

Different cytokine production and activation marker profiles in circulating cutaneous-lymphocyte-associated antigen⁺ T cells from patients with acute or chronic atopic dermatitis

C. Antúnez*, M. J. Torres*, C. Mayorga*, J. A. Cornejo-García*, L. F. Santamaría-Babi† and M. Blanca*

*Research Unit for Allergic Diseases, Allergy Service, Carlos Haya Hospital, Málaga, Spain and †Almirall Prodesfarma, Barcelona, Spain

Summary

Background Atopic dermatitis (AD) is an inflammatory skin disease whose lesions can have two stages: acute and chronic. In skin biopsies a biphasic pattern of cytokine expression has been shown, Th2 in acute lesions and Th1 in chronic AD lesions.

Objective We investigated the expression of an activation marker and a homing receptor, as well as cytokine production, in different peripheral blood T cell subpopulations from AD patients with chronic (Group A) and acute lesions (Group B) and controls.

Methods We evaluated 26 adult AD patients (12 Group A, 14 Group B) and 14 non-atopic controls. IgE was measured by immunoassay. CD4, CD8, cutaneous-lymphocyte-associated antigen (CLA) and human leucocyte antigen (HLA)-DR expression, and cytokine production (IL-2, IL-13, IFN- γ , TNF- α , IL-10, IL-4) were analysed in mononuclear cells by flow cytometry.

Results In Group B there was a significant increase in eosinophil levels and a non-significant increase in IgE. In Group A we found an increase in CLA⁺CD4⁺ cells (8.19 ± 1.84) compared with controls (4.83 ± 0.53) ($P < 0.05$) and CD4⁺HLA-DR⁺ cells in the CLA⁺ subpopulation (45.54 ± 15.40) compared with controls (30.49 ± 6.07) ($P < 0.05$). In the CLA⁺CD4⁺ subpopulation, there was a significant increase in IL-4, IL-13 and TNF- α production in Group B (12.46 ± 7.7 , 11.26 ± 5.97 , 43.92 ± 15.55) compared with controls (5.34 ± 3.50 , 4.54 ± 1.78 , 19.29 ± 9.97) with no differences in Group A.

Conclusion Greater immunological differences were detected in peripheral blood from patients with acute compared with chronic lesions, especially in the circulating T cell-subset with skin tropism that preferentially responded to cutaneous allergens. This is the first demonstration of phenotypic changes in circulating CLA⁺ T cells between AD patients with acute and chronic lesions.

Keywords acute, atopic dermatitis, chronic, CLA, cytokines, homing, T cells

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Introduction

Atopic dermatitis (AD) is a chronic, relapsing, highly pruritic, inflammatory skin disease that frequently predates the development of allergic rhinitis or asthma [1]. There are two stages in AD lesions: acute and chronic. AD with acute lesions is characterized by intensely pruritic, erythematous papules over erythematous skin, associated with extensive excoriations, erosions, and serous exudate and AD with chronic lesions by thickened skin, lichenification and fibrotic papules.

Despite multiple studies of AD, its pathogenesis is still not completely understood. The efficacy of treatment acting directly on T cells underlies the relevance of these cells in the pathology of AD [2]. The most of T cells homing to the skin are of the CD45RO⁺ memory/effector phenotype and they

express the selective skin-homing receptor, cutaneous-lymphocyte-associated antigen (CLA) [3]. Moreover, T cell specificity for skin-related allergens is confined to the CLA⁺ T cell subpopulation [4] and peripheral blood CLA⁺CD45RO⁺ T cells demonstrate typical features of *in vivo* activation [4, 5]. A significant increase in CLA amongst CD4⁺ T cells has been found in AD patients compared with controls and psoriatic patients, with no differences in CLA expression in the CD8⁺ T cells between the three groups [6]. These changes in the immune response may be due to an imbalance of the cytokine network.

Although Th2-type cells are thought to play a key pathogenic role in AD [7–11] Th1 cytokines also contribute to the pathogenesis of skin inflammation [12–14]. Data from skin biopsies obtained from uninvolved skin and acute and chronic skin lesions of AD patients indicate that acute skin lesions contain significantly more cells producing IL-4, IL-5 and IL-13, but not IFN- γ compared with chronic skin lesions. In AD with chronic lesions cells expressing IL-4 and IL-13 were significantly decreased but cells expressing IL-5 and

Correspondence: Miguel Blanca, Allergy Service, Hospital Civil, Plaza del Hospital Civil s/n, 29009 Málaga, Spain.

E-mail: mariaj.torres.sspa@juntadeandalucia.es

IFN- γ were increased [15, 16]. This biphasic pattern of cytokine expression in AD has also been demonstrated in studies of skin biopsy specimens from atopic patch-test reaction sites [4, 15, 17]. These studies indicate that in skin lesions provoked by epicutaneous application of allergens in AD patients, the majority of allergen-specific T cells produce predominantly Th2 cytokines such as IL-4, IL-5 and IL-13, as do skin homing peripheral blood T cells. Recent studies have shown that IFN- γ predominates over IL-4 in chronic skin lesions and older patch-test reactions in AD even though IL-5 and IL-13 remain at high levels [18–21].

Most comparisons between AD patients with acute and chronic lesions have been performed in skin biopsies but few data are available from analysis of peripheral blood mononuclear cells (PBMC). We therefore investigated the expression of different T cell subpopulations, an activation marker and a skin-homing receptor, as well as cytokine expression in peripheral blood T cells from AD patients with acute and chronic lesions and controls.

Materials and methods

Patients

Twenty-six patients with AD according to the criteria of Hanifin and Rajka [22] were included in the study. None had asthma or any other skin disease or had used any systemic steroids or therapies such as tacrolimus or psoralen-ultraviolet A for at least 6 months before peripheral blood was drawn. Clinical activity of the skin lesions was graded according to the severity of AD (3–4, mild; 4.5–7.5, moderate; 8–9, severe) [23]. Patients were classified into two groups according to the time elapsed between the beginning of the relapse and collection of the blood sample:

- Group A: Formed by patients with chronic lesions (lesion onset 2 weeks or more before the study); 12 patients (seven women and five men, with a mean age of 31 years, ranging from 27 to 37). The mean severity score was 6.8.
- Group B: Formed by patients with acute lesions (lesion onset less than 3 days before the study); 14 patients (10 women and four men, with a mean age of 23 years, ranging from 18 to 26). The mean severity score was 7.2.

Fourteen healthy non-atopic persons with no skin diseases formed a control group, nine women and five men, with a mean age of 29 years, ranging from 27 to 33.

A peripheral blood cell count was performed in all patients and controls. Total serum IgE was quantified by fluoroenzymeimmunoassay (UniCAP, Pharmacia Diagnostic AB, Uppsala, Sweden), according to the manufacturer's instructions.

The study was approved by the institutional review board, and informed consent for all the diagnostic procedures was obtained from all patients and controls.

Phenotypic immunofluorescence analysis

PBMC from each patient were isolated by density gradient centrifugation (Nycomed, Oslo, Norway) from 6 mL of heparinized venous blood for immunofluorescence staining, as previously described [24]. Briefly, 5×10^5 cells were sequentially stained with different MoAbs at 4 °C. Stained

cells were fixed in 1% paraformaldehyde in phosphate-buffered saline. Cell subsets were assessed with CD3-PerCP, CD4-APC and CD8-APC, the homing receptor expression with CLA-fluorescein isothiocyanate and the activation state with HLA-DR-phycoerythrin (Becton-Dickinson, San Jose, CA, USA).

Cytokine production was determined in cells stimulated for 4 h at 37 °C with phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO, USA) and ionomycin (Sigma) at a final concentration of 25 ng/mL and 1 μ g/mL of cell suspension, respectively, in which the intracellular protein transport of cytokines was disrupted with monensin (Sigma) at 10 μ g/mL of cell suspension. After subset cell surface staining for 15 min, cells were fixed for 15 min using Fix and Per cell permeabilization kit (Caltag Laboratories, Burlingame, CA, USA) and permeabilized with the same kit and stained intracellularly with monoclonal antibodies to cytokines (IL-2-FITC, IFN- γ -PE, TNF- α -PE, IL-10-FITC, IL-4-PE and IL-13-PE, all provided by Becton-Dickinson) for 30 min at room temperature. After washing, cells were analysed on the flow cytometer. A disadvantage of PMA activation is the down-regulation of certain surface proteins, including CD4. Therefore, as most CD4⁺ T cells are contained within the CD3⁺/CD8[−] population, in the analysis of the cytokine production, when we refer to the CD4⁺ population we in fact measured the CD3⁺/CD8[−] population.

Six parameters were analysed on a FacsCalibur flow cytometer using Cell Quest software. Negative isotype controls were used to verify the staining specificity of the antibodies used.

Statistical study

The data analysed, expressed as the number of cells expressing the different markers, are continuous variables with normal distribution. Comparison of means for these variables was performed in two ways: (a) between AD patients and controls using the Student's *t*-test and (b) between Group A, Group B and controls using ANOVA with the Bonferroni test. All reported *P*-values represented two-tailed tests, with values of 0.05 or less considered statistically significant. The statistical analysis was performed using the SPSS program, version 9.0.

Results

Eosinophil levels were significantly increased in AD patients (0.45 ± 0.17) compared with controls (0.21 ± 0.19 , $P < 0.05$). However, after separating the patients according to skin lesion activity, this increase was only significant in Group B ($P < 0.05$) (Group A 0.43 ± 0.18 ; Group B 0.49 ± 0.15). There was also a significant increase in total IgE levels in AD patients (1732 ± 1500) compared with controls (300 ± 82.95 , $P < 0.05$) (data not shown).

Cell surface expression

Evaluation of the CLA expression in peripheral blood T cells from AD patients and controls (Fig. 1a) showed significant differences (AD patients 8.62 ± 3.17 ; controls 6.02 ± 0.9 ;

$P < 0.05$). Classification of AD patients according to skin lesion activity showed a significant increase in just AD patients from Group A (9.87 ± 2.21) compared with controls ($P < 0.05$).

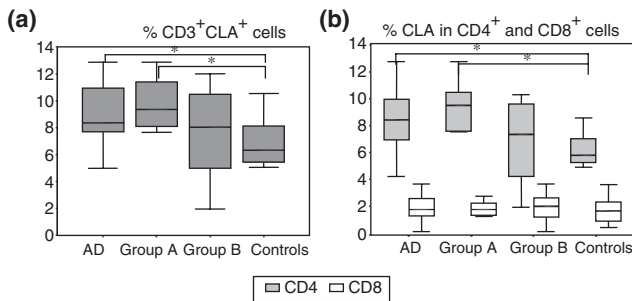


Fig. 1. (a) Box plot of the percentage of cutaneous-lymphocyte-associated antigen (CLA)⁺ expression in CD3⁺ cells. (b) Box plot of the percentage of CLA⁺ expression in both CD4⁺ and CD8⁺ cells. The study was performed in patients with atopic dermatitis (AD), in Group A (patients with chronic lesions), in Group B (patients with AD acute lesions) and in controls. Statistical differences (*) are considered significant with a $P < 0.05$.

Original data of CLA⁺CD4⁺ and CLA⁺CD8⁺ of patients from Groups A and B and controls are shown on the left of Tables 1–3, respectively. Levels of CLA⁺CD4⁺ cells were significantly higher in AD patients (6.91 ± 2.57) compared with controls (4.83 ± 0.53) ($P < 0.05$), but there were no differences in levels of CLA⁺CD8⁺ cells (AD patients 1.71 ± 0.82 ; controls 1.68 ± 0.54). Comparisons of the CLA⁺CD4⁺ expression between the three groups showed that these levels were significantly increased only in Group A (Group A: 8.19 ± 1.84 ; Group B: 5.84 ± 1.84 ; controls 4.83 ± 0.53 ; $P < 0.05$), whereas there were no significant differences in CLA expression among CD8⁺ T cells (Group A: 1.68 ± 0.54 ; Group B: 1.73 ± 1.05 ; controls 1.18 ± 0.59) (Fig. 1b). Figure 2 shows the CLA⁺CD4⁺ and CLA⁺CD8⁺ flow cytometry results of two patients (case 1 in Group A, case 5 in Group B) and control number 5.

Regarding T cell activation, we found a significant increase in CD4⁺HLA-DR⁺ cells in the CLA⁺ subpopulation in AD patients from Group A (45.54 ± 15.40) compared with controls (30.49 ± 6.07) ($P < 0.05$) with no differences between Group B (34.41 ± 9.42) and controls or between AD patients

Table 1. Original data of each individual in Group A (formed by patients with chronic lesions)

Patients	CLA		IL-4		IL-13		IL-2		IL-10		IFN- γ		TNF- α	
	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
1	8.95	2.44	1.80	1.80	1.85	1.73	5.03	4.48	7.26	10.67	9.26	15.62	42.39	29.19
2	6.49	1.16	1.91	4.19	1.89	2.29	3.31	2.03	2.36	1.96	3.73	7.70	11.41	10.96
3	6.50	1.60	2.30	3.00	1.69	3.59	2.87	2.70	1.43	3.96	4.12	6.58	5.91	10.41
4	10.87	2.00	1.74	10.12	0.84	3.51	1.94	5.02	1.44	5.41	3.22	5.49	14.60	20.29
5	8.13	1.22	2.73	4.69	0.77	1.30	2.59	4.50	1.06	3.57	10.63	18.30	15.24	20.70
6	8.65	1.68	2.09	4.35	1.20	1.62	5.23	4.25	3.20	9.56	9.45	12.30	17.85	28.65
7	9.85	2.03	1.66	3.65	1.75	2.35	3.10	2.69	2.75	1.63	3.28	20.32	19.65	10.75
8	7.24	1.26	1.65	3.56	0.85	3.85	1.85	2.10	1.85	4.52	4.65	7.45	6.25	20.65
9	5.11	1.85	2.13	9.97	1.96	3.66	5.45	4.25	1.78	5.47	3.62	5.62	17.98	1.54
10	8.95	2.14	2.36	4.12	0.74	1.47	5.23	5.29	1.33	3.75	9.87	10.23	20.32	22.10
11	6.45	1.26	2.87	2.96	1.85	1.98	6.52	4.75	2.56	5.21	9.85	18.52	18.98	20.29
12	11.05	1.85	1.85	4.75	1.98	3.65	3.02	1.98	1.40	2.10	9.56	7.45	19.65	11.32

Percentage of CLA and the different cytokines in both CD4 and CD8 subsets. CLA, cutaneous-lymphocyte-associated antigen.

Table 2. Original data of each individual in Group B (formed by patients with acute lesions)

Patients	CLA		IL-4		IL-13		IL-2		IL-10		IFN- γ		TNF- α	
	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
1	8.80	3.19	3.99	3.76	5.93	4.95	3.65	2.63	2.36	7.56	10.65	17.65	47.54	52.89
2	5.43	2.32	2.11	3.43	2.20	2.81	3.99	3.60	3.33	5.67	18.57	18.55	23.08	21.08
3	8.21	2.26	2.88	5.38	3.51	2.98	4.39	1.88	1.70	1.64	9.08	20.99	19.16	11.66
4	7.21	1.13	3.95	3.56	3.74	1.85	3.44	3.08	2.66	3.24	5.85	15.08	21.80	12.76
5	3.68	1.32	2.64	4.42	4.52	1.33	4.49	3.90	2.75	2.68	8.16	15.54	13.74	8.96
6	1.75	0.21	3.32	4.96	1.94	1.46	3.58	3.21	2.48	7.15	8.90	29.96	13.01	8.15
7	7.56	1.75	3.56	3.67	2.03	4.98	3.65	3.01	2.56	2.32	10.32	20.36	47.63	51.32
8	4.65	1.76	2.16	3.90	1.92	3.02	3.96	3.52	2.65	5.65	9.96	20.16	25.63	25.62
9	4.96	1.28	2.78	4.56	3.97	2.85	4.16	3.22	2.35	3.72	11.32	21.65	11.32	10.25
10	7.34	2.63	3.98	5.63	3.25	3.76	4.36	3.02	2.22	3.56	10.72	20.56	18.65	9.65
11	4.65	0.63	2.56	3.65	4.86	1.65	4.56	3.65	2.96	3.65	8.34	17.65	22.30	8.65
12	5.76	2.88	3.82	4.89	5.98	1.26	3.46	3.32	2.75	1.65	9.65	22.32	12.32	11.52
13	5.85	1.62	2.60	3.72	2.90	2.95	4.38	3.70	2.96	6.05	13.15	23.91	35.39	20.63
14	5.96	1.78	3.95	4.63	4.38	2.30	3.56	2.64	2.16	2.03	7.09	16.15	10.63	19.63

Percentage of CLA and the different cytokines in both CD4 and CD8 subsets. CLA, cutaneous-lymphocyte-associated antigen.

Table 3. Original data of each individual control

Patients	CLA		IL-4		IL-13		IL-2		IL-10		IFN- γ		TNF- α	
	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
1	4.27	1.76	1.19	2.91	0.53	1.24	6.22	3.46	3.33	3.00	9.83	18.57	9.42	13.61
2	5.63	1.85	2.58	3.74	1.80	2.02	4.85	5.85	3.00	5.69	17.06	28.48	14.01	16.60
3	4.65	0.48	0.34	2.12	1.30	3.14	1.23	2.96	1.87	12.47	10.23	17.56	2.55	2.51
4	5.83	2.33	2.34	2.40	1.13	1.19	1.52	1.13	2.16	3.25	4.75	6.25	5.22	7.13
5	4.57	0.50	1.38	1.69	0.64	1.37	5.24	1.02	1.00	1.49	9.27	24.46	10.94	12.94
6	4.52	1.28	1.35	5.10	1.19	3.87	4.02	4.61	1.49	2.01	5.13	10.71	10.64	14.73
7	5.34	1.26	1.19	2.91	0.53	1.24	6.22	3.46	3.33	3.00	9.83	18.57	9.42	13.61
8	4.41	0.73	2.58	3.74	1.80	2.02	4.85	5.85	3.00	5.69	11.62	28.48	14.01	16.60
9	4.13	1.07	3.25	1.17	0.97	1.56	6.25	7.89	2.36	4.65	9.86	7.85	14.32	13.32
10	4.98	0.95	0.35	4.74	2.30	2.51	5.36	2.63	1.32	3.65	8.65	25.36	6.52	16.53
11	4.56	1.92	2.66	4.69	1.95	1.02	7.96	4.65	2.65	6.78	8.54	12.56	10.32	3.65
12	4.48	0.51	0.45	1.02	1.13	1.85	1.26	5.56	3.26	2.16	6.52	17.63	11.62	7.69
13	5.24	1.10	0.98	5.32	0.82	3.82	5.42	5.98	1.65	1.86	9.65	15.15	10.32	12.32
14	5.25	0.85	2.94	5.09	2.70	0.85	3.26	2.45	3.65	4.12	5.63	9.36	3.25	13.25

Percentage of CLA and the different cytokines in both CD4 and CD8 subsets. CLA, cutaneous-lymphocyte-associated antigen.

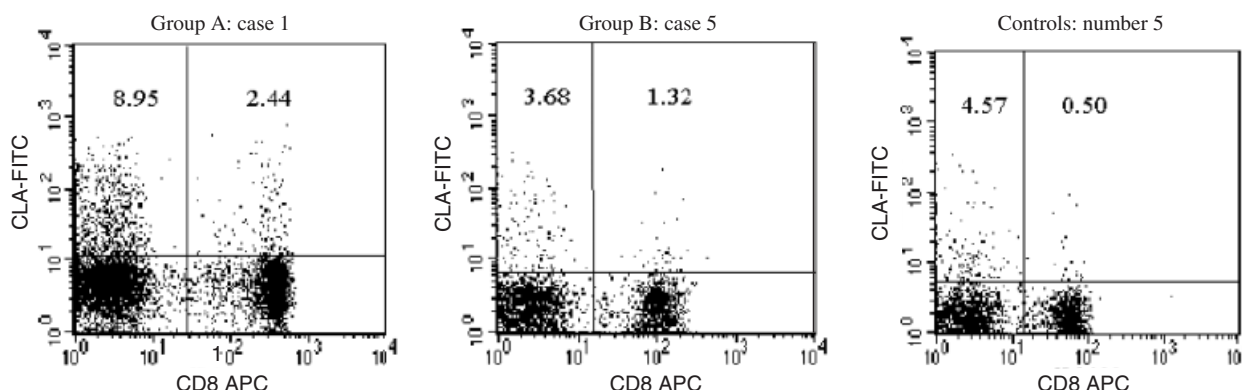


Fig. 2. Flow cytometric expression of cutaneous-lymphocyte-associated antigen (CLA)⁺CD4⁺ and CLA⁺CD8⁺. When we refer to the CD4⁺ population we in fact measured the CD3⁺/CD8[−] population. Example of one patient in Group A (case 1), one patient in Group B (case 5) and one control (number 5).

(38.86 ± 12.71) and controls (Fig. 3a). There were no significant differences in the CD8⁺HLA-DR⁺ cells in the CLA⁺ subpopulation between the groups (AD: 33.58 ± 12.16; Group A: 39.26 ± 13.25; Group B: 29.80 ± 10.85; controls 30.49 ± 6.07) (Fig. 3b).

Intracellular cytokine production

Original data of the different cytokine production in both CD4 and CD8 subpopulations in patients from Groups A and B and controls are shown in Tables 1–3, respectively. Comparison of cytokine production between CD4⁺ and CD8⁺ cells is shown in Fig. 4. The mean cytokine production values of IL-4, IL-13 and TNF- α in the CD4⁺ subpopulation in patients with AD were 2.67 ± 0.8, 2.62 ± 1.6 and 20.71 ± 12.96, respectively; in Group A it was 2.09 ± 0.41, 1.40 ± 0.55 and 17.9 ± 14.17; in Group B it was 3.14 ± 0.74, 3.64 ± 1.48 and 23.05 ± 12.7; and in controls it was 1.68 ± 1.01, 1.34 ± 0.66 and 9.64 ± 4.07. The increases compared with controls in these three cytokines were significant in patients with AD ($P=0.03$, 0.03 and 0.02) and in patients from Group B ($P<0.05$, 0.002 and <0.05),

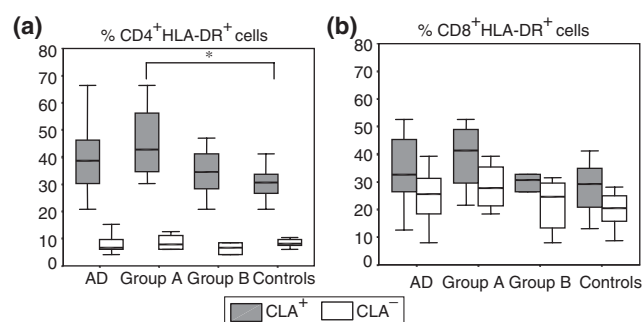


Fig. 3. (a) Box plot of the percentage of HLA-DR expression in CLA⁺ and CLA[−] in CD4⁺ subsets. (b) Box plot of the percentage of HLA-DR expression in CLA⁺ and CLA[−] in CD8⁺ subsets. Comparisons have been made between patients with atopic dermatitis (AD), Group A (patients with chronic lesions), Group B (patients with acute lesions) and controls. Statistical differences (*) are considered significant with a $P<0.05$.

but not in patients from Group A. There were no significant differences for IL-4, IL-13 and TNF- α in the CD8⁺ subpopulation and for IL-2, IL-10 and IFN- γ in either CD4⁺ or CD8⁺ subpopulations. Figure 5 shows the flow

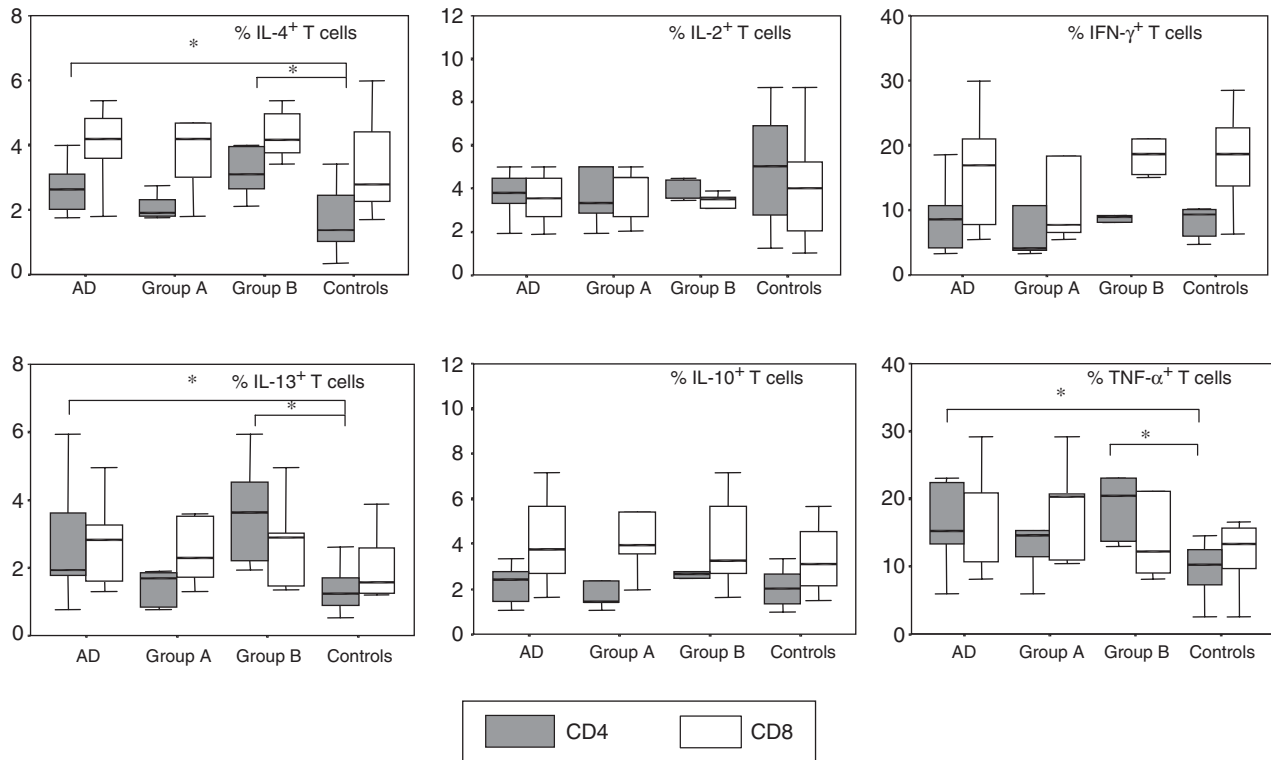


Fig. 4. Box plot of the percentage of both CD4⁺ and CD8⁺ cell subsets producing IL-4, IL-13, IL-2, IL-10, IFN- γ and TNF- α . Comparisons have been made between patients with atopic dermatitis (AD), Group A (patients with chronic lesions), Group B (patients with acute lesions) and controls. Statistical differences (*) are considered significant with a $P < 0.05$.

cytometry of the cytokine production values of IL-4, IL-13, and TNF- α in the CD4⁺ and CD8⁺ subpopulations of two patients (case 11 in Group A, case 4 in Group B) and control number 5.

Comparison of cytokine production between CLA⁺ and CLA⁻ cells in both CD4⁺ and CD8⁺ subpopulations showed that in CD4⁺CLA⁺ cells in patients from Group B the production of IL-4 (12.46 ± 7.7), IL-13 (11.26 ± 5.97) and TNF- α (43.92 ± 15.55) was significantly increased compared with controls (IL-4: 5.34 ± 3.50 ; IL-13: 4.54 ± 1.78 ; TNF- α : 19.29 ± 9.97 ; $P < 0.05$). The production in patients from Group B of IL-13 (3.15 ± 1.56) was also elevated in CD4⁺CLA⁻ cells (controls: 1.04 ± 0.45 , $P = 0.008$) and in CD8⁺CLA⁺ cells (10.68 ± 4.96 ; controls: 4.02 ± 1.51 , $P < 0.05$) (Fig. 6).

Discussion

Skin homing T cells play an important role in the pathogenesis of AD [25]. However, little is known about the phenotype of circulating CLA⁺ T cells during the acute or chronic stages of AD. In the present study we analysed PBMC from AD patients at different stages of the disease, acute and chronic, and compared them with non-atopic controls. We investigated different T lymphocyte subsets, especially CLA⁺ T cells, because previous studies have implicated these cells in the immuno-inflammatory process of AD [3–6]. We found an increase in CLA⁺ cells in peripheral blood in AD patients compared with controls (Fig. 1a). These cells were CD4⁺, with no changes in the CD8⁺ subpopulation (Fig. 1b), which

is in agreement with previous reports [3, 4, 6, 26, 27]. However, when we classified AD patients by lesion stage, acute (Group B) or chronic (Group A), the CLA⁺CD4⁺ cells were only significantly increased in those with chronic lesions. The frequency of circulating CLA⁺ T cells has been shown to reflect changes taking place in the lesional skin in AD and similar correlations have been shown for different cutaneous diseases such as anti-convulsant-induced toxic epidermal necrolysis [28], bullous pemphigoid [29] and psoriasis [30].

In addition, we also found an increased expression of HLA-DR on CLA⁺ T cells of patients with chronic lesions compared with those with acute lesions and non-atopic controls. This difference in expression was only significant in CLA⁺CD4⁺ T cells, with no differences in the CLA⁺CD8⁺ or CLA⁻CD4⁺ T cell subpopulations (Fig. 3). Similar results have been described in relation with disease severity [31] but in our patients there were no important differences in severity between groups. The fact that in our patients with chronic lesions T cells expressed HLA-DR, the late T cell activation marker, probably indicates that these cells come from the skin where they are activated. Indeed, phototherapy treatment in AD patients has been shown to induce a significant decrease in the number of circulating HLA-DR⁺ T cells [32]. Moreover, long-lived T cell clones have been detected in blood and skin by their TCR-hyper-variable regions [33], which suggest the existence of a recirculation of memory T cells between blood and secondary organs like the skin [34].

In AD, tissue eosinophilia is normally found in skin lesions and elevated IgE levels in peripheral blood, reflecting an increased expression of Th2 cytokines [25]. In our study we found important increases in eosinophils and total IgE in

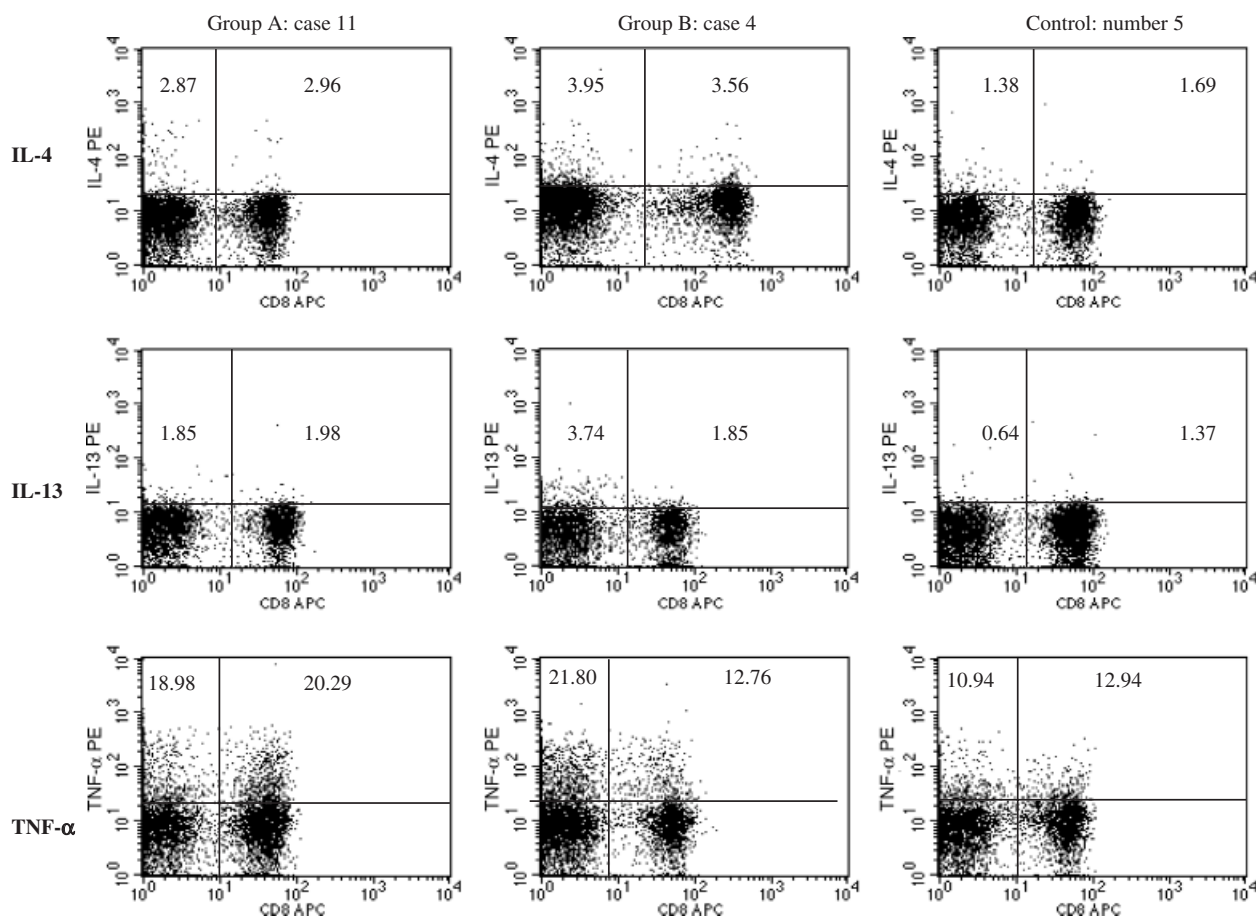


Fig. 5. Flow cytometry of the cytokine production values of IL-4, IL-13 and TNF- α in the CD4⁺ and CD8⁺ subpopulations. When we refer to the CD4⁺ population we in fact measured the CD3⁺/CD8⁺ population. Example of one patient in Group A (case 11), one patient in Group B (case 4) and one control (number 5).

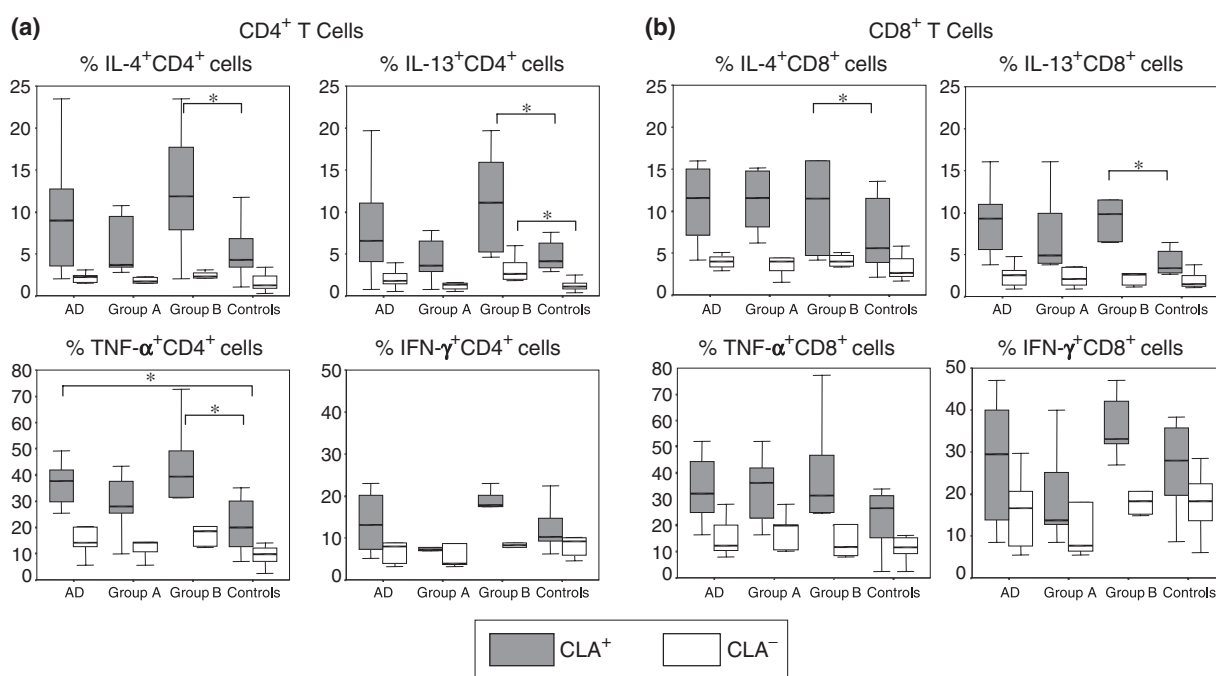


Fig. 6. Box plot of the percentage of cutaneous-lymphocyte-associated antigen (CLA)⁺ and CLA⁻ subsets in (a) CD4⁺ and (b) CD8⁺ cell subpopulations producing IL-4, IL-13, TNF- α and IFN- γ . Comparisons have been made between patients with atopic dermatitis (AD), Group A (patients with chronic lesions), Group B (patients with acute lesions) and controls. Statistical differences (*) are considered significant with a $P < 0.05$.

peripheral blood from patients with acute lesions, indicating that this stage probably reflects a Th2 response. Moreover, eosinophils are present in high numbers in late-phase reactions, which may explain why we were unable to detect these cells in peripheral blood from AD patients with chronic lesions, because these cells are located in the skin.

Many studies indicate that a Th2 mechanism is involved in acute AD lesions compared with chronic lesions, where a Th1 mechanism seems to take part [15–21]. Most of these studies have been performed in skin biopsies and to our knowledge no comparisons have been undertaken in peripheral blood T cells. The present study showed that in peripheral blood from AD patients with acute lesions there is an increased percentage of T cells secreting IL-13, IL-4 and TNF- α and in those with chronic lesions there was no increased percentage in these cytokines, with no changes in the other cytokines. It is of note that all these increases in cytokine production were only detected in the CD4⁺ subpopulation but not in the CD8⁺ cells (Fig. 4). Moreover, these changes could only be detected in the CD4⁺CLA⁺ subsets except for IL-13, which was also increased in the CLA⁻ subpopulation. These results suggest that circulating CLA⁺ T cells may reflect changes that take place in the acute and chronic cutaneous lesions of AD patients: a Th2 cytokine pattern that becomes Th0.

We conclude that immunological differences can be detected in peripheral blood from AD patients with acute and chronic lesions, especially when studying the subset of circulating T cells with skin tropism that preferentially respond to cutaneous allergens. This is the first study demonstrating phenotypic changes in the circulating CLA⁺ T cell subset in AD patients with acute and chronic lesions. These data will contribute to understanding the role of skin homing T cells in the different stages of AD.

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