

Cutaneous lymphocyte-associated antigen expression in children with atopic dermatitis and non-atopic healthy children

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Cutaneous lymphocyte-associated antigen (CLA) is a cell surface glycoprotein which has been implicated in the homing of lymphocytes to cutaneous sites. It is postulated to play an important role in T-cell migration to skin in atopic dermatitis; however, the expression of CLA in both normal children and children with atopic dermatitis has not been extensively studied. If CLA expression on T cells were important in the traffic of lymphocytes to atopic dermatitis skin lesions, it might be expected that the proportion of CLA⁺ T cells in unstimulated peripheral blood from children with atopic dermatitis would be elevated. We have examined the proportion of CLA⁺ T cells in children with atopic dermatitis and non-atopic age-matched controls. The proportion of CLA⁺ T cells in non-atopic children was highly associated with and increased with increasing age ($r = 0.88$, $p < 0.001$). There was no difference between the proportion of T cells expressing CLA in the unstimulated peripheral blood mononuclear cells from children with severe ($p = 0.18$) or with mild/moderate ($p = 0.3$) atopic dermatitis and age-matched non-atopic controls. Despite this, children with atopic dermatitis did show evidence of perturbation of CLA expression, as unlike the non-atopic children the proportion of CLA⁺ T cells in the atopic children did not correlate with age. These findings suggest that while CLA expression may play a role in atopic dermatitis, other as yet undefined surface markers are likely to principally determine the migration of T cells to skin in atopic dermatitis.

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Cutaneous lymphocyte-associated antigen (CLA) is a surface glycoprotein which is expressed on a relatively small percentage of peripheral blood lymphocytes, as well as monocytes and granulocytes. It has been postulated that CLA acts as a skin homing molecule for T cells, controlling lymphocyte migration to cutaneous sites (1). CLA is expressed exclusively on memory T cells (2) and is reported to be induced by carbohydrate modification of the ubiquitously expressed p-selectin glycoprotein ligand-1 (PSGL-1) (3), which is found on all human peripheral blood T cells. CLA-positive T cells are able to bind to E-selectin and P-selectin, whereas CLA-negative T cells can only bind to P-selectin (4). Thus, it has been postulated that CLA may play an important

role in the selective migration of T cells to sites of cutaneous inflammation by interaction with E-selectin on activated endothelium.

Whether expression of CLA plays an important role in atopic dermatitis in either adults or children is currently unclear. CLA⁺ T cells have been reported to be increased in the skin of subjects with atopic dermatitis (1), although it has been recently suggested this may relate to an increase in total T cells in skin rather than to selective migration of CLA⁺ T cells *per se* (5). Other evidence that CLA may play a role includes the finding of *in vitro* antigen-specific proliferation in the CLA⁺ T-cell subset in peripheral blood mononuclear cells (PBMC) from atopic dermatitis subjects in response to dust mite (6) and

caesin (7). The proportion of CLA⁺ cells in unmanipulated PBMC from children with milk-sensitive atopic dermatitis was reported to be enhanced compared to PBMC from children with milk-induced enteritis; however, non-atopic healthy children were not examined in this study (7). In contrast, no difference was found in the proportion of CLA⁺ T cells in unstimulated PBMC from a small number of adults with atopic dermatitis and non-atopic controls (5).

Thus, if CLA expression on T cells determines migration of lymphocytes to the skin in atopic dermatitis, it might be expected that unstimulated PBMC from children with atopic dermatitis would have an expanded population of CLA⁺ T cells. We have examined the expression of CLA on unstimulated PBMC from children with atopic dermatitis and age-matched non-atopic healthy controls.

Methods

Subjects

Heparinized blood was obtained from 16 children with mild/moderate atopic dermatitis and 10 children with severe atopic dermatitis. Subjects were diagnosed according to the criteria of Hanifin and Rajka (8). The severity of atopic dermatitis was determined by the clinical severity score and was defined by the sum of the individual scores, graded as 0 (none), 1 (mild), 2 (moderate), 3 (severe), for each of six parameters (pruritus, erythema, lichenification, scaling, weeping and edema/papulation) (9). Children were defined as having severe atopic dermatitis if they had a severity score of at least 8, and involvement of at least 20% of their body surface area. The mean IgE of the children with severe atopic dermatitis was 1306 IU/ml (range 94–6600 IU/ml) and that of the children with mild/moderate atopic dermatitis was 395 IU/ml (5–1510 IU/ml). The mean age of the children with severe atopic dermatitis was 23 months, and that of the children with mild/moderate atopic dermatitis was 47 months.

Heparinized blood was obtained from 22 age-matched healthy non-atopic children, who were undergoing routine day surgery procedures and had serum IgE in the normal range for age. Controls with no history of atopy but serum IgE above the normal range were excluded. Blood was taken on anesthetic induction, following parental consent. Heparinized blood was also obtained from six non-atopic adults and four cord blood samples for age association studies. The studies were approved by the Royal

Children's Hospital Research Foundation Ethics Committee.

Cell staining

PBMC were separated from heparinized blood by ficoll–hypaque density gradient centrifugation as previously described (10). Unstimulated PBMC (2.5×10^5 per tube) were stained with combinations of 25 µl of anti-human CLA–fluorescein isothiocyanate (FITC) (Pharmingen, San Diego, California, USA), anti-CD3–peridinin chlorophyll protein (PerCP), anti-CD4–phycoerythrin (PE) (Becton Dickinson, San José, California, USA) or isotype controls IgG₁–FITC/PE/PerCP (Becton Dickinson) in the dark for 15 min, washed with PBS and then fixed with 150 µl per tube of 1% ultrapure methanol-free formaldehyde (Polysciences Inc., Warrington, PA, USA).

Flow cytometry analysis

PBMC were analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometric Systems, Palo Alto, California, USA) equipped with a 15 mW argon ion laser with filter settings for FITC (530 nm), PE (585 nm) and PerCP (630 nm). A total of 20 000 gated events were collected in list-mode files using a Cicero interface, and subsequently analyzed using CyCLOPS for Cicero 3.14 software (Cytomation, Fort Collins, Colorado, USA). Dead cells and monocytes were excluded by forward and side-scatter characteristics, and confirmed on set-up by surface staining with CD14/CD45 and propidium iodide (PI). The cut of fluorescence for CLA positivity was defined by the fluorescence of the FITC-isotype control for each sample (background fluorescence). The numbers of CLA⁺ T cells were calculated by the percentage of CLA⁺/CD3⁺ cells as a proportion of total CD3⁺ cells.

Statistical methods

Kolmogorov–Smirnov normality testing was performed on groups, with *post hoc t*-test or Mann–Whitney rank sum test depending on whether data was normally distributed. Correlations were carried out using the Pearson's correlation coefficient.

Results

CLA expression in unstimulated PBMC

CLA expression in healthy non-atopic children. The percentage of CD3⁺ T cells which expressed CLA in unstimulated PBMC from non-atopic healthy children was closely associated with and increased with age ($r = 0.88$, $p < 0.001$ Fig. 1).

CLA expression in children with atopic dermatitis. The percentage of CD3⁺ T cells which expressed CLA in unstimulated PBMC obtained from children with severe and mild/moderate atopic dermatitis did not differ significantly from that of age-matched non-atopic controls ($p = 0.18$, $p = 0.3$, respectively) (Fig. 2). Unlike CLA expression in non-atopic controls, CD3⁺CLA⁺ expression in unstimulated PBMC from children with atopic dermatitis did not correlate with age ($r = 0.04$, $p = 0.8$, Fig. 3). CLA expression in CD3⁺ T cells from children with atopic dermatitis did not correlate with serum IgE ($r = 0.22$, $p = 0.3$).

Discussion

There are two principle findings of this study. Firstly, the *in vivo* expression of CLA on T cells increased with age in healthy non-atopic controls. Secondly, there was no difference between the proportion of CLA⁺CD3⁺ T cells in unstimulated PBMC from children with atopic dermatitis and age-matched non-atopic children.

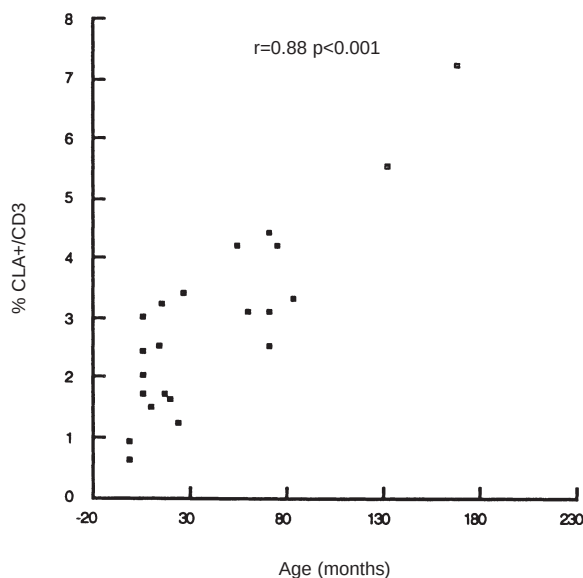


Fig. 1. Relationship between the age of the child (months) and proportion of CD3⁺ T cells expressing CLA in unstimulated PBMC from non-atopic control children ($n = 22$) and CBMC ($n = 4$).

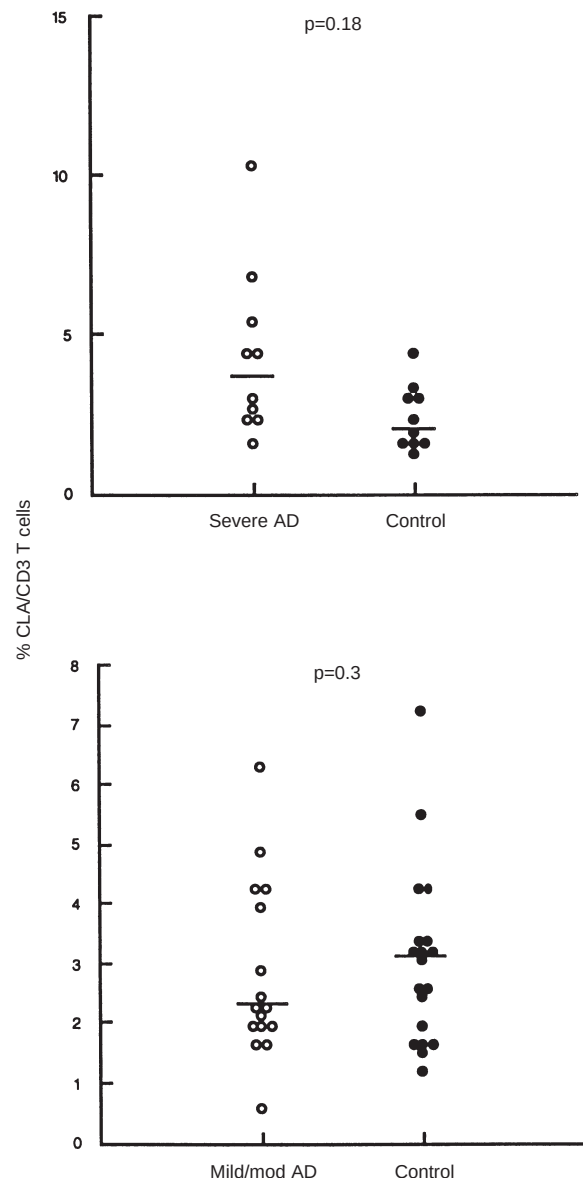


Fig. 2. Percentage of CD3⁺ T cells expressing CLA in unstimulated PBMC from children with severe atopic dermatitis (AD) ($n = 10$), moderate/mild atopic dermatitis ($n = 16$) and age-matched non-atopic controls. The bold line represents the median values.

There have been no previous reports concerning the ontogeny of CLA expression. We found that the expression of CLA on T cells increased from $< 1\%$ in cord blood to over 10% in adults. The CLA expression in T cells from non-atopic adults of $10\text{--}13\%$ in this study is similar to previous reports of approximately 15% (5,11). As CLA is reported to be expressed exclusively on memory T cells, and memory T cells increase in proportion throughout childhood, it is possible that the reduced CLA expression in infancy and childhood reflects a relative lack of antigenic exposure, and therefore reduced memory T cells. Thus CLA expression in lymphocytes may

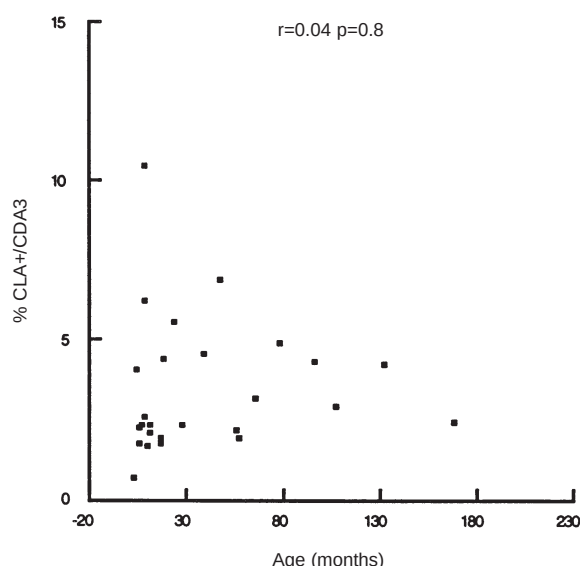


Fig. 3. Relationship between the age of the child (months) and proportion of CD3⁺ T cells expressing CLA in unstimulated PBMC from children with atopic dermatitis (n = 26).

parallel acquisition of T cell memory, as the expression of CD45 RO increases from 5% in the neonatal period to approximately 40% in adults (12). There is evidence that other T-cell surface markers, such as the adhesion molecule CD11b and the activation markers CD25 and HLA-DR, also increase with age; however, the degree of increase is not as marked as the 10-fold increase observed for the change in proportion of CLA⁺ T cells from neonates to adults. Thus, the proportion of T cells expressing CD11b increases 2.5-fold from 6% in neonates to 15% in young adults (13), CD25 increases 2.5-fold from 10% to 25%, and HLA-DR increases 5-fold from 5 to 25% (14). Our finding that the proportion of T cells expressing CLA increases with age indicates that it is essential to use age-matched controls when examining CLA expression in diseases of childhood.

There was no difference in the proportion of CD3⁺ T cells which expressed CLA in the atopic and non-atopic age-matched groups. This finding is in keeping with that of de Vries et al. (5), who also found no difference in the CLA expression in unstimulated PBMC from six adults with atopic dermatitis and six non-atopic adult controls. By contrast, Abernathy-Carver et al. (7) found that children with milk-induced atopic dermatitis (n = 7) had a higher expression of CLA on unstimulated PBMC than children with milk-induced enteritis (n = 10). Unfortunately, there were no age-matched control children, and only adults controls were used in this study. Furthermore, the children with enteritis had a

lower median age (3 years) than those with atopic dermatitis (5 years), and may have had a lower CLA expression for this reason alone. The current findings in children and the findings of de Vries et al. in adults suggest that the proportion of CLA⁺ T cells is not increased in the peripheral blood of subjects with atopic dermatitis. An alternative explanation for the current findings is that there was an increase in the production of T cells expressing CLA in the atopic subjects, but that these cells were preferentially taken up by the skin. However, if CLA expression is a mechanism by which T cells migrate preferentially to the skin, it would be likely that this would be reflected in the circulating blood pool.

An interesting finding was that the close association between age and an increasing proportion of CLA⁺ T cells in normal children was not seen in children with atopic dermatitis. This suggests a perturbation of CLA⁺ T cell expression in atopic dermatitis. It is possible that the dynamics of the circulating and skin CLA⁺ lymphocyte populations differ in atopic dermatitis and healthy subjects. Children with atopic dermatitis may produce more CLA⁺ T cells because of activation of naive T cells with antigens and/or superantigens in peripheral lymph nodes. Children with atopic dermatitis have marked lymphadenopathy in areas which drain cutaneous sites, and it is in these lymph nodes that acquisition of CLA expression is postulated to occur (2). Atopic dermatitis also results in activated local endothelium with enhanced expression of adhesion molecules, such as E-selectin (15,16), which would favor increased migration of CLA⁺ T cells to the skin. Thus increases in the number of CLA⁺ T cells in lymphoid tissue and in blood, coupled with enhanced uptake of CLA⁺ T cells into the skin could result in a greater flux and variation of the proportion of CLA⁺ T cells in the peripheral T-cell pool of children with atopic dermatitis. This could possibly account for the loss of the association between age and CLA expression observed in the atopic children. Although the median proportion of CD3⁺ T cells expressing CLA was higher in the severe atopic dermatitis than age-matched control group, this did not reach significance and the proportions of CLA⁺ T cells in the children with mild/moderate atopic dermatitis and age-matched controls were equivalent. Thus it is possible that CLA⁺ T-cell production is increased in atopic dermatitis; however, increased removal of CLA⁺ T cells from the circulation could result in a steady state which does not significantly differ from that of controls.

In this study, we demonstrate that CLA expression on T cells from non-atopic normal children is highly associated with age. Although there was not an increased proportion of CLA⁺ T cells in PBMC from children with atopic dermatitis compared to age-matched normal controls, we show a loss of the association of CLA expression with age in children with atopic dermatitis, implicating CLA T-cell expression in the immunological response of childhood atopic dermatitis. These findings suggest that T-cell surface markers other than CLA are likely to play an important role in traffic of T cells to skin in atopic dermatitis, and that further investigation of T-cell surface receptors in atopic dermatitis is warranted.

Acknowledgments

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References

- PICKER 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.
- PICKER LJ, TREER JR, FERGUSON-DARNELL B, COLLINS PA, BERGSTRESSER PR, TERSTAPPEN LW. 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.
- FUHLBRIGGE RC, KIEFFER JD, ARMERDING D, KUPPER TS. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature* 1997; 389: 978–81.
- PICKER LJ, KISHIMOTO TK, SMITH CW, WARNOCK RA, BUTCHER EC. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 1991; 349: 796–9.
- DE VRIES IJ, LANGEVELD-WILDSCHUT EG, VAN REIJSEN FC, BIHARI IC, BRUIJNZEEL-KOOMEN CA, THEPEN T. Nonspecific T-cell homing during inflammation in atopic dermatitis: expression of cutaneous lymphocyte-associated antigen and integrin alphaE beta7 on skin-infiltrating T cells. *J Allergy Clin Immunol* 1997; 100: 694–701.
- SANTAMARIA BABI LF, PICKER LJ, PEREZ SOLER MT, 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, PICKER 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.
- HANIFIN J, RAJKA G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980; 92: 44–7.
- HANIFIN JM. Standardized grading of subjects for clinical research studies in atopic dermatitis: workshop report. *Acta Derm Venereol* 1989; 144: 28–30.
- CAMPBELL DE, KEMP AS. Proliferation and production of interferon-gamma and IL-4 in response to *Staphylococcus aureus* and Staphylococcal superantigen in childhood atopic dermatitis. *Clin Exp Immunol* 1997; 107: 392–7.
- LEUNG DY, GATELY M, TRUMBLE A, FERGUSON-DARNELL B, SCHLIEVERT PM, PICKER 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.
- AKBAR AN, TERRY L, TIMMS A, et al. Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J Immunol* 1992; 140: 2171–8.
- HOSHINO T, YAMADA A, HONDA J, et al. Tissue specific distribution and age dependent increase of human CD11b⁺ T cells. *J Immunol* 1993; 151: 2237–46.
- AMLOT PL, TAHIMI F, CHINN D, RAWLINGS E. Activation antigen expression on human T cells. Analysis by two colour flow cytometry of umbilical cord blood, adult blood, and lymphoid tissue. *Clin Exp Immunol* 1996; 105: 176–82.
- WAKITA H, SAKAMOTO T, TOKURA Y, TAKIGAWA M. E-selectin and vascular cell adhesion molecule-1 as critical adhesion molecules for infiltration of T lymphocytes and eosinophils in atopic dermatitis. *J Cutan Pathol* 1994; 21: 33–9.
- CZECH W, SCHOPF E, KAPP A. Soluble E-selectin in sera of patients with atopic dermatitis and psoriasis – correlation with disease activity. *Br J Dermatol* 1996; 134: 17–21.