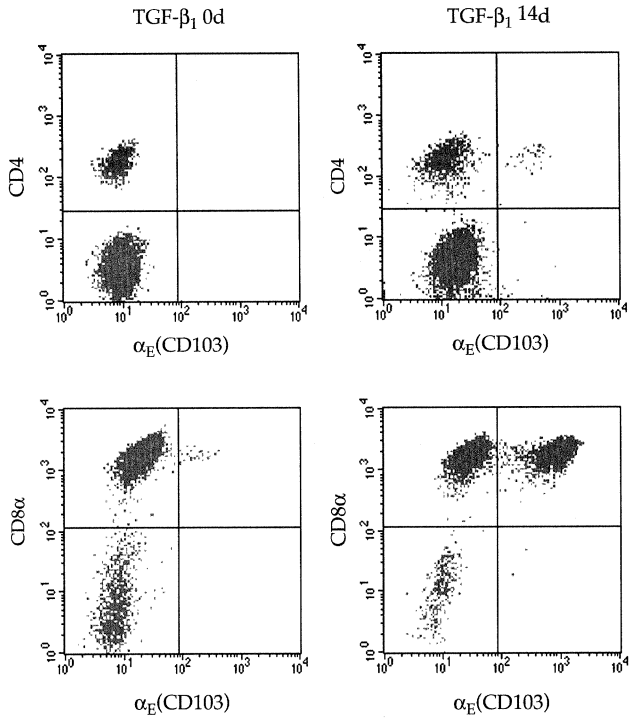

Figure 2. Integrin $\alpha_E\beta_7$ can be selectively induced on activated $CD8^+$ T lymphocytes by TGF-β_1 *in vitro*. PBL were cultured in the presence of PHA (0,5 μg per ml) and interleukin-2 (20 U per ml) for 10 d. Thereafter, TGF-β_1 (2 ng per ml, *right panels*) or control medium (*left panels*) was added for 14 d, and the cells were subjected to two-color FACS analysis as outlined in *Materials* and *Methods*. The subset distribution was assessed by staining with CD4-RPE/CD103-FITC (*upper row*) or CD8-RPE/CD103-FITC (*lower row*), respectively. Double-positive cells are located in the upper right quadrant (0.2% in *upper left panel*; 1.8% in *lower left panel*; 4.9% in *upper right panel*; 48.7% in *lower right panel*).

psoriasis patients (0.51%; SD ±0.18%). This observation is consistent with the hypothesis that integrin $\alpha_E(CD103)\beta_7$ expression is induced *in situ* in psoriatic lesions.

Integrin $\alpha_E(CD103)\beta_7$ is selectively induced on $CD8^+$ T cells *in vitro* *In vitro*, the expression of integrin $\alpha_E(CD103)\beta_7$ on PBL can be induced by TGF-β_1 (Kilshaw and Murant, 1991; Cepek *et al*, 1993; Russell *et al*, 1994) or, as reported in one study, by culture with PHA and interleukin-2 (Brew *et al*, 1995). In this study, experiments were performed with lymphoblasts from more than 30 different blood donors. Culture in the presence of PHA and interleukin-2 alone resulted in the induction of $\alpha_E(CD103)\beta_7$ expression by up to 10% of cultured T cells from six donors. This phenotype, however, was observed only in the first week of culture and was not maintained during long-term culture (data not shown). In contrast, $\alpha_E(CD103)\beta_7$ expression on T lymphoblasts was strongly upregulated and maintained by incubation of PHA-activated T lymphocytes with 2 ng per ml TGF-β_1. Interestingly, two-color FACS analyses (n = 12 donors) revealed that 52.8% (± 16.2%) of the $CD8^+$ T cells but only 6.1% (± 2.3%) of $CD4^+$ T cells expressed integrin $\alpha_E(CD103)\beta_7$ (**Fig 2**). Maximum expression of $\alpha_E(CD103)$ was seen after 5–14 d of TGF-β_1 treatment, suggesting interindividual differences in the TGF-β_1 responsiveness of T lymphocytes (**Fig 2**). PBL isolated from psoriasis patients (n = 7 donors) were similarly responsive to TGF-β_1 ($CD8^+/CD103^+$ 40.5% ± 8.8% *vs* $CD4^+/CD103^+$ 7.6% ± 4.6%).

Given that TGF-β_1 has been reported to upregulate expression of CLA, which is thought to act as a skin-specific homing receptor of T cells (mostly $CD4^+$) (Picker *et al*, 1991), we assessed whether $\alpha_E(CD103)$ and CLA might be coexpressed by some T cells. CLA was expressed by 8.2% (±3.1%) of freshly isolated PBL from psoriasis patients (n = 8) and by 8.0% (±3.4%) of PBL from healthy donors (n = 9), suggesting that there are no significant differences in CLA expression between normal and psoriatic individuals *in vivo*. As expected (Picker *et al*, 1994), CLA was primarily expressed by $CD4^+$ T cells. As assessed by two-color FACS analysis, only 0.3% (±0.1%, n = 9) of PBL from healthy donors and 0.2% (±0.1%) of PBL from psoriasis donors (n = 8) coexpressed integrin $\alpha_E(CD103)$ and CLA.

In contrast to the strong *in vitro* induction of $\alpha_E(CD103)\beta_7$, only 6.5% (±2.7%) of T lymphoblasts from psoriasis patients (n = 4) and 8.5% (±4.6%) of T lymphoblasts from healthy donors (n = 9) expressed CLA when cultured in the presence of 2 ng per ml TGF-β_1 for 14 d. In addition, as assessed by two-color FACS analysis, TGF-β_1 did not significantly increase the proportion of T lymphoblasts coexpressing $\alpha_E(CD103)\beta_7$ and CLA [1.08% (±0.39%) for psoriatic donors (n = 4) *versus* 0.67% (±0.8%) for healthy donors (n = 9)], suggesting that expression of $\alpha_E(CD103)$ and of CLA on T cells are regulated differentially.

Integrin $\alpha_E(CD103)\beta_7$ mediates adhesion of activated $CD8^+$ T cells, but not $CD4^+$ T cells, to cultured human keratinocytes In order to assess the functional relevance of $\alpha_E(CD103)\beta_7$, PHA-, and TGF-β_1- stimulated, unfractionated T lymphoblasts (>90% $CD3^+$), the $CD3^+/CD4^+$ subset (>98% pure) and the $CD3^+/CD8^+$ subset (>96% pure) were metabolically labeled with ^{35}[S]-methionine and allowed to adhere to a matrix of cultured normal human keratinocytes. Based upon quantitation of bound radioactivity in three independent experiments, all three lymphoblast populations strongly adhered to the monolayer of cultured keratinocytes. In a representative experiment performed in sextuplicate, blocking of integrin $\alpha_E(CD103)$ by MoAb Ber-Act8 reduced the binding of mixed T lymphoblasts [$\alpha_E(CD103)^+/CD8^+$ 39.55%] by 42.7% [±3.4%, p = 0.0005 comparing adhesion of T cells after incubation with isotype control MoAb and anti-$\alpha_E(CD103)$], and the adhesion of the $CD8^+$ T cells subset by 25.9% (±4.2%, p = 0.01) (**Fig 3*A***). Similarly, the anti-E-cadherin MoAb, E4.6, reduced binding of mixed lymphocytes by 35.4% (±5.1%, p = 0.002) and binding of the $CD3^+/CD8^+$ subpopulation by 17.1% (±11%, p = 0.01), compared to the isotype-treated controls. When both $\alpha_E(CD103)$ and E-cadherin-specific MoAb were used, adhesion of mixed lymphoblasts was reduced by 43.42% (±1.34%, p = 0.0003) and adhesion of the $CD8^+$ subset was reduced by 28.9% (±7.9%, p = 0.002 compared to isotype controls). In contrast, adhesion of $CD4^+$ T cells was not affected significantly by either anti-$\alpha_E(CD103)$ or anti-E-cadherin (**Fig 3*A***). These results were confirmed in two independently performed subsequent experiments showing similar results. As the surface-binding control MoAb, BT15, VF18, or Ber-H8, did not significantly affect T cell binding to cultured keratinocytes (data not shown), the inhibition of binding by both anti-α_E- and anti-E-cadherin MoAb was not due to unspecific effects following surface binding of MoAb. Of note, the relative inhibition of cellular adhesion appeared to correlate with the proportion of $\alpha_E(CD103)^+$ cells in the cultures. These results suggested that $\alpha_E(CD103)$ is important for adhesive interactions of $CD8^+$ T cells and keratinocytes. More than 90% of TGF-β_1-stimulated T lymphoblasts expressed LFA-1, however, and incubation with anti-LFA-1 MoAb almost completely (84% in one experiment and 91% in a confirmatory experiment) inhibited binding of these cells to cultured keratinocytes. MoAb specific for $\alpha_E(CD103)$ did not significantly increase this effect.

Integrin $\alpha_E(CD103)\beta_7$ mediates adhesion of $CD8^+$ T cells to psoriatic epidermis To directly assess the role of $\alpha_E(CD103)\beta_7$ in adhesive interactions between T lymphocytes and psoriatic epidermis, modified Stamper–Woodruff assays were performed. In these experiments, TGF-β_1-stimulated unfractionated lymphoblasts (>90% $CD3^+$), $CD8^+$ T cells (>96% $CD3^+/CD8^+$), or $CD4^+$ T

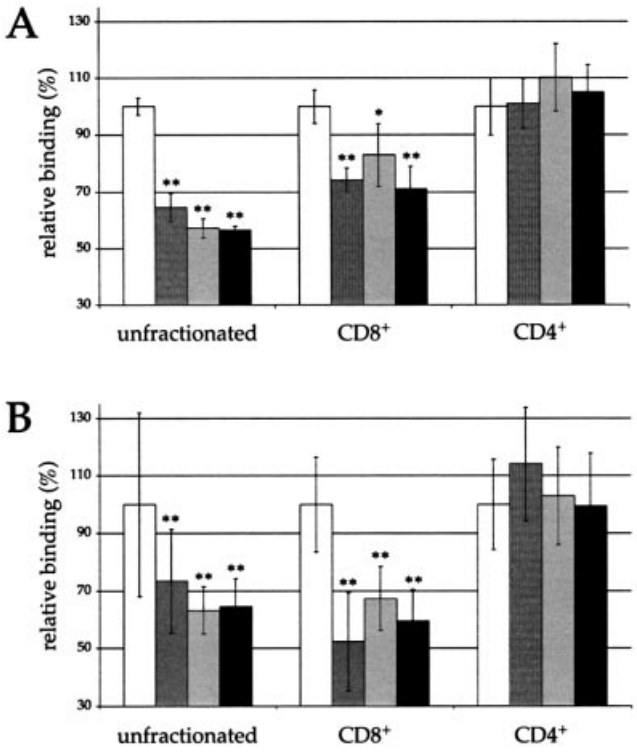

Figure 3. Integrin α_E(CD103)β_7 and E-cadherin mediate binding of activated CD8$^+$ T lymphoblasts to cultured keratinocytes and to psoriatic epidermis. (*A*) After stimulation with 2 ng per ml TGF-β_1 for 14 d, unfractionated lymphocytes (>90% CD3$^+$, *left panel*), purified CD3$^+$/CD8$^+$ (>96% pure, *middle panel*), or purified CD3$^+$/CD4$^+$ (>98% pure, *right panel*) lymphoblasts were radiolabeled with ^{35}[S]-methionine. Thereafter, T lymphoblasts were overlayered onto subconfluent cultures of human keratinocytes in 96-well plates. For antibody-mediated blocking, the T lymphoblasts and keratinocytes were incubated with isotype-matched control MoAb (*left columns*), anti-α_E(CD103) MoAb (second from left), anti-E-cadherin MoAb (third from left), or a combination of anti-α_E- and anti-E-cadherin MoAb (*right columns*). The lymphoblasts then adhered for 45 min in the presence of 1 mM $CaCl_2$ and 2 mM $MgCl_2$. Bound cells were lyzed in a 2% sodium dodecyl sulfate buffer, and bound radioactivity was quantitated. *Bars* represent the means (±SD) of sextuplicates. *$p < 0.05$; **$p < 0.01$ comparing adhesion after MoAb treatment with isotype controls. The experiment shown is representative for three independent experiments showing similar results. (*B*) T lymphoblasts were separated and labeled as outlined above. Cryostat-cut frozen sections of psoriatic skin were rehydrated in AAB containing 1 mM $CaCl_2$ and 2 mM $MgCl_2$ and, after incubation with isotype control MoAb (*left columns*), anti-α_E(CD103) MoAb (second from left), anti-E-cadherin MoAb (third from left), or a combination of anti-α_E(CD103) and anti-E-cadherin MoAb (*right columns*), overlayered with T lymphoblasts for 45 min as described in (*A*). Bound cells were quantitated per millimeter DEJ in at least 16 microscopic fields. *Bars* represent mean values (±SD), **$p < 0.01$ comparing adhesion of MoAb-treated samples and isotype controls. The experiment shown is representative for eight different psoriatic tissues and five different blood donors.

cells (>98% CD3$^+$ $n = 5$ blood donors in each case) were allowed to adhere to unfixed cryostat-cut frozen sections of psoriatic skin ($n = 8$ donors). All T lymphocyte populations examined adhered well to the epidermal compartment of the skin. In a representative experiment with T lymphoblasts expressing α_E(CD103) on 41.6% of CD8$^+$ T cells, MoAb Ber-Act8 reduced the adhesion of unfractionated lymphoblasts significantly by 26.5% (±18.0%, $p < 0.0001$ compared to the isotype-matched control), and adhesion of the CD8$^+$ enriched population was inhibited by 47.7% (±17.3%, $p < 0.0001$ comparing binding inhibition of CD8$^+$ and isotype control MoAb) (**Fig 3*B***). Likewise, preincubation of the skin sections with the E-cadherin-specific MoAb E4.6 reduced the epidermal binding of unfractionated T cells by 36.7% (±8.2%, $p < 0.0001$) and binding of purified CD8$^+$ T cells by 32.6% (±10.4%, $p < 0.0001$). Simultanous incubation with both MoAb did not increase the inhibitory effect (unfractionated lymphoblasts, 35.4% ± 9.8%, $p < 0.0001$; CD8$^+$ T cells, 11.19% ± 1.2%, $p < 0.0001$, compared to the isotype control). Epidermal adhesion of TGF-β_1-stimulated CD4$^+$ T cells, however, was not negatively affected by either MoAb (**Fig 3*B***). Incubation with the surface-binding MoAb, BT15, VF18, or Ber-H8, did not significantly affect epidermal T cell binding (not shown). In addition, T lymphoblast binding to dermal components was not affected by either anti-α_E(CD103) or anti-E-cadherin MoAb (data not shown). Similar results were obtained in three independent experiments strongly suggesting a role of both α_E(CD103) and E-cadherin in adhesive interactions of some CD8$^+$ T cells and the psoriatic epidermis.

Focal overexpression of TGF-β_1 in psoriatic skin How is α_E(CD103)β_7 induced on T lymphocytes localizing to the epidermis? To approach this as yet unanswered question, we assessed expression of TGF-β_1 in psoriatic and normal skin to estimate its potential role for the induction of α_E(CD103)β_7 *in vivo*. Thus far, TGF-β_1 is the only known stimulator for integrin α_E(CD103)β_7 expression on T lymphocytes (Kilshaw and Murant, 1991; Cepek *et al*, 1993). Real-time PCR (TaqMan) was performed to quantitatively assess TGF-β_1 mRNA expression in normal ($n = 7$) and psoriatic ($n = 24$) skin. Both groups showed considerable interindividual variation, but the average expression of TGF-β_1 mRNA was not significantly different [159 fg per 100 ng cDNA (±57.4) in normal *versus* 133 fg per 100 ng cDNA (±72.3) in psoriatic skin] (**Fig 4*A***). These results suggested that the induction of α_E(CD103)β_7 on epidermal T cells within psoriatic lesions was not due to a general increase of TGF-β_1 expression.

Interestingly, however, immunohistochemical analysis revealed a marked focal expression of total TGF-β_1 in psoriatic skin in some dermal papillae directly underneath the hyperplastic epidermis. This was not seen in normal skin (**Fig 4*B***). To assess the potential colocalization of α_E(CD103)β_7-expressing T cells and TGF-β_1, immunohistochemical studies were performed using sequential sections of psoriatic tissue ($n = 7$). As TGF-β_1 was located in the dermis, however, and α_E(CD103)β_7-expressing cells resided within the epidermis, a clear-cut demonstration of such a colocalization was not possible. Overall, it is conceivable that focal overexpression of TGF-β_1 contributes to the induction of α_E(CD103)β_7 on T lymphocytes approaching the epidermis within the psoriatic microenvironment.

A limited role of T cell–keratinocyte interactions for induction of α_E(CD103)β_7 As α_E(CD103) expression was almost exclusively seen on intraepidermal T cells in psoriatic epidermis, we sought to approach the question whether epidermis-associated factors might contribute to α_E(CD103) expression by T lymphocytes. To assess such putative additional factors, unfractionated T lymphoblasts were co-cultured with epidermal keratinocytes under various conditions as outlined in *Materials and Methods*. In four initial experiments, it appeared that co-culture of PHA blasts with viable keratinocytes induced α_E(CD103) expression marginally on approximately 5% of T cells, superimposing on the induction achieved by TGF-β_1. We therefore examined several conditions including co-culture with fixed keratinocytes, co-culture with or without keratinocyte-conditioned medium, co-culture in the presence of TGF-β_1-neutralizing MoAb, and combinations of these conditions. It was found that the additional induction of α_E(CD103) by cultured normal keratinocytes under the conditions tested was not consistent and appeared to have considerable interindividual variations (data not shown). These experiments do not formally rule out the possibility, however, that keratinocytes contribute to α_E(CD103) expression *in vivo*.

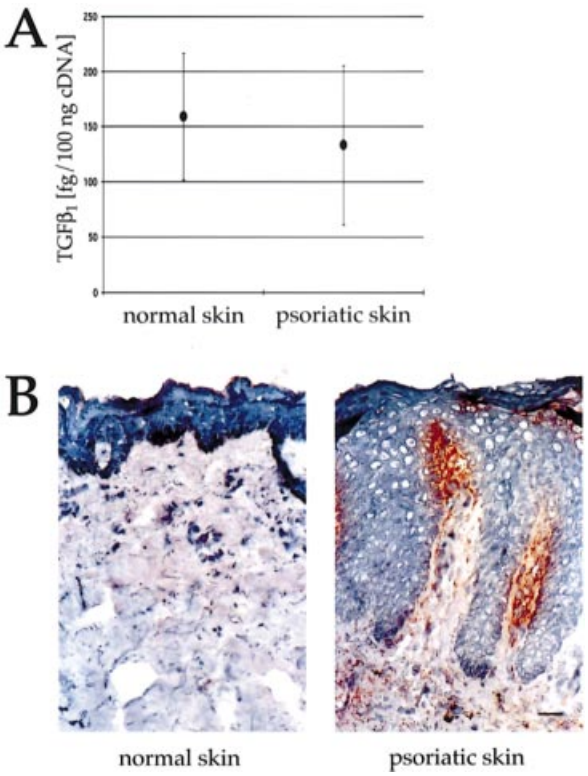

Figure 4. TGF-β_1 is focally overexpressed in psoriatic skin, whereas total amounts of TGF-β_1 mRNA are similar in psoriatic and normal skin. (*A*) Total RNA was extracted from skin biopsies obtained from psoriatic lesions (n = 24) and normal skin (n = 7), reverse transcribed, and analyzed using quantitative real time PCR (TaqMan®). The data were normalized against the internal control and presented as fg TGF-β_1-specific probe per 100 ng cDNA. (*B*) Frozen sections of normal (*left panel*) and psoriatic (*right panel*) skin were stained by the ABC immunoperoxidase method using a TGF-β_1-specific MoAb. Positive signals are visualized by the red color. *Scale bar*: 20 μm. The photomicrographs shown are representative of six different tissue specimens each from normal and psoriatic skin.

DISCUSSION

Given the importance of selective therapies, molecular mechanisms guiding tissue-specific compartmentalization of T cells in inflammatory disorders are a major research focus. We have identified the integrin α_E(CD103)β_7 as a receptor mediating adhesive interactions between CD8$^+$ T lymphocytes and epidermal keratinocytes, thus presumably contributing to epidermal localization and/or retention of a specific T cell subset. The first steps of T cell localization, including rolling, firm adhesion, extravasation, and transmigration through connective tissues, are relatively well understood (Groves and Kupper, 1996). Leukocyte rolling on the vessel wall is mediated primarily by selectins, including P-, E-, and L-selectin (Groves *et al*, 1991; Smith *et al*, 1993). Glycoproteins bearing the Sialyl-Lewis^X moiety function as E-selectin ligands (Varki, 1994), including CLA, which is thought to be involved in tissue-specific localization of cutaneous T cells (Picker *et al*, 1991). Rolling leukocytes then firmly attach and transmigrate using β_2- and β_1-integrins (Dustin *et al*, 1986; Hemler, 1990; Hynes, 1992), and CD44, a hyaluronate receptor (Camp *et al*, 1993).

In contrast to endothelial and dermal localization, we know little about epidermal localization of T cells. Many ligands for T cell adhesion receptors, such as β_1-integrin ligands, are not expressed beyond the epidermal basement membrane (Konter *et al*, 1989). Induced by proinflammatory cytokines, there is *de novo* expression of intercellular adhesion molecule 1 (ICAM-1) in inflamed epidermis (Dustin *et al*, 1988; Griffiths *et al*, 1989). Indeed, *in vitro* studies suggested that ICAM-1–LFA-1 interactions mediate binding of activated T cells to inflamed epidermis (Kashihara-Sawami and Norris, 1992). This may not be the only mechanism, however, as constitutive epidermal expression of ICAM-1 in transgenic mice did not result in cutaneous T cell infiltration (Williams and Kupper, 1994), and expression and spatial distribution of ICAM-1 and LFA-1 do not correlate in many cases (Griffiths *et al*, 1989; Konter *et al*, 1989; Olivry *et al*, 1995). The recently identified glycoprotein LEEP-CAM (lymphocyte endothelial–epithelial cell adhesion molecule), whose ligand on T has not been identified yet, may also be involved in epidermal T cell localization (Shieh *et al*, 1999). Overall, molecular mechanisms guiding epidermal localization of T lymphocytes still remain somewhat obscure. In this setting, the integrin α_E(CD103)β_7 is an interesting candidate molecule mediating epidermal localization of a particular T cell subset, namely CD8$^+$ T cells, possibly complementing other mechanisms. As epidermal CD8$^+$ T cells are thought to play a pathogenic role in common inflammatory skin disorders, such as psoriasis (Hammar *et al*, 1984; Bos and de Rie, 1999; Prinz, 1999), it is conceivable that compounds interfering with the α_E(CD103)β_7 function may specifically alleviate such disorders. Indeed, expression of α_E(CD103)β_7 has been described in several inflammatory skin conditions, such as lichen planus and atopic dermatitis (de Vries *et al*, 1997; Walton *et al*, 1997), as well as in some cutaneous T cell lymphomas (Simonitsch *et al*, 1994; Dietz *et al*, 1996; Schechner *et al*, 1999). CLA and integrin α_E(CD103)β_7 may not act synergistically on a given T cell subset, as these adhesion receptors were not coexpressed by most T cells in our experiments.

Expression of α_E(CD103)β_7 was detected on very few dermal T cells in psoriatic lesions as well as in the peripheral blood, which is consistent with previous reports (Cerf-Bensussan *et al*, 1987; Parker *et al*, 1992). Thus, α_E(CD103)β_7 appears to be induced on CD8$^+$ T cells *in situ* upon entering the epidermis, consistent with the focal expression of TGF-β_1 directly underneath the epidermis. There is no experimental proof thus far, however, directly demonstrating such a role of TGF-β_1 *in vivo*. Although direct interactions of T cells and keratinocytes resulted only in marginal induction of α_E(CD103)β_7 on some T cells *in vitro*, it cannot be formally ruled out that such mechanisms or other keratinocyte-derived factors may contribute to α_E induction *in vivo*. In addition, it is possible that α_E(CD103)β_7 also mediates adhesion to other as yet unidentified ligands within the epidermis, as one recent study suggested an alternative ligand for α_E(CD103)β_7 within the epidermis (Brown *et al*, 1999). Given that the α_E(CD103)β_7 ligand, E-cadherin, is constitutively expressed throughout all viable layers of the epidermis (Furukawa *et al*, 1994), it is possible that the α_E(CD103)β_7–E-cadherin interaction is regulated via alternate functional states of E-cadherin (Higgins *et al*, 1998) and/or on the level of α_E(CD103)β_7 itself. In any case, based upon our observation that TGF-β_1 is focally upregulated within psoriatic skin, it is possible that this heterogenous expression contributes to site-specific induction and function of α_E(CD103)β_7 in the cutaneous microenvironment *in vivo*, similar to the intestinal mucosal microenvironment (Kilshaw, 1999). Consistent with previous circumstantial evidence that α_E(CD103) expression is associated with T cells of the CD8$^+$ lineage (Kilshaw and Murant, 1990; Lefrancois *et al*, 1994), our experiments demonstrated a selective inducibility of α_E(CD103)β_7 on CD8$^+$ T cells. This mechanism may, at least in part, contribute to the spatial compartmentalization of T cells in psoriasis. Expression of α_E(CD103)β_7 may explain (at least in part) adhesion but not necessarily directed migration of this T cell subset to the epidermis. The α_E(CD103)-related action of TGF-β_1 on epidermis-entering T cells may be 2-fold: on the one hand, TGF-β_1 might modulate the preferential association of the α_E and β_7 subunits by down-regulating α_4(CD49d)β_7 (Lim *et al*, 1998); on the other hand, TGF-β_1-responsive elements have been identified in the 5′ proximal promoter regions of both the β_7 and α_E encoding gene regions (Lim *et al*, 1998).

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