

Nonspecific T-cell homing during inflammation in atopic dermatitis: Expression of cutaneous lymphocyte-associated antigen and integrin $\alpha\text{E}\beta 7$ on skin-infiltrating T cells

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Atopic dermatitis (AD) is a chronic skin disorder, characterized by infiltration of activated memory CD4^+ T cells into skin. A model to study the onset of allergic inflammation in a patient with AD is the atopy patch test (APT), in which, by epicutaneous application of aeroallergen, an eczematous reaction is induced in 50% of sensitized patients with AD. Extravasation of T cells into skin is thought to be critically dependent on expression of the surface molecule cutaneous lymphocyte-associated antigen (CLA), which recognizes and binds its ligand E-selectin on endothelium. We studied the dynamics of expression of CLA and the gut homing receptor $\alpha\text{E}\beta 7$ (HML-1) on T cells in the skin of patients with AD and in APT reactions and nickel and sodium lauryl sulfate patch test reactions by means of immunohistochemical double staining of skin biopsy specimens. The results show an increase in the number of CD3^+ T cells in the lesional skin of patients with AD, APT reactions, and nickel and sodium lauryl sulfate patch test reactions as compared with non-lesional skin of the same patients and nonatopic individuals. In contrast, the percentages of CLA^+ T cells in the lesional skin of patients with AD, in the APT reactions, and in sodium lauryl sulfate and nickel patch test reactions were decreased. In addition, we found a marked expression of $\alpha\text{E}\beta 7$ by T cells present in skin, indicating a nonspecific influx of T cells during allergic skin inflammation. We propose that during allergic skin inflammation CLA expression is not a prerequisite for cutaneous T-cell infiltration. CLA expression may be important for T cells to extravasate from blood into skin during immune surveillance or for retention of allergen-specific T cells in skin. (*J Allergy Clin Immunol* 1997;100:694-701.)

Key words: Atopic dermatitis, contact dermatitis, irritant reaction, cutaneous lymphocyte-associated antigen, atopy patch test, lymphocyte extravasation, cutaneous inflammation, T-cell homing

Abbreviations used

AD:	Atopic dermatitis
APT:	Atopy patch test
CLA:	Cutaneous lymphocyte-associated antigen
mAb:	Monoclonal antibody
NHoS:	Normal horse serum
NHuS:	Normal human serum
NRS:	Normal rabbit serum
PBMCs:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
SLS:	Sodium lauryl sulfate
TGF:	Transforming growth factor

Atopic dermatitis (AD) is a chronic, inflammatory skin disorder with an important allergic component. Together with allergic asthma and rhinitis, it is one of the major clinical manifestations of the atopy syndrome. The skin lesions result from an allergic skin response to foreign proteins, which is often aggravated by bacterial superinfections.¹ Histologic examination of lesional skin of patients with AD reveals a dermal infiltrate consisting mainly of activated (HLA-DR^+), memory (CD45RO^+), and helper (CD4^+) T cells and monocytes.²

To respond adequately to foreign antigens that affect the integrity of the body, the immune system has developed an elaborate system for the direction of immune cells to effector sites and different lymphoid compartments. Under physiologic flow conditions, leukocytes interact with endothelial cells through constitutively functional (i.e., activation-independent) leukocyte homing receptors and their ligands.³ This selectin-based initial adhesion, however, is unstable and not sufficient for extravasation. Functional activation of integrins mediated by locally produced activation factors and chemoattractants leads to firm attachment and subsequently to extravasation.⁴ The need for these factors increases the complexity of the mechanism by which immune cells are directed to certain locations in the body, hence augmenting the specificity of the immune system.

T cells at cutaneous sites were shown to be phenotyp-

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ically different from T-cell populations at noncutaneous sites because of their expression of the cutaneous lymphocyte-associated antigen (CLA),⁵ identified by the rat monoclonal antibody (mAb) HECA-452. This homing receptor on memory T cells binds its ligand E-selectin on endothelium.⁶ In lesional skin of patients with AD, the majority of T cells express CLA.⁵ CLA is expressed on approximately 10% to 15% of peripheral blood T cells and on about 45% of the T cells in normal skin from nonatopic control subjects.⁷ As yet, it is unclear whether CLA expression is a prerequisite for blood T cells to extravasate into cutaneous sites or whether there is a nonhoming-specific T-cell influx during inflammation, followed by CLA upregulation in skin. Recently, the importance of CLA has been shown. In patients with AD and in patients with contact allergy, allergen-dependent responses of peripheral blood lymphocytes are largely confined to the CLA⁺ T-cell subset.⁸ Furthermore, casein stimulation of T cells derived from blood of allergic children with milk-induced eczema resulted in expansion of CLA⁺ T cells, whereas this was not the case in children with milk-induced gastroenteropathy.⁹

Expression of homing receptors on T cells can be both induced and upregulated. Induction of CLA expression, which occurs during the transition from naive to memory state, is mediated by various cytokines, among which are transforming growth factor (TGF)- β 1 and IL-12.^{10, 11} Furthermore, these two cytokines are also capable of upregulating CLA expression on memory T cells.^{10, 12} In addition, however, TGF- β 1 can upregulate expression of integrin α E β 7 on T lymphocytes.¹³ This molecule has been related to the localization and adhesion of T cells at mucosal sites.¹⁴ α E β 7 is expressed by more than 95% of intraepithelial lymphocytes located in the intestine, by a substantial number of lymphocytes within the bronchial epithelium, by some alveolar wall lymphocytes, and by bronchial alveolar lavage fluid T cells.¹⁵ In contrast, less than 2% of peripheral blood T cells express α E β 7.

To study homing receptor expression on infiltrating skin T cells, we used the atopy patch test (APT), in which aeroallergens, epicutaneously applied to clinically noninvolved skin of patients with AD, induce an eczematous reaction after 24 to 48 hours.¹⁶ APT-induced eczematous skin lesions are characterized by a dermal influx of CD4⁺ T cells, eosinophils, and CD1⁺ cells.¹⁷ Histologic appearance of late APT sites (72 hours) closely resembles the lesional skin of patients with AD.¹⁸

We studied homing receptor expression on T cells in the lesional skin of patients with AD, clinically noninvolved skin of patients with AD, the skin of APT sites of patients with AD, and nickel and sodium lauryl sulfate (SLS) patch test reactions. Therefore we performed immunohistochemical double staining with anti-CD3 and antibodies against CLA or α E β 7, which allows specific identification of homing receptor expression on T cells.

METHODS

Patients and test procedures

Patients with AD. We studied 16 patients with AD, diagnosed according to the criteria of Hanifin and Rajka,¹⁹ who were selected on the demonstration of a positive APT response at 24 hours, a positive skin prick test response (defined as >3 mm induration 20 minutes after intradermal allergen challenge), and a positive RAST response to house dust mite allergen. APTs were performed as described previously.¹⁶ In short, clinically noninvolved skin of the back was stripped 10 times with adhesive tape, after which house dust mite allergen (0.08 ml, 10,000 AU/ml) was applied with Leucotest patches (Beiersdorf AG, Hamburg, Germany). APT results were read after 24, 48, and 72 hours and recorded as: +, erythema with slight induration; 2+, erythema with papules; and 3+, erythema, papules, and vesicles. The diluent was applied as a negative control in all patients. Punch biopsy specimens (3 mm) were taken after local anesthesia (1% lidocaine) from clinically noninvolved skin ($n = 6$) and lesional skin of patients with AD ($n = 5$), and from APT sites at 24 ($n = 5$), 48 ($n = 5$), and 72 hours ($n = 5$).⁶ Biopsy specimens from early APT sites were taken at 0 hours ($n = 4$), 20 minutes ($n = 2$), 2 hours ($n = 2$), 4 hours ($n = 2$), 6 hours ($n = 1$), 8 hours ($n = 1$), 16 hours ($n = 1$), and 24 hours ($n = 3$).

Patients with contact dermatitis. Six patients with allergic contact dermatitis, who were known to have positive patch test reactions to nickel sulfate, were studied. Patch tests were performed on the back with 5% nickel sulfate dissolved in white petrolatum by using Finn chambers (Epitest, Norgesplaster, Norway). Patch tests were read after 24, 48, or 72 hours. In all patients strong reactions were seen with erythema and papules (2+). Punch biopsy specimens (3 mm) were taken after local anesthesia (1% lidocaine) at 0 ($n = 6$), 48 ($n = 3$), and 72 hours ($n = 3$).

Irritant reactions. Irritant reactions were induced in four patients with AD with 1% SLS in distilled water by using Leucotest patches (Beiersdorf AG). Patch tests were removed after 24 hours. All individuals showed erythema and induration at that time point. Punch biopsy specimens (3 mm) were taken after local anesthesia (1% lidocaine) at 0 ($n = 4$) and 24 hours ($n = 4$).

Nonatopic controls. Control skin biopsy specimens were taken from healthy nonatopic volunteers ($n = 3$), with negative skin prick test and RAST results.

All participants in this study gave their informed consent. All biopsy specimens were snap-frozen in liquid nitrogen and stored at -70°C before use. Sequential sections (6 μm) were cut on a freezing microtome and mounted on 3-aminopropyl tri-ethoxy silane (Sigma A3648) coated slides.

Double staining. To establish the expression of α E β 7 on T cells, double staining was performed with HML-1, an mAb recognizing α E β 7, and anti-CD3 mAb. Sections were fixed in dry acetone with H_2O_2 (30%, 100 μl /100 ml) for 7 minutes and air dried and preincubated for 20 minutes in 10% normal horse serum (NHoS), 10% normal human serum (NHuS), 10% normal rabbit serum (NRS) (Dako X-903), after which the sections were incubated with anti- α E β 7 (HML-1, Immunotech, 0318; 1:50 in phosphate-buffered saline [PBS] with 1% NHoS, 1% NHuS) for 60 minutes. Subsequently biotinylated horse-anti-mouse (1:800 in PBS 1% NHoS, 1% NHuS, 1% NRS) was added for 30 minutes, and sections were blocked for 30 minutes with polyclonal rabbit-anti-mouse antibody (Dako Z-109; 1:50 in PBS 1% NHuS, 1% NRS). Anti-CD3 (Leu-4, Becton Dickinson 668; 1:50 in PBS 1% NHuS, 1% NRS) was then applied

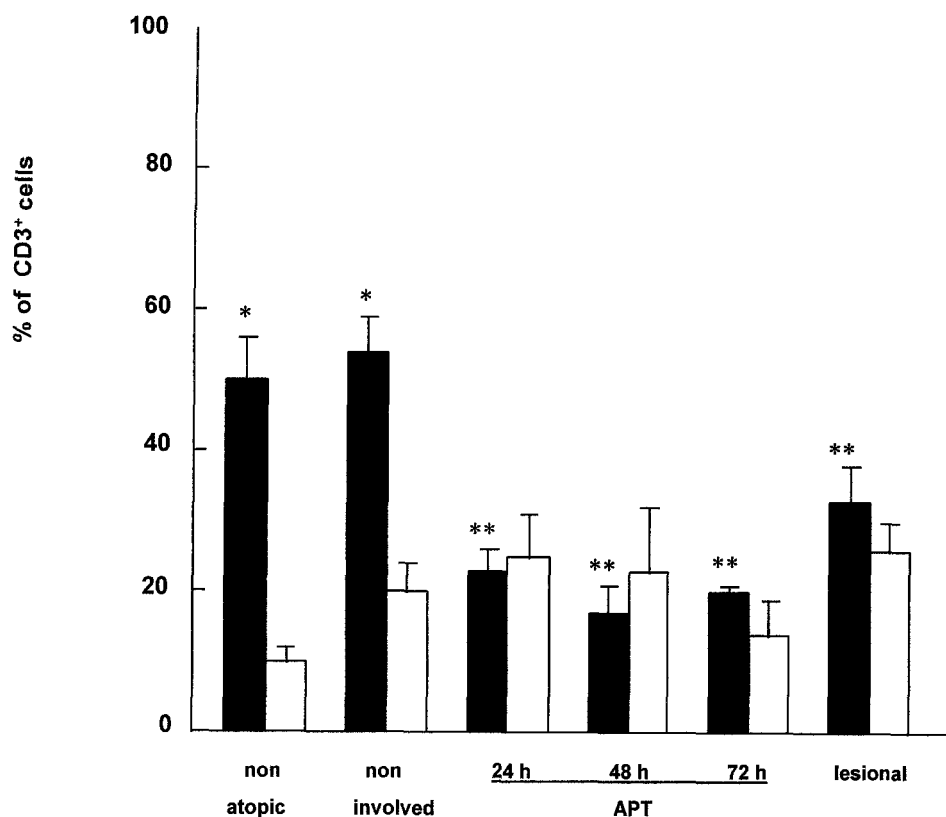


FIG. 1. Percentage of CD3⁺ T cells expressing CLA or αEβ7. CLA (shaded column) and αEβ7 (open column) were double stained with CD3 in nonatopic skin, clinically noninvolved skin, and lesional skin of patients with AD and in 24, 48, and 72 hour APT sites of patients with AD who underwent APT at 24, 48, and 72 hours. The data represent the mean (±SEM) of the percentage of double-stained cells. Significant differences between the percentages CLA⁺/CD3⁺ cells and αEβ7⁺/CD3⁺ cells are indicated by an asterisk. The percentage of CLA⁺/CD3⁺ cells at all APT timepoints significantly differs when compared with clinically noninvolved skin of patients with AD as indicated by a double asterisk.

for 60 minutes. Sections were then incubated with a mixture of streptavidine-conjugated alkaline phosphatase (Dako, D-396; 1:300) and peroxidase-conjugated rabbit-anti-mouse (Dako P-161; 1:50) in PBS 1% NHuS, 1% NRS for 30 minutes. In between incubations, sections were washed with PBS-Tween (three times for 5 minutes), and before substrate incubation, slides were washed with Tris-HCL (0.1 mol/L, pH 8.5; three times for 5 minutes).

First, alkaline phosphatase activity was demonstrated by using naphthol AS MX phosphate (Sigma N-4875) as the substrate and fast blue BB base (Sigma F-0125) as the chromogen, resulting in blue staining.²⁰ After rinsing twice with PBS and once with NaAc (0.1 mol/L, pH 5.0), peroxidase activity was revealed with 3-amino-9-ethylcarbazole (Sigma A-5754) as the substrate, resulting in a red color.²¹ In this way cells positive for CD3 stain red, cells expressing αEβ7 stain blue, and αEβ7-expressing T cells appear as purple cells.

The specificity of the double staining was established by using the following controls. Anti-αEβ7 was replaced by IgG2a isotype control (Southern Biotechnology Associates, Inc., Birmingham, Ala.), which resulted in red-staining cells only. Replacement of anti-CD3 by IgG1 isotype control (Southern Biotechnology Associates, Inc.) resulted in blue-staining cells only.

To determine whether αEβ7 was expressed on CD8⁺ cells, anti-CD3 was replaced by anti-CD8 (DAKO T8, M707; 1:50).

In this way CD8⁺ cells stain red, αEβ7⁺ cells stain blue, and double-positive cells stain purple.

To determine CLA expression on T cells, sections were double stained with HECA-452, a rat mAb,⁵ and anti-CD3 mAb. After fixation with dry acetone with H₂O₂, sections were preincubated for 20 minutes in PBS with 10% NHuS, 10% NRS, then sections were incubated with anti-CD3 (1:50) in combination with HECA-452 (kindly donated by R. Scheper, Amsterdam; 1:5) in PBS with 1% NHuS, 1% NRS. The sections were incubated with a mixture of peroxidase-conjugated rabbit-anti-mouse (1:50) and biotinylated rabbit-anti-rat (Dako, EO468; 1:2000) in PBS with 1% NHuS, 1% NRS, after which streptavidine-conjugated alkaline phosphatase was applied. In between incubations, sections were washed with PBS-Tween (three times for 5 minutes). Enzyme reactivity was demonstrated as described above. With this method CD3⁺ cells stain red, whereas CLA-expressing cells stain blue. CLA-expressing T cells appear as purple cells.

The specificity of the double staining was established by using the following controls. HECA-452 was replaced by IgM isotype control (clone G42-6.1; Pharmingen, San Diego Calif.), which resulted in red-staining cells only. Replacement of anti-CD3 by IgG1 isotype control (Southern Biotechnology Associates) resulted in blue-staining cells only.

Microscopic evaluation. Sections of skin biopsy specimens were examined by light microscopy at ×400 magnification.

TABLE I. Expression of CLA and α E β 7 by CD3⁺ cells

	CD3 ⁺ (mm ²)	CD3 ⁺ CLA ⁺ (mm ²)	CD3 ⁺ α E β 7 ⁺ (mm ²)
Nonatopic control skin (n = 3)	17 $\pm$ 3 ^{a,b}	8 $\pm$ 1 ^g	3 $\pm$ 1
Clinically noninvolved skin of patients with AD (n = 6)	45 $\pm$ 5 ^{b,c,d,e,f}	27 $\pm$ 3 ^{g,h}	17 $\pm$ 5 ⁱ
24 hr APT (n = 5)	116 $\pm$ 2 ^d	31 $\pm$ 8	25 $\pm$ 5
48 hr APT (n = 5)	196 $\pm$ 29 ^e	42 $\pm$ 16	28 $\pm$ 13
72 hr APT (n = 5)	173 $\pm$ 41 ^f	34 $\pm$ 10 ⁱ	22 $\pm$ 6
Lesional skin of patients with AD (n = 5)	211 $\pm$ 25 ^{a,c}	73 $\pm$ 11 ^{h,i}	65 $\pm$ 18 ^j

All values are given as cells per square millimeter, mean $\pm$ SEM.
Statistical analyses of corresponding letters: $p < 0.05$.

Before examination of the double staining, sections were compared with their control-stained countersections. For quantification of cell numbers and homing receptor expression, all double- and single-stained cells between the dermal-epidermal junction and the base of the hair follicles were counted independently by two observers. The mean interobserver coefficient of variation was 10%. In fields containing sweat ducts and hair shafts, only intervening dermal regions were counted. Of all biopsy specimens, at least two sections (surface area >1.5 mm² per section) were stained and counted. Results are expressed as the number of positive cells per square millimeter. Percentages in Fig. 1 were calculated per patient, after which the mean was taken.

Surface marker analysis by flow cytometry. Surface marker analysis on peripheral blood mononuclear cells (PBMCs) was performed by indirect immunofluorescence with flow cytometric assessment. PBMCs were isolated from peripheral blood obtained from nonatopic control subjects and from patients with AD. Briefly, heparinized blood (diluted 1:1 in PBS) was centrifuged on Ficoll-Paque (20 minutes, 2100 rpm, room temperature, no brake). The interphase (PBMC) was collected and washed twice with PBS. PBMCs [1×10^5 in PBS/2% FCS/5 mmol/L sodium azide (NaN₃)] were incubated for 30 minutes at 4°C with HECA-452 (1:5), α E β 7 (HML-1; 1:50), or control antibody of the appropriate isotype class (rat IgM or mouse IgG2a, as control for HECA-452 and α E β 7, respectively) in 96-well round-bottom plates. Cells were then washed (three times in PBS/FCS/NaN₃ solution) and incubated for 30 minutes at 4°C with biotinylated rabbit-anti-rat (1:2000) (HECA-452/rat IgM) or with biotinylated horse-anti-mouse (1:800) (HML-1/IgG2a). After washing the cells, samples were incubated with a mixture of phycoerythrin-labeled streptavidin (1:20) and fluorescein isothiocyanate-conjugated CD3 (Becton Dickinson, Leu 4; 1:10) or fluorescein isothiocyanate-labeled IgG1 (Becton Dickinson; 1:10) as isotype-matched control for CD3. Finally, cells were fixed with 2% paraformaldehyde and stored at 4°C until they were analyzed. Cytofluorography (Becton Dickinson FACScan) was performed with laser excitation at 488 nm. CLA expression and α E β 7 expression on T cells were determined by gating fluorescein isothiocyanate-positive cells (CD3⁺), and individual markers (CLA and α E β 7) were measured from the amount of phycoerythrin binding. Specific binding was determined by subtraction of the isotype control antibody.

Statistical analysis

Statistical analysis was performed by using the Wilcoxon rank sum test for nonparametric distributions. Probability (p) values of less than 0.05 were considered significant.

RESULTS

Increased numbers of CD3⁺ cells in lesional and clinically noninvolved skin of patients with AD

Skin biopsy specimens from nonatopic control subjects and patients with AD were immunohistochemically stained for the pan-T cell marker CD3. In lesional skin of patients with AD, the number of CD3⁺ cells was significantly increased when compared with clinically noninvolved skin of patients with AD, which in turn contained significantly higher numbers of CD3⁺ cells than skin of nonatopic subjects (Table I).

Higher percentage of CLA⁺ T cells in clinically noninvolved skin of patients with AD than in lesional skin of patients with AD

To study the expression of homing receptors on T cells in skin of patients with AD and nonatopic control subjects, we double stained skin sections with mAbs against CD3 and CLA (Fig. 2).

In both clinically noninvolved skin of patients with AD and skin of nonatopic subjects, CLA⁺ cells were located in small perivascular infiltrates in the dermis. In lesional skin they were found in much larger infiltrates scattered throughout the dermis, in particular in the papillary region, and occasionally in the epidermis. A few single-stained CLA⁺ cells were found only in lesional skin, all localized in the dermis.

A significantly larger number of CD3⁺ cells expressing the skin-homing receptor CLA was seen in clinically noninvolved skin of patients with AD than in skin from nonatopic subjects (Table I). The percentages in these groups, however, were comparable (Fig. 1). Lesional skin showed an increased number of CLA⁺/CD3⁺ cells compared with clinically noninvolved skin from patients with AD (Table I). Importantly, however, and seemingly in contrast, the percentage of CD3⁺ cells expressing CLA was significantly lower in the lesional skin of patients with AD (Fig. 1).

By means of fluorescence-activated cell sorter analysis, the percentage of CD3⁺ cells expressing CLA in blood was determined. In blood, both from nonatopic subjects and from patients with AD, the percentage of CLA⁺/CD3⁺ cells was significantly lower than in the

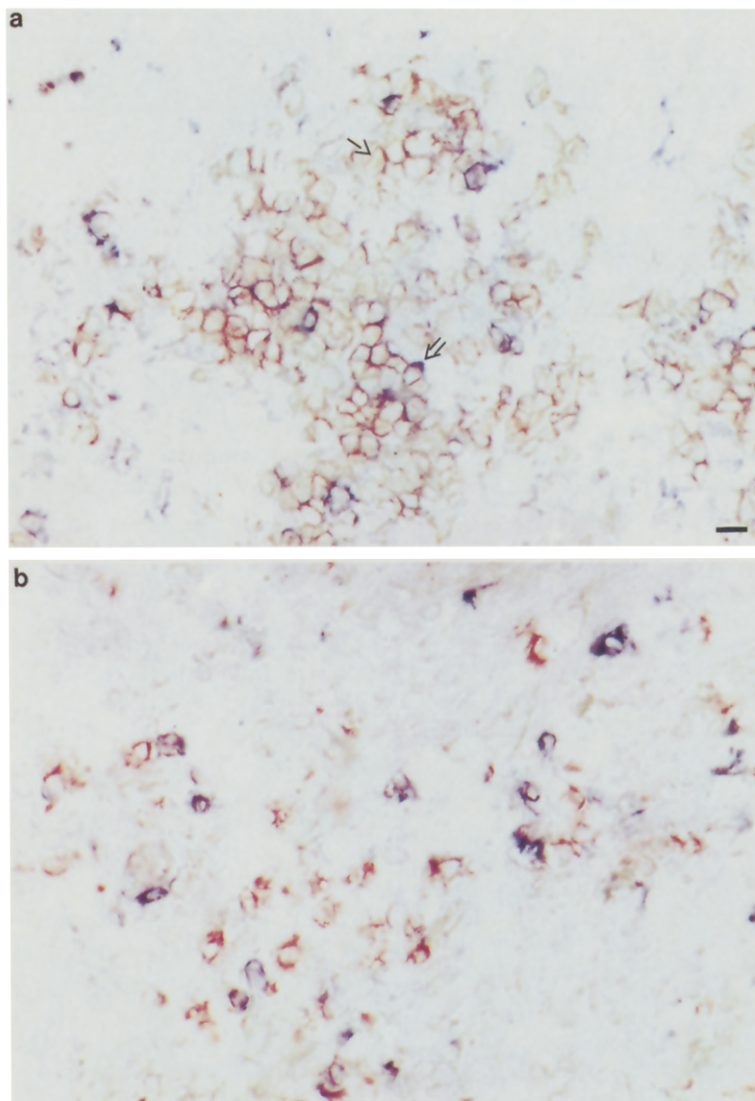


FIG. 2. Immunohistochemical double staining of T cells in lesional skin of patients with AD. **a**, Double staining with anti-CD3 (red) and anti-CLA (blue). Double-positive cells ($CD3^+/CLA^+$) are indicated by purple staining. Arrows indicate a double-positive cell (*double arrow*) and a single red positive cell (*single arrow*). No single blue-staining cells are seen. **b**, Double staining with anti-CD3 (red) and anti- $\alpha E\beta 7$ (blue). Double-positive cells ($CD3^+/\alpha E\beta 7^+$) are indicated by purple staining. Scale bar, 10 μm .

skin. No difference was observed between the percentage of $CLA^+/CD3^+$ cells in the blood of nonatopic control subjects ($14.2\% \pm 5\%$ [mean $\pm$ SD], $n = 6$) and blood of patients with AD ($16.8\% \pm 7\%$, $n = 6$).

Increased numbers of $\alpha E\beta 7$ -expressing $CD3^+$ cells in lesional skin of patients with AD

A substantially increased number of $CD3^+$ cells expressing the gut-homing receptor $\alpha E\beta 7$ was observed in the lesional skin of patients with AD compared with clinically noninvolved skin of patients with AD (Fig. 2). In addition, the number of double-positive cells in noninvolved skin of patients with AD was significantly higher than in the skin of nonatopic subjects (Table I). The distribution of $\alpha E\beta 7^+/CD3^+$ cells was comparable

to that of $CLA^+/CD3^+$ cells, although no single $\alpha E\beta 7^+$ cells were observed.

Immunofluorescence double staining of peripheral blood obtained from patients with AD ($n = 6$) and nonatopic control subjects ($n = 6$) with $\alpha E\beta 7$ and CD3 showed that no significant difference existed between the two groups with regard to the percentages of $CD3^+/\alpha E\beta 7^+$ cells ($1.6\% \pm 0.8\%$ and $1.4\% \pm 0.6\%$, respectively).

Increasing numbers of $CD3^+$ cells found in the APT, the majority expressing neither CLA nor $\alpha E\beta 7$

All patients with AD showed positive APT reactions with scores of 1+ or 2+. In the APT there was a strong

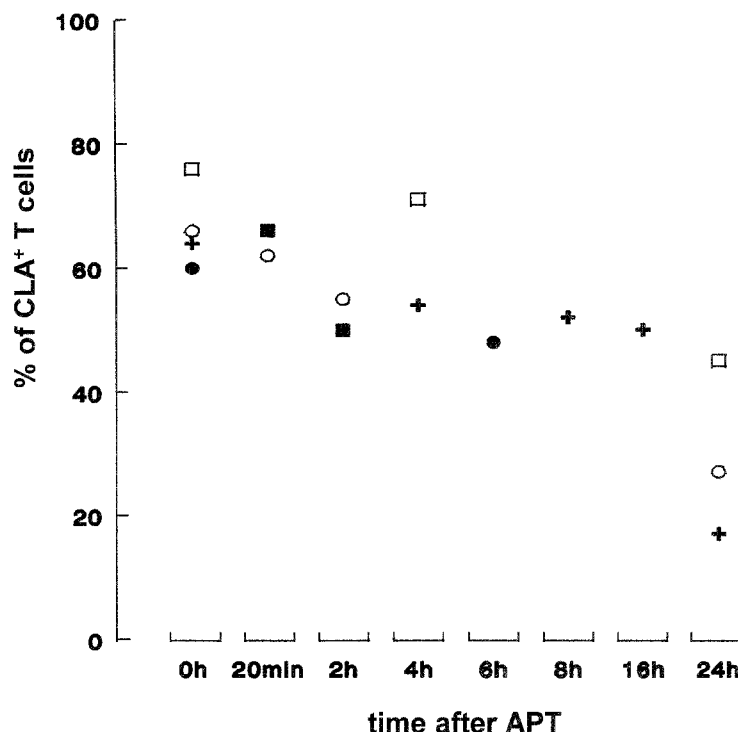


FIG. 3. Percentage of CD3⁺ T cells expressing CLA during the early APT timepoints. Data are depicted per patient; different symbols represent individual patients.

influx of CD3⁺ cells during the first 48 hours after allergen application (Table I). In contrast, no increase in CD3⁺ T cells was seen after either tape stripping only or buffer occlusion.

After patch testing for either 24, 48, or 72 hours, neither the number of CLA⁺/CD3⁺ cells nor the number of α E β 7/CD3⁺ cells changed as compared with clinically noninvolved skin (Table I). Because the number of infiltrating CD3⁺ cells increased during patch testing, there was a significant decrease in the percentage of CLA⁺/CD3⁺ cells at all three APT time points (Fig. 1).

To study whether CD3⁺ cells infiltrating in the early APT were CLA⁺, serial biopsy specimens were taken from the skin of patients with AD between 0 and 24 hours of the APT time points. As is shown in Fig. 3, there was no increase in the percentage of CLA⁺/CD3⁺ cells between 0 and 24 hours after allergen application; there is even a gradual decrease reaching a minimum at 24 hours.

At all APT time points, the CLA⁺/CD3⁺ cells were found in perivascular infiltrates and papillary regions in the dermis and occasionally in the epidermis, whereas no CLA single positive cells were observed. As in the lesional skin of patients with AD, the majority of the α E β 7⁺/CD3⁺ cells were localized in perivascular infiltrates, but some were localized in papillary regions.

Previously, α E β 7 expression was predominantly observed on CD8⁺ cells.¹⁴ To determine whether in skin α E β 7⁺ cells are of the CD4 or CD8 T-cell subset, APT

skin sections were double stained for α E β 7 and CD8. A minority of α E β 7⁺ cells were of the CD8⁺ subset (34% $\pm$ 9% [mean $\pm$ SEM] n = 3), but not all CD8⁺ cells expressed α E β 7.

In noninvolved and lesional skin of patients with AD and in skin from nonatopic subjects, the majority of the CD3⁺ cells expressed either CLA or α E β 7. During APT this percentage decreased dramatically and the majority of the CD3⁺ cells expressed neither CLA nor α E β 7.

We did not find a correlation between the intensity of the macroscopic skin reaction and the number of CLA⁺ T cells.

Increasing numbers of CD3⁺ cells after patch testing with nickel sulfate or SLS, the majority expressing neither CLA nor α E β 7

To study homing receptor expression on T cells during other skin inflammatory reactions, CLA/CD3 double staining was performed in biopsy specimens from nickel patch test reactions after 48 and 72 hours and in biopsy specimens obtained from 24 hours SLS patch test reaction sites. The number of infiltrating CD3⁺ cells varied considerably between the two groups. In the nickel patch test reactions, the mean number of CD3⁺ cells was 837 per square millimeter at 72 hours, ranging from 532 to 1028 per square millimeter. In SLS patch test reactions, the mean number of CD3⁺ cells was 218 per square millimeter at 24 hours, ranging from 98 to 424 per square millimeter.

In nickel patch test reactions, the percentage of

CLA⁺/CD3⁺ cells was significantly decreased both at 48 hours ($16\% \pm 3\%$; mean $\pm$ SEM) and at 72 hours ($26\% \pm 4\%$) as compared with nonpatch-tested skin ($74\% \pm 7\%$). The slight increase at 72 hours was not significant compared with the increase at 48 hours. In SLS patch test reactions, the percentage of CLA⁺/CD3⁺ ($36\% \pm 5\%$) was also significantly decreased compared with non-patch-tested skin ($64\% \pm 7\%$), albeit not as dramatically as in nickel patch test reactions.

DISCUSSION

To perform their effector and immunoregulatory functions, T cells must extravasate from blood into tissue. A specific mechanism by which T cells extravasate is called selective homing. In this process T cells expressing homing receptors are able to migrate from blood into discrete tissue sites by local expression of homing receptor ligands on vascular endothelium. This concept is based on the observation that under normal, noninflammatory conditions, the expression of homing receptors on T cells shows a tissue-specific distribution.²²

There are several explanations for the relatively restricted tissue expression of putative homing receptors. One is that they play a role in the regulation of T-cell extravasation during immune surveillance under normal circumstances. Another function could be retention of specific T-cell subsets in tissue, possibly after local induction.

The expression of skin-homing receptor CLA and gut-homing receptor α E β 7 on T cells is, however, not exclusive to their alleged specific tissue site,^{15, 23, 24} indicating that other factors may play a role in T-cell extravasation as well. Extravasation of T cells is thought to be a multiple-step process, involving activation-independent adhesion (mediated by selectins) and activation-dependent adhesion (mediated by integrins and members of the immunoglobulin gene superfamily).³ Therefore homing receptors are almost certainly not the only adhesion molecules mediating T-cell extravasation at sites of cutaneous inflammation.¹⁰

In this study we set out to determine at what stage of cutaneous inflammation T-cell homing receptor expression plays a significant role in extravasation of these cells. To that end, skin biopsy specimens of nonatopic subjects and noninvolved skin, lesional skin, and APT reaction sites of patients with AD and skin from nickel and SLS patch test sites were immunohistochemically double stained for CD3 and either α E β 7 or CLA.

It was previously demonstrated that in noninflamed skin, both nonatopic skin and clinically noninvolved skin of patients with AD, a large proportion of T cells expresses CLA.^{5, 7, 23} Our data are in agreement with these results but also show that a minority of cutaneous T cells expresses α E β 7. Together these results indicate that CLA is involved in specific homing of T cells to skin. However, during the APT reaction and the nickel and SLS patch test reactions, the number of T cells increases, whereas the percentage of T cells expressing CLA diminishes. This therefore suggests that CLA expression

is not a prerequisite for blood T cells to extravasate into skin during eczematous reactions.

Allergen-specific Th₂ clones have been detected in clinically noninvolved skin of patients with AD²⁵ (Van Reijssen et al. Unpublished data). Furthermore, these T cells have been shown to express CLA *in vitro*.²⁶ Similar allergen-specific T cells may well be part of the CLA⁺ T cells we observe in clinically noninvolved skin. We propose that stimulation of these Th₂ cells in an allergen-specific manner induces a cytokine cascade, upregulating adhesion molecules on endothelial cells and in this way facilitating, or even inducing, extravasation of circulating blood T cells, which may express nonskin-specific homing receptors.²⁷ Monitoring T-cell influx during APT reactions showed that the phenotype of T cells, with regard to their cytokine production, changes from an initial Th₂ into a Th₁/Th₀ type.¹⁸ This indicates that in acute allergic skin inflammation, the allergen-specific T-cell response is followed by a nonspecific bystander response. Our observation that T cells infiltrating during APT are CLA⁺ suggests that these might be the Th₁/Th₀ bystander cells.

Expression of CLA on memory T cells can be upregulated by IL-2, IL-6, TGF- β 1, and IL-12 in a superantigen-dependent way.^{10, 12} IL-2 is available in an autocrine fashion, whereas IL-6, TGF- β 1, and IL-12 are paracrine fashion provided by other cells in the skin.^{10, 28-30} Furthermore, Hamid et al.³¹ recently showed that there was a significant increase in IL-12 messenger RNA-positive cells in chronic skin lesions compared with acute lesions and clinically noninvolved skin of patients with AD. However, CLA upregulation by the above-mentioned cytokines takes 2 to 6 days *in vitro* and is likely to take as much time *in vivo*.^{10, 12} This would explain why we did not observe an upregulation of CLA⁺ T cells 72 hours after nickel and house dust mite patch testing, although the number of CLA⁺ T cells was increased in lesional skin.

α E β 7 has been described to be not only a homing receptor for the gut but also an activation marker.^{24, 32} The increased percentage of α E β 7⁺ T cells in the lesional skin of patients with AD indicates that cells are activated because of continuous stimulation. Indeed, the majority of α E β 7-positive T cells also expressed CD25, an activation antigen on T cells, as was determined by double staining (data not shown). In addition, it was shown that a considerable number of T cells in lesional skin expresses the activation marker HLA-DR.³³

In summary, we propose that CLA expression is not a prerequisite for cutaneous T-cell infiltration during acute or chronic inflammation in skin, although a role for CLA in determining tissue specificity of T cells under other circumstances cannot be excluded. Accordingly, expression may be important for T cells previously activated in skin or skin-related lymphoid tissue to extravasate from blood into the skin during immune surveillance. This important feature of CLA will enable the immune system to respond adequately on contact with antigen through the skin and thus provide protec-

tive immunity against such antigens at the most likely site of repeated encounter. During acute inflammation, however, other mechanisms may permit extravasation of other, non-CLA-expressing T cells to equip the inflamed skin with an immune defense system that is as broad as possible to combat a wide array of potentially harmful antigens.

REFERENCES

- Leung DYM, Harbeck RJ, Bina P, Hanifin JM, Reiser RF, Sampson HA. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. *J Clin Invest* 1993;92:1374-80.
- Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg ML. Predominance of "memory" T cells (CD4+, CD29+) over naive T cells (CD4+, CD45R+) in both normal and diseased human skin. *Arch Dermatol Res* 1989;281:24-30.
- Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;76:301-14.
- Colditz IG. The induction of plasma leakage in skin by histamine, bradykinin, activated complement, platelet-activating factor and serotonin. *Immunol Cell Biol* 1991;69:215-9.
- Pickler LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* 1990;136:1053-68.
- Shimizu Y, Shaw S, Graber N, Gopal TV, Horgan KJ, Van Seventer GA, et al. Activation-independent binding of human memory T cells to adhesion molecule ELAM-1. *Nature* 1991;349:799-802.
- Bos JD, De Boer OJ, Tibbosch E, Das PK, Pals ST. Skin-homing T lymphocytes: detection of cutaneous lymphocyte-associated antigen (CLA) by HECA-452 in normal human skin. *Arch Dermatol Res* 1993;285:179-83.
- Santamaria Babi LF, Pickler LJ, Perez Soler MT, Drzimalla K, Flohr P, Blaser K, et al. Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. *J Exp Med* 1995;181:1935-40.
- Abernathy-Carver KJ, Sampson HA, Pickler LJ, Leung DY. Milk-induced eczema is associated with the expansion of T cells expressing cutaneous lymphocyte antigen. *J Clin Invest* 1995;95:913-8.
- Pickler LJ, Treer JR, Ferguson-Darnell B, Collins PA, Bergstresser PR, Terstappen LWM. Control of lymphocyte recirculation in man. II. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. *J Immunol* 1993;150:1122-36.
- Liu L, Foer A, Sesterhenn J, Reinhold U. CD2-mediated stimulation of the naive CD4+ T-cell subset promotes the development of skin-associated cutaneous lymphocyte antigen-positive memory cells. *Immunology* 1996;88:207-13.
- Leung DYM, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Pickler LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med* 1995;181:747-53.
- Barnard JA, Beauchamp RD, Coffey RJ, Moses HL. Regulation of intestinal epithelial cell growth by transforming growth factor type beta. *Proc Natl Acad Sci U S A* 1989;86:1578-86.
- Cerf-Bensussan N, Jarry A, Brousse N, Lisowska-Grospierre B, Guy-Grand D, Griscelli C. A monoclonal antibody (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *Eur J Immunol* 1987;17:1279-85.
- Erle DJ, Brown T, Christian D, Aris R. Lung epithelial lining fluid T cell subsets defined by distinct patterns of beta 7 and beta 1 integrin expression. *Am J Respir Cell Mol Biol* 1994;10:237-44.
- Langeveld-Wildschut EG, Van Marion AMW, Thepen T, Mudde GC, Bruijnzeel PLB, Bruijnzeel-Koomen CAFM. Evaluation of variables influencing the outcome of the atopy patch test. *J Allergy Clin Immunol* 1995;96:66-73.
- Reitamo S, Visa K, Kahonen K, Kyhk K, Stubb S, Salo O. Eczematous reactions in atopic dermatitis patients caused by epicutaneous testing with inhalant allergens. *Br J Dermatol* 1986;114:303-9.
- Thepen T, Langeveld-Wildschut EG, Bihari IC, Van Wichen DF, Van Reysen FC, Mudde GC, et al. Biphasic response against aeroallergen in atopic dermatitis showing a switch from initial T_{H2} into T_{H1} response in situ: an immunocytochemical study. *J Allergy Clin Immunol* 1996;97:828-37.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44-7.
- Burstone MS. Histochemical comparison of naphthol-phosphatases for the demonstration of phosphatases. *J Natl Cancer Inst* 1958;20:601-4.
- Claassen E, Adler LT, Adler EL. Double immuno-cytochemical staining for the in situ study of allotype distribution during an anti TNP immune response in chimeric rabbits. *J Histochem Cytochem* 1986;34:989-98.
- Pickler LJ. Mechanisms of lymphocyte homing. *Curr Opin Immunol* 1992;4:227-86.
- Pickler LJ, Martin RJ, Trumble A, Newman LS, Collins PA, Bergstresser PR, et al. Differential expression of lymphocyte homing receptors by human memory/effector T cells in pulmonary versus cutaneous immune effector sites. *Eur J Immunol* 1994;24:1269-77.
- Brew R, West DC, Burthem J, Christmas SE. Expression of the human mucosal lymphocyte antigen, HML-1, by T cells activated with mitogen or specific antigen in vitro. *Scand J Immunol* 1995;41:533-62.
- Van Reysen FC, Mudde GC, De Weger RA, Bruijnzeel-Koomen CAFM. Retention of long-lived, allergen-specific T cells in atopic dermatitis skin. *J Invest Dermatol* 1997;108:530.
- Rossiter H, Van Reysen FC, Mudde GC, Kalthoff FS, Bruijnzeel-Koomen CAFM, Pickler LJ, et al. Skin disease-related T cells bind to endothelial selectins: expression of cutaneous lymphocyte antigen (CLA) predicts E-selectin but not P-selectin binding. *Eur J Immunol* 1994;24:205-10.
- Springer TA. Adhesion receptors of the immune system. *Nature* 1990;346:425-34.
- Seghal PB. Interleukin 6: molecular physiology. *J Invest Dermatol* 1990;94:2-6S.
- Kane CJM, Knapp AM, Mansbridge JN, Hanawalt PC. Transforming growth factor-beta1 localization in normal and psoriatic epidermal keratinocytes in situ. *J Cell Physiol* 1990;144:144-52.
- Grewe M, Walther S, Gyufko K, Czech W, Schöpf E, Krutmann J. Analysis of the cytokine pattern expressed in situ in inhalant allergen patch test reactions of atopic dermatitis patients. *J Invest Dermatol* 1995;105:407-10.
- Hamid OA, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DYM. In vivo expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 1996;98:225-31.
- Schieferdecker HL, Ullrich R, Weiss-Breckwoldt AN, Schwarting R, Stein H, Riecken E, et al. The HML-1 antigen of intestinal lymphocytes is an activation antigen. *J Immunol* 1990;144:2541-9.
- Leung DYM, Bhan AK, Schneeberger EE, Geha RS. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* 1983;71:47-56.