

The role of skin-homing T cells in extrinsic atopic dermatitis

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Summary

Background: T cells that express Cutaneous Lymphocyte-Associated antigen (CLA) have the potential of migrating to the skin, and are hypothesized to play a role in cutaneous atopic disease.

Aim: To investigate the immune phenotype and cytokine responses to Der p 1 stimulation of CLA+ T cells in extrinsic atopic dermatitis (EAD).

Design: *In vitro* testing, with controls.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from EAD patients ($n=27$) and non-atopic healthy individuals ($n=22$). Phenotypic analysis of naive, CLA+ and non-CLA+ memory/effector CD4+ and CD8+ T cells used markers of cell activation, differentiation, adhesion, apoptosis and chemokine receptor

expression. Cytokine responses in these cells were studied following Der p 1 stimulation.

Results: CLA+ T cells from EAD patients expressed significantly higher levels of CD25, HLA-DR, CD38, CD71, CXCR1, CXCR2 and lower levels of bcl2, CCR5, CCR7, CXCR3, and CD62L ($p<0.05$).

Discussion: In EAD patients, CLA+ T cells express increased levels of markers associated with activation, adhesion and apoptosis, show differences in the level of expression of differentiation markers and display a distinct chemokine receptor preference, compared with cells from healthy controls. These data suggest a significant role for CLA+ T cells in the pathogenesis of cutaneous atopic disease.

Introduction

The combined prevalence of atopic dermatitis (AD), asthma and allergic rhinitis is up to 20–30% in the Western world.¹ The Hanifin and Rajka Diagnostic Criteria for AD have been refined and validated in many populations. When used with AD disease-scoring systems, they allow the rapid and reproducible identification and characterization of affected individuals.^{2,3}

Individuals with AD have an increased tendency to react to common environmental antigens. For example, up to 75% of AD patients have skin-prick test reactivity and/or specific IgE to house dust mite, cat and dog dander and grass.⁴ Patch test challenge with house dust mite extract leads to the development of a clinical and histological eczematous response in many individuals with AD.⁵ Furthermore, careful house dust mite

avoidance measures have been associated with improvement in disease severity scores.⁶

T cells are the dominant cells infiltrating AD skin lesions, and include a significant proportion of CD8+ T cells.^{7,8} Dust-mite- and cat-specific CD4+ and CD8+ T-cell responses have been identified in blood, lesional skin and following dust mite patch test challenge.^{7,9–15}

The controlled expression of vascular selectins and their ligands is thought to be essential for regulated recruitment of T lymphocytes to lymphoid tissues and sites of inflammation. In its commonest form within the periphery, cutaneous lymphocyte-associated (CLA) antigen is carbohydrate-modified P-selectin glycoprotein ligand-1 (PSGL1). It is believed to be a marker on cells able to interact with E-selectin,^{16–18} and thus migrate to the skin.

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E-selectin is expressed on venular endothelial cells of inflamed skin, oral mucosa and the female genital tract, and provides initial signals that trigger rolling of CLA-positive T cells along endothelium. CLA expression by T cells is believed to associate with the transition from the naive to an antigen-experienced state.¹⁹

The CD45RA and CD45RO isoforms are used to broadly identify naive and memory/effector T cells, respectively. However within CD8+ T cells, CD45RA appears to be re-expressed and CD27 lost during terminal differentiation. Thus naive CD8+ T cells can be identified by the expression of both CD45RA and CD27. The pool of memory/effector T cells consists of CLA+ and CLA- subsets.

In previous phenotypic studies, CLA+ T cells were enriched for expression of CD45RO, HLA-DR, CD40L and CD25, with relative loss of CCR7.^{20–22} However, no comprehensive analysis comparing expression of markers of activation, differentiation, adhesion, apoptosis and chemokine receptors on naive, CLA+ and non-CLA+ effector/memory CD4+ and CD8+ T cells from extrinsic atopic dermatitis (EAD) patients and healthy controls has been done. Such an analysis would further refine our understanding of the potential role for CLA expression on both CD4+ and CD8+ T cells in disease pathogenesis, and could potentially contribute to the identification of novel subset treatment targets.

We tested the hypothesis that CLA+ T cells from patients with EAD would show differences in phenotype and cytokine production when compared to those from non-atopic healthy controls.

Methods

Atopic dermatitis was defined according to the UK AD diagnostic criteria, which are both specific and sensitive.^{2,3} These criteria are as follows: an itchy skin condition plus three of more of the following: onset below 2 years of age; history of skin crease involvement; history of generally dry skin; visible flexural dermatitis; personal history of another atopic disease (or history of atopic disease in a first degree relative).

Patients

Symptomatic EAD patients ($n = 27$, mean $\pm$ SD age 24 ± 7 years; F:M 11:16) and non-atopic healthy controls ($n = 22$, mean $\pm$ SD age 27 ± 6 years; F:M 9:13) were recruited through the Department of Dermatology, Churchill Hospital, Oxford, under ethical approval from the Oxfordshire Clinical Research Committee. All individuals were

Caucasian. The age and sex distributions of the two groups were not statistically different ($p > 0.05$). All EAD patients had positive skin prick tests and IgE RAST tests to house dust mite. All of the patients had moderate to severe atopic dermatitis, as defined by the SASSAD disease scoring system. None of the patients or controls had received systemic immunosuppressive treatments during the preceding 6 months. All were using topical steroids and emollients. All individuals are likely to have continued exposure to the ubiquitous allergen Der p 1, as none were practicing house dust mite avoidance measures. Previous reports also show that both atopics and non-atopics may have allergen-specific CD4+ T cells.²³ All individuals were also exposed to the Der p 1-allergen through skin prick testing.

Flow cytometry

Four-colour flow cytometric analysis was performed using a FACS Calibur (Becton Dickinson) with CellQuest software (Becton Dickinson). Some 10^6 peripheral blood mononuclear cells (PBMCs) were centrifuged at 300g for 5 min and resuspended in a volume of 50 μ l R10 (RPMI 1640 medium, Gibco BRL, supplemented with 10% fetal calf serum (Gibco), 2 mM L-glutamine, 50 U/ml penicillin and 50 μ g/ml streptomycin). Directly-conjugated antibodies were added and the samples incubated for 60 min at 4°C. After two washes in cold phosphate-buffered saline (PBS), the samples were fixed in 2% formaldehyde.

Antibodies were sourced from BD Bioscience (CD3, CD4, CD8, CD38, CD45RO, CD45RA, bcl2) and Pharmingen (CLA, Mouse IgG1, Mouse IgG2b, Rat IgM, CD11a, CD11b, CD25, CD27, CD28, CD49d, CD62L, CD69, CD71, CD95, CD162, CCR4, CCR7, CXCR1, CXCR2, HLA-DR, Integrin β 7, CD103, $\alpha\beta$ TCR, $\gamma\delta$ TCR; R&D CCR1, CCR2, CCR3, CCR5, CCR6, CCR9, CXCR3, CXCR5, CXCR4, CXCR6). Antibodies specific for the cytokines IL-4, IL-5, IL-13 and TNF- α were obtained from BD Bioscience. We did a preliminary analysis with a pilot patient group to validate the stains before proceeding to analysis of the full cohort.

PBMCs were stimulated using recombinant Der p 1 allergen (Indoor Biotechnologies) used at 10 μ g/ml. The optimal stimulation time was found to be 8 h, with brefeldin 10 mg/ml added for the last 6 h. Incubation for >12 h caused significant cell death. Thus, Der p 1 experiments were performed using 8 h of incubation, which, as might be anticipated, is longer than the time interval in peptide-stimulated assays.²⁴ The cells were then washed twice with phosphate-buffered saline (PBS)

containing 0.1% bovine serum albumin (BSA), incubated in 0.5 ml permeabilizing solution (Becton Dickinson) at room temperature for 10 min, washed with PBS/BSA, and then incubated for 25 min with the relevant monoclonal antibody-fluorochrome conjugates. After staining, the cells were washed and resuspended in 0.5 ml 1% paraformaldehyde in PBS before flow cytometric analysis. Negative control reagents were used to verify staining specificity of experimental monoclonal antibodies, and as guides for setting markers to delineate positive and negative populations. In these experiments, cytokine staining was confirmed to be intracellular, as <0.2% of cells were positive with surface staining (i.e. staining performed prior to permeabilization). In addition, specificity of antibody reactivity was supported by showing that preincubation of the conjugated antibody with 1 µg of the appropriate recombinant cytokine (Peprotech) for 30 min at 4°C, could abolish staining.

The following T-cell subsets were gated to identify naive and memory/effector CD4+ and CD8+ T cell subsets: CD4+CD45RA+ (naive), CLA+CD4+CD45RO+ (CLA+ memory/effector CD4+ T cells), Non-CLA+CD4+CD45RO+ (Non-CLA+ memory/effector CD4+ T cells); CD8+CD45RA+CD27+ (naive), CLA+CD8+CD45RA+ and CLA+CD8+CD45RA+CD27+ (CLA+ memory/effector CD8+ T cells), Non-CLA+CD8+CD45RA+ and Non-CLA+CD8+CD45RA+CD27+ (Non-CLA+ memory/effector CD8+ T cells). Levels of expression of markers of activation, differentiation, adhesion, apoptosis and chemokine receptor expression on these different T cell subsets were assessed in EAD patients and healthy controls.

Statistics

Statistical analyses used the χ^2 , Fisher's exact and t-tests, $p < 0.05$ being accepted as significant.

Results

Higher percentages of T cells from EAD patients express CLA

CD3+ T cells from EAD patients expressed significantly higher percentages of CLA, compared to those from healthy controls (19.6% vs. 9.6%, $p < 0.05$). This was also true for CD4+ (26.3% vs. 12.5%, $p < 0.05$) and CD8+ (9.2% vs. 5.1%, $p < 0.05$) T-cell subsets. In both groups, 99% of CLA+ T cells expressed the $\alpha\beta$ T cell receptor, consistent with the finding that $\gamma\delta$ T cells are rare in human skin.

Phenotypic analysis of T cells in EAD patients and healthy controls

No differences were found between naive T cells in the two groups of subjects (data not shown). Naive T cells expressed very low levels of activation markers. Mean $\pm$ SD levels of expression of activation, differentiation, adhesion, apoptotic markers and chemokine receptors on CLA+ and non-CLA+ CD4+ and CD8+ T cells from healthy controls and EAD patients are shown in Tables 1–4.

Among healthy controls, significantly higher percentages of memory/effector CLA+CD4+ and CLA+CD8+ T cells expressed CD62L, CD95 and CCR4 ($p < 0.05$), while significantly lower percentages expressed CD11b, CD103, CCR9, CXCR1, CXCR5 and CXCR6, when compared with memory/effector non-CLA+ CD4+ and non-CLA+ CD8+ T cells, respectively ($p < 0.05$) (Tables 1–4). Furthermore, CLA+CD8+ T cells from healthy controls also expressed higher levels of CCR7 and lower levels of CCR5 when compared with non-CLA+ CD8+ T cells ($p < 0.05$ for both comparisons) (Tables 2 and 4).

The expression of activation markers CD25, HLA-DR, CD38 and CD71 on CLA+CD4+ and CLA+CD8+ T cells was significantly ($p < 0.05$) greater in EAD patients than in healthy controls (Tables 1 and 2). No difference in levels was seen between non-CLA+ and naive T cells in the two groups. T cells with higher intensity CLA staining had higher levels of HLA-DR, CD38 and CD71 expression. CD69 was virtually absent on CLA+ T cells, presumably because it is an early marker of activation that is only briefly expressed.

Based on the expression of CCR7, CD62L, CD28 and CD27, CD4+ and CD8+ T cells could be divided into subsets showing early, intermediate and late differentiation programmes. CLA+ T cells in healthy controls expressed high levels of all four differentiation markers (Tables 1 and 2); >95% of CLA+ T cells from healthy control subjects expressed both CD28 and CD27. This suggests that CLA+ memory/effector T cells in healthy controls are at an earlier stage of differentiation than non-CLA memory/effector T cells. In the EAD patients, changes in these markers were seen only in the CLA+ T cell subset (Tables 1 and 2), and appeared to be greater among CD4+ T cells. Levels of CCR7 on CLA+ T cells in EAD patients were lower than in healthy controls, but the majority still expressed CCR7. Levels of expression of CD62L by CLA+ T cells were significantly ($p < 0.05$) lower in EAD patients compared with healthy controls. However, the magnitude of these differences was

Table 1 Phenotypic analysis of CLA+ and non-CLA+ memory/effector CD4+ T cell subsets in healthy controls and EAD patients

Marker	Healthy controls		EAD patients		<i>p</i> *		
	A: CLA+	B: Non-CLA+	C: CLA+	D: Non-CLA+	A vs. B	A vs. C	C vs. D
<i>Activation</i>							
CD25	11.6 (3.8)	10.9 (3.6)	40.2 (5.6)	11.4 (4.7)	NS	<0.01	<0.01
HLA-DR	6.2 (2.3)	8.2 (2.5)	27.7 (6)	9.1 (3.1)	NS	<0.01	<0.01
CD38	6.5 (3.7)	5.9 (3.2)	16.6 (3.2)	5.8 (2.90)	NS	<0.01	<0.01
CD71	15.5 (5.8)	13.5 (5.7)	38.2 (5)	13.1 (6.3)	NS	<0.01	<0.01
<i>Differentiation</i>							
CD28	98 (2.9)	99 (2.8)	84 (4)	99 (3.7)	NS	<0.05	<0.05
CD27	97 (3.2)	91 (3.4)	77 (7)	92 (3.1)	NS	<0.05	<0.05
CD62L	80 (8.9)	52.3 (9.2)	52.6 (8)	54.1 (10.3)	<0.01	<0.01	NS
CCR7	86.3 (7)	85 (6.4)	63.8 (5)	86 (7.2)	NS	<0.05	<0.05
<i>Adhesion and apoptosis</i>							
CD11a (high)	41 (7)	37.1 (7.2)	60.6 (7)	39.3 (8.4)	NS	<0.05	<0.05
CD11a (low)	59 (6)	62.9 (5.4)	39.4 (5)	61.7 (7.3)	NS	<0.05	<0.05
CD11b	0.1 (0.1)	12 (2.9)	1.4 (0.4)	10 (4.5)	<0.01	NS	<0.01
CD49d	82 (9)	86 (6.9)	92 (7)	88 (6.1)	NS	<0.05	NS
Beta-7-Integrin	1.4 (0.5)	60.3 (7.4)	10 (3)	57 (6.9)	<0.01	<0.05	<0.01
CD103	0.6 (0.3)	5 (2.1)	0.7 (0.2)	5 (3.2)	<0.05	NS	<0.05
CD95	97 (6)	52.5 (6.3)	97 (4)	56.9 (5.4)	<0.01	NS	<0.01
Bcl2	94 (4)	96 (4.5)	66 (8)	97 (4.1)	NS	<0.01	<0.01

Data are means (SD). *For comparisons between mean values in the columns indicated. NS: not significant $p > 0.05$.

Table 2 Phenotypic analysis of CLA+ and non-CLA+ memory/effector CD8+ T cell subsets in healthy controls and EAD patients

Marker	Healthy controls		EAD patients		<i>p</i> *		
	A: CLA+	B: Non-CLA+	C: CLA+	D: Non-CLA+	A vs. B	A vs. C	C vs. D
<i>Activation</i>							
CD25	12.2 (4)	11.3 (4.5)	32.2 (4.3)	11.9 (5.3)	NS	<0.01	<0.01
HLA-DR	6 (2.1)	7.6 (2.4)	21.1 (6.2)	9.2 (3.2)	NS	<0.01	<0.01
CD38	7.5 (3.5)	7.4 (2)	25.2 (1.9)	8 (2)	NS	<0.01	<0.01
CD71	14.3 (6)	16.7 (4.1)	30 (4.3)	15.9 (3.7)	NS	<0.01	<0.05
<i>Differentiation</i>							
CD28	96 (3)	81.7 (4)	80 (5.4)	83 (5)	NS	<0.05	NS
CD27	95 (3.8)	90 (3.8)	82.6 (6.2)	90.8 (4.1)	NS	<0.05	NS
CD62L	72.8 (5.1)	23.5 (5.3)	65 (7.6)	25.7 (6.2)	<0.01	<0.05	<0.01
CCR7	81.1 (7)	31 (4.9)	72.8 (8.7)	35 (4.2)	<0.01	NS	<0.01
<i>Adhesion and apoptosis</i>							
CD11a (high)	39 (6)	42 (4.3)	53.4 (5.4)	43 (5.9)	NS	<0.05	<0.05
CD11a (low)	61 (5.4)	58 (6)	46.6 (4.7)	57 (6.3)	NS	<0.05	<0.05
CD11b	0.1 (0.1)	14.1 (2.8)	1.6 (0.5)	15.2 (3.6)	<0.01	NS	<0.01
CD49d	74 (7)	77 (6.2)	88 (6)	80 (4.8)	NS	<0.05	NS
Beta-7-Integrin	1.6 (0.3)	58 (8.4)	8 (2)	54.7 (7.8)	<0.01	<0.05	<0.01
CD103	1.4 (0.2)	14.3 (3.5)	1.3 (0.5)	12.9 (4)	<0.05	NS	<0.05
CD95	95 (5)	58 (7)	95 (5.6)	59 (6.3)	<0.01	NS	<0.01
Bcl2	94 (4)	96 (4)	74 (7.5)	96 (3)	NS	<0.01	<0.01

Data are means (SD). *For comparisons between mean values in the columns indicated. NS: not significant $p > 0.05$.

Table 3 Chemokine receptor expression of CLA+ and non-CLA+ memory/effector CD4+ T cell subsets in healthy controls and EAD patients

Chemokine receptor	Healthy controls		EAD patients		<i>p</i> *		
	A: CLA+	B: Non-CLA+	C: CLA+	D: Non-CLA+	A vs. B	A vs. C	C vs. D
CCR1	6.8 (2.6)	4.6 (1.9)	7 (3.1)	5.2 (3)	NS	NS	NS
CCR2	5.1 (3.4)	16 (3.9)	5.4 (2.4)	14.7 (4.5)	NS	NS	NS
CCR3	1.7 (1.1)	1.3 (0.8)	1.5 (1.9)	1.3 (0.5)	NS	NS	NS
CCR4	98 (6)	20.2 (4.3)	97 (5.4)	36.3 (6.6)	<0.01	NS	<0.01
CCR5	6.7 (2)	15 (3.2)	0.2 (0.1)	17 (4.1)	NS	<0.05	<0.01
CCR6	41 (7)	42.1 (6.1)	18 (4.7)	40.3 (7)	NS	<0.01	<0.01
CCR9	0.1 (0.1)	6 (1.6)	0.1 (0.1)	4 (1.3)	<0.05	NS	NS
CXCR1	2.3 (1.2)	14 (4.3)	13.1 (5.4)	13.4 (5)	<0.01	<0.01	NS
CXCR2	0.8 (0.4)	4.1 (2.1)	4.6 (2.1)	4.5 (1.7)	NS	<0.05	NS
CXCR3	32 (6)	39 (6.7)	10 (3)	34 (5.9)	NS	<0.01	<0.01
CXCR4	85 (7)	94.6 (3.7)	85 (6.8)	95.5 (4.5)	NS	NS	NS
CXCR5	0.1 (0.1)	17 (2.1)	4.3 (2.1)	15.8 (3.9)	<0.01	NS	<0.05
CXCR6	0.7 (0.2)	8.1 (4.5)	0.7 (0.3)	13.4 (5.7)	<0.05	NS	<0.05

Data are means (SD). *For comparisons between mean values in the columns indicated. NS: not significant $p > 0.05$.

Table 4 Chemokine receptor expression of CLA+ and non-CLA+ memory/effector CD8+ T cell subsets in healthy controls and EAD patients

Chemokine receptor	Healthy controls		EAD patients		<i>p</i> *		
	A: CLA+	B: Non-CLA+	C: CLA+	D: Non-CLA+	A vs. B	A vs. C	C vs. D
CCR1	6 (2.3)	8.1 (2.6)	6.2 (2.8)	7.5 (3.2)	NS	NS	NS
CCR2	4.9 (2)	19 (4.2)	5.2 (2.1)	18.5 (4.1)	NS	NS	NS
CCR3	1.7 (1)	1.1 (0.6)	1.3 (0.7)	1.2 (0.4)	NS	NS	NS
CCR4	96 (5)	8 (2.1)	97 (7.8)	19.6 (4.4)	<0.01	NS	<0.01
CCR5	5.7 (1.5)	51.2 (8.9)	0.4 (0.2)	49.4 (7.5)	<0.01	<0.05	<0.01
CCR6	47 (4)	26 (6.8)	22 (2.9)	28 (5.7)	NS	<0.01	NS
CCR9	0.1 (0.1)	4 (0.9)	0.1 (0.1)	4 (0.7)	NS	NS	NS
CXCR1	2.1 (1)	18 (6.3)	10.5 (4.6)	19.1 (5.7)	<0.01	<0.05	NS
CXCR2	0.6 (0.5)	1.1 (0.2)	4 (2.3)	1.3 (0.7)	NS	<0.05	NS
CXCR3	26 (4.3)	52 (8.5)	12 (4.2)	46 (7.3)	NS	<0.01	<0.01
CXCR4	83 (6.4)	82 (9.1)	87 (6.7)	84 (10.1)	NS	NS	NS
CXCR5	0.1 (0.1)	5 (0.8)	3.5 (1.7)	5 (1.2)	<0.05	NS	NS
CXCR6	0.3 (0.1)	28.2 (5.9)	0.3 (0.2)	33.9 (5.8)	<0.01	NS	<0.01

Data are means (SD). *For comparisons between mean values in the columns indicated. NS: not significant $p > 0.05$.

smaller when compared with differences in levels of expression of the activation markers.

Levels of expression of markers relating to adhesion [CD11a (LFA-1), CD11b (Mac-1), VLA-4 α (CD49d), β 7 integrin and CD103] and apoptosis on CLA+CD4+ and CLA+CD8+ T cells from patients with EAD and healthy controls are shown in Tables 1 and 2. Although a sizeable percentage of CLA+ T cells expressed CD11a and CD49d, most did not express CD11b or β 7 integrin and CD103 (markers typically linked to gut homing). Levels of

expression of the anti-apoptotic protein bcl2 by CLA+ T cells were significantly lower in EAD patients than healthy controls ($p < 0.05$) (Tables 1 and 2). Most CLA+ T cells (mean 96%) from both groups of subjects expressed CD95, with no significant differences between the groups.

Levels of expression of chemokine receptors CCR1–9 and CXCR1–6 by CLA+CD4+ and CLA+CD8+ T cells in EAD patients and healthy controls are shown in Tables 3 and 4. Both CLA+CD4+ and CLA+CD8+ T cells from EAD

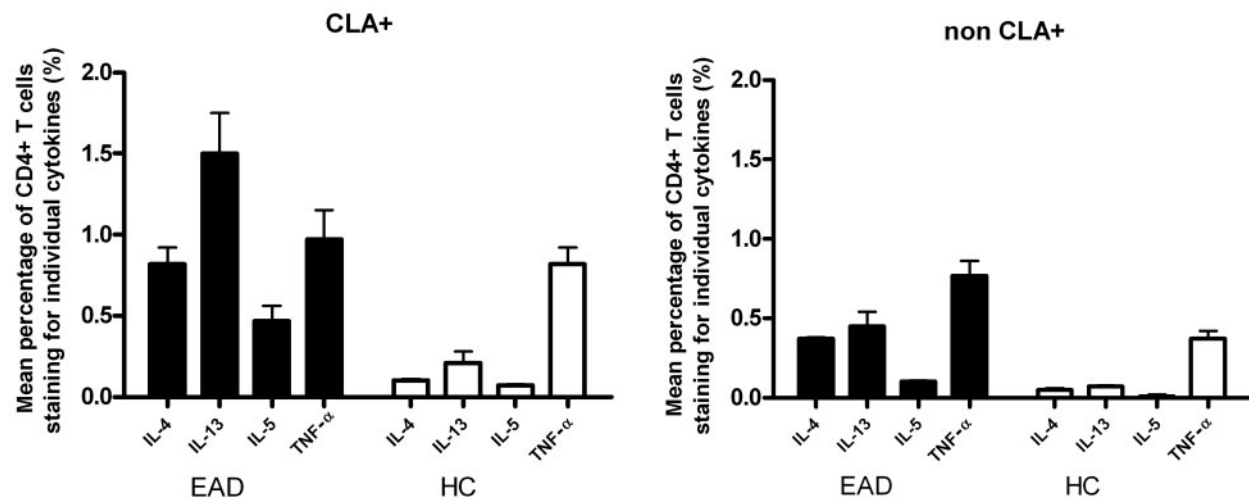


Figure 1. Production of cytokines by CLA+ or non-CLA+ T cells in response to stimulation with Der p 1 allergen (10 µg/ml) for 8 h, with brefeldin 10 mg/ml added for the last 6 h. EAD, extrinsic atopic dermatitis; HC, healthy controls.

patients expressed significantly lower levels of CCR5, CCR6, CCR7 and CXCR3 and significantly higher levels of CXCR1 and CXCR2 compared with healthy controls (both $p < 0.05$). Levels of expression of other chemokine receptors were not significantly different between the groups of subjects.

Differences in cytokine production by CLA+ and CLA– T cells

On the basis of previous studies,^{9,20,21} we had predicted that the CLA+ T cell subset would be enriched for cells specific for cutaneous antigens. We investigated whether this was the case in our patients. Naïve T cells did not produce significant amounts of any of the cytokines measured in these studies. Differences were seen in levels of cytokine production by CLA+ and non-CLA+ memory/effector T cells in response to Der p 1-allergen stimulation (Figure 1). Der-p-1-specific CLA+ T cells from both groups were able to produce TNF-α. However, the Th2 cytokines (IL-4, IL-13 and IL-5) were predominantly produced by Der-p-1-specific CLA+ T cells from EAD patients. Non-CLA+ T cells did contain cells responsive to Der p 1, but levels were significantly lower than in the CLA+ T cell subset.

Discussion

Higher percentages of T cells expressed the skin homing marker CLA in our EAD patients compared with non-atopic healthy controls. In addition, CLA+CD4+ and CLA+CD8+ T cells from our

EAD patients showed increased expression of markers of activation, adhesion and apoptosis, and preferential chemokine receptor usage. Levels of expression of differentiation markers differed between EAD patients and healthy controls. Der-p-1-specific CLA+ T cells from EAD patients produced both type 1 and type 2 cytokines, with the CLA+ T cell subset containing cells with Der-p-1 specificity. These data suggest a significant role for CLA+ T cells in the pathogenesis of EAD.

High frequencies of CLA-positive functional MelanA-specific CD8+ T cells are found in the peripheral blood of individuals with vitiligo, consistent with a role for CD8+ T cells in the pathogenesis of melanocyte destruction.²⁵ T cells specific for house-dust-mite-derived Der p 1 are found in the blood of individuals with atopic disease, and can express CLA.^{9,20,21} Herpes simplex virus (type II)-specific CD8+ T cells express high levels of CLA, consistent with their specificity for a skin- or genital-tropic virus.²⁶ Taken together with our present findings that the CLA+ subset contains T cells with Der p 1 specificity, this suggests that CLA identifies a functionally relevant population of cells that may play a role in the pathogenesis of skin disease.

The activated phenotype in EAD patients may reflect their specificity for persistent and ubiquitous atopic antigens. Our data confirm and extend previous findings that CLA+ T cells are broadly susceptible to apoptosis, and that this may increase in individuals with severe atopic dermatitis.²⁷ Our result also suggest that differential Th1 and Th2 susceptibility to apoptosis may contribute to Th2 persistence, and although we have not compared Th1/Th2 cell expression of apoptotic markers,

our finding that CD95 is present at high levels on CLA+ T cells would be compatible with such data.

Based on the expression of differentiation markers CD28, CD27, CD62L and CCR7, CLA+ T cells in healthy controls were largely at an early stage of differentiation. Furthermore, most CLA+ T cells in EAD patients and healthy controls were CCR7+CD62L+, suggesting that they could maintain lymph node homing despite being able to home to skin. Such continued dual skin and lymph node homing may contribute to persistent T-cell activation and cutaneous inflammation. Progression along the intermediate and late differentiation programme is believed to be associated with acquisition of rapid effector function,²⁸ which may be altered in the case of CLA+ T cells. Thus even after showing evidence of antigen encounter and activation, the CLA+ cells unexpectedly maintain the expression of markers associated with lymph node homing.

CCR7 binds MIP-3 β /ELC (CCL19) and SLC (CCL21), and is thought to be important in lymph-node homing. Expression of CCR7 on T cells tends to be lost during the transition from naive to antigen-experienced state. Although we predicted that the acquisition of the tissue-specific homing receptor CLA would be accompanied by the loss of CCR7 (believed to be a marker of cells that can home to lymph nodes), our findings did not confirm this. Thus in EAD patients, although CLA+ T cells are activated, and susceptible to apoptosis, they largely do not enter the typical intermediate and late differentiation programme.

The differential expression of chemokine receptors by CLA+ T cells may contribute to the differences in migration of these cells to different inflammatory or immune compartments. CCR4 and CCR10 expression on CLA+ T cells has been suggested to play a role in chemotaxis to inflamed epidermis via TARC/CCL17 and CTACK/CCL27, respectively. Little is known of other chemokine receptor expression on CLA+ T cells. Our EAD patients had preferential expression of CXCR1 and CXCR2 and lower levels of expression of CCR5, CCR6, CCR7 and CXCR3 by CLA+CD3+ T cells, compared with healthy controls.

Most CLA+ T cells in both EAD patients and healthy controls expressed CCR4. This argues in favour of a contributory role for CCR4 in homing of these cells to the skin. CCR3 and CCR4 have been previously linked to a Th2 phenotype. We found that CLA expression was associated with production of cytokines in response to Der p 1 (Figure 1). However we did not find significant differences between EAD patients and healthy controls as regards CCR3 or CCR4 expression by CLA+ T cells.

CCR5 and CXCR3 have been suggested as being associated with a Th1 phenotype,²⁹ and with antigen-experience and effector differentiation in Th1 cells. Reduced levels of expression of CCR5 and CXCR3 on CLA+ T cells from EAD patients thus are in keeping with the Th2-type cytokine profiles in these patients. CCR6 binds MIP-3 α (CCL20) and possibly the antimicrobial β -defensins. Its expression is thought to be associated with differentiation of T cells into effector cells.³⁰ The lower levels of expression of CCR6 on CLA+ T cells from EAD patients may mean that in addition to these patients having reduced levels of defensins, they also have altered levels of these antimicrobial peptide ligands.³¹

CXCR1 and CXCR2 bind a number of chemokines, including IL-8 (CXCL8), GRO α (CXCL1), GRO β (CXCL2), GRO γ (CXCL3), ENA-78 (CXCL5), GCP-2 (CXCL6), and NAP-2 (CXCL7). Previous studies suggest IL-8 may be important for transendothelial migration of CLA+ T cells.³² Thus increased levels of expression of CXCR1 and CXCR2 (receptors for IL-8) on CLA+ T cells from EAD patients suggests this receptor-ligand pair may have an important role in disease pathogenesis.

Increased frequencies of both CD4+ and CD8+ T cells producing type-2 cytokines (including IL-4, IL-5, IL-13) circulate in the peripheral blood of individuals with AD.^{20,33,34} While we and others have previously shown that CLA+ T cells contain cells with Der p 1 specificity and that Der-p-1-specific T cells can produce both type 1 and type 2 cytokines,^{9,20,21} we felt it important to confirm in the current cohort that CLA+ T cells did indeed include T cells recognizing putative cutaneous antigens.

In summary, we compared the phenotypic and cytokine-producing functions of CLA+ T cells in EAD patients and non-atopic healthy controls. CLA+ T cells from EAD patients had increased expression of markers of activation, adhesion and apoptosis, differences in the level of expression of differentiation markers and preferential chemokine receptor usage. These findings suggest a significant role for CLA+ T cells in the pathogenesis of atopic dermatitis, and this population of cells may represent a useful future therapeutic target.

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