

Cutaneous CD30+ Cells in Children with Atopic Dermatitis

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Key Words

CD30 · Skin biopsy · Atopic dermatitis

Abstract

Background: CD30 expression can be considered a marker of Th2 cells. We investigated the presence of CD30+ cells in the lesional skin of children with atopic dermatitis (AD). We also analyzed the possible relationship between CD30+ cells and serum soluble CD 30 (sCD30) levels, and IgE, soluble interleukin-2 (IL-2) receptor (sIL-2R) or soluble CD23 (sCD23) levels in the blood, and clinical score. **Methods:** Ten eczematous children (4 males, 6 females; median age: 4 years and 5 months; range: 11 months to 14 years), 9 sex- and age-matched control children and an adult control group were studied. A clinical score (SCORAD index), was given to eczematous lesions. Blood was taken for the determination of IgE, sCD30, sIL-2R and sCD23 levels. Punch biopsies of lesional skin were stained with hematoxylin and eosin or incubated with anti-CD30 monoclonal antibodies. Skin prick tests (SPTs) were also performed. **Results:** In the biopsy specimens, CD30 expression was observed in high proportions of infiltrating cells. In children with AD, total serum IgE, sCD30, sIL-2R, sCD23 and eosinophils were significantly elevated compared to controls. CD30+ cells were not associated with serum IgE, sCD30, sIL-2R, sCD23, or SPT results, score of inflammatory cells in

lesional skin or clinical score. Children with AD who had high total IgE and specific IgE antibodies did not differ from those with normal total IgE and negative specific IgE in respect of age, clinical score, number of CD30+ cells, sCD30, sIL-2R and sCD23 levels, score of inflammatory cells in skin or clinical score. **Conclusion:** Our results showed remarkable numbers of CD30+ cells in the lesional skin and high sCD30 in the serum of children with AD. CD30+ cells did not correlate with systemic markers of IgE reaction.

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Introduction

In atopic dermatitis (AD), lesional skin is characterized by infiltrates, which mainly consist of CD4+ T helper (Th) cells [1, 2] and eosinophils [3]. Th cells can be classified by their cytokine profile into two subsets: Th1 and Th2. In vitro studies have demonstrated that cytokines derived from Th2 cells, such as interleukin-4 (IL-4), IL-10 and IL-13, are involved in IgE production and in the development of immediate hypersensitivity reactions [4]. In AD, a large proportion of skin-infiltrating Th cells express a Th2-like phenotype [5–7]. The production of Th2-type cytokines is associated with membrane expression of the glycoprotein CD30 in both CD4+ and CD8+ cell clones [8], and CD30 expression is regulated by IL-4

[9]. The extracellular soluble portion of CD30 (sCD30) can be released by CD30-expressing T lymphocytes [8, 10, 11]. CD30 molecules stimulate Th2 development and proliferation [8, 12]. Based on these findings, expression of the CD30 molecule has been considered a marker for Th2 cells. However, CD30 expression has recently been found to occur also on Th1-type clones [13–15].

Increased production of sCD30 [16] and upregulation of CD30 expression have been demonstrated in circulating cutaneous lymphocyte-associated antigen (CLA)+ T cells [17] from patients with AD. We are aware of only one study [18] which has investigated the expression of CD30 antigen in the lesional skin of adults with AD. In that study, both the number of CD30+ cells and the level of sCD30 were increased. Moreover, T cell clones derived from the lesional skin secreted Th2-type cytokines and expressed membrane CD30.

To gain further information on CD30+ cells, we studied the expression of CD30 in infiltrating cells in the lesional skin of children with AD. Furthermore, we analyzed the possible relationship between CD30+ cells and serum sCD30 levels and IgE, soluble IL-2 receptor (sIL-2R) or soluble CD23 (sCD23) levels in the blood.

Materials and Methods

Subjects

Ten children (4 males and 6 females; age range: 11 months to 14 years, mean: 5.9 ± 4 years) with AD who fulfilled the diagnostic criteria of Hanifin and Rajka [19] were consecutively included in the study. None of the children had asthma or other atopic conditions. Children with immunologic disorders or who had had infectious diseases in the preceding month were excluded from the study. Children were also excluded if they had been treated with antibiotics, systemic steroids, anti-inflammatory drugs, antihistamines or immunosuppressive drugs in the preceding month. The severity of AD was assessed according to the SCORAD system [20], and a median score of 7 was recorded (range: 2–10).

In order to ascertain whether levels of sCD30 may be influenced by age, two control groups were selected. The first consisted of 9 sex- and age-matched healthy children; the second included 13 healthy adults (5 males, 8 females; age range: 29–48 years, mean: 41.3 ± 16 years). Both control groups were composed of subjects with no atopic or immunologic diseases and no infectious episodes in the preceding month. None of the controls had been on antibiotics, steroids, antihistamines, anti-inflammatory drugs, immunomodulators or immunosuppressive drugs in the preceding month. The control subjects had no family history of atopy. Family history was considered positive if a mother, father, brother or sister had one of the following diseases: AD, asthma, allergic rhinoconjunctivitis or other IgE-mediated diseases.

Informed consent was obtained from adult subjects or the parents of child subjects.

Immunologic Tests

Children with AD were given skin prick tests (SPTs) using commercial extracts (Lofarma, Milano, Italy) for the following allergens: grasses, house dust mites, *Parietaria*, cow's milk, β -lactoglobulin, α -lactalbumin, casein, egg, soy, cod, salmon, wheat, lamb, beef, chicken, peanut, potato, tomato. The positive control was histamine (1 mg/ml). The negative control consisted of the diluent. The results were evaluated after 15 min. Weals at least 3 mm greater in diameter than weals at the site of the negative control were considered positive. Total serum IgE levels were determined according to conventional assay (PRIST; Pharmacia, Uppsala, Sweden).

Circulating IgE antibodies (RAST Cap System; Pharmacia) to grass, house dust mites, *Parietaria*, cow's milk, β -lactoglobulin, α -lactalbumin, casein, egg, soy, cod, salmon, wheat, lamb, peanut, potato and tomato were measured. sCD30 serum levels were determined using a commercially available sandwich ELISA assay (Dako, Glostrup, Denmark). CD30 (Ki-1 antigen; Dako), sIL-2R (T Cell Diagnostic, Cambridge, Mass., USA) and sCD23 (Serotec, Oxford, UK) were measured in the blood according to the manufacturer's recommendations. The absolute number of circulating eosinophils was also determined (Vega ABX; Roche, Milano, Italy). Levels of circulating eosinophils higher than 400 cells/mm³ were considered elevated.

Immunohistochemistry

Punch biopsies (4 mm) were taken from lesional skin of the upper limb in children with AD, after parents had given their informed consent. The specimens were immediately frozen in liquid nitrogen and stored at -80°C . Sequential cryostat sections (5 μm) were cut from each specimen, air dried for 16–18 h and fixed in 100% acetone. Serial sections were washed in Tris-buffered saline and then incubated with monoclonal antibodies anti-CD3, anti-CD4, anti-CD8 (Becton Dickinson, Mountain View, Calif., USA), anti-CD20 (L26/CD20; Dako) or anti-CD30 (BerH2; Dako) at decreasing dilutions (1:20, 1:50, 1:100) in Tris at pH 8.2 for 45 min. After washing for 10 min, the sections were incubated for 30 min with rabbit anti-mouse Ig antiserum (RAM, 1:30; Dako). After washing, the sections were incubated for 30 min with streptavidin-biotin-peroxidase complex (Dako). After washing in Tris, binding of the complex was revealed by 3-aminoethylcarbazole H₂O₂ in acetate buffer at pH 5.8 for 8–10 min. Negative control sections were obtained by excluding the primary antibody.

The sections were counterstained with Mayer's hematoxylin and mounted in glycerin. The number of CD30+ cells in the whole biopsy tissue ($\times 40$) was counted by an observer in a blind fashion.

Conventional Histology

Part of the skin biopsy was fixed in B5 and embedded in paraffin. Adjacent tissue sections were stained with hematoxylin and eosin or with periodic acid-Schiff stain. The whole section was examined at $\times 40$ magnification by an observer in a blind manner. A score was assigned to inflammatory cells, including neutrophils, eosinophils, metachromatic cells and mononuclear cells. A semiquantitative grading was used, as follows: 0 = none, 1 = a few scattered cells (mild inflammation), 2 = a moderate or large number of cells (moderate to severe inflammation).

Statistical Analysis

Data were presented as mean $\pm$ SEM. The Mann-Whitney test was used for comparison of the data. Correlations were examined by the Spearman rank correlation test.

Results

Skin biopsies were performed in 9 of the 10 child subjects, since for 1 child we did not obtain the parents' consent. In the lesional skin of children with AD, most of the infiltrating cells were CD3+ T cells, with a mean CD4+ to CD8+ ratio of 2:1. CD30 expression was observed in a high proportion of infiltrating cells, and predominantly on CD4+ lymphocytes, as indicated by studies on serial sections. CD20+ cells were very rare. Inflammatory infiltration was moderate to severe in 6 cases and mild in 3.

In children with AD, total serum IgE, eosinophils, sCD30, sIL-2R and sCD23 were significantly elevated as compared to controls (table 1). The adult control group had significantly lower levels of sCD30 than the control group of healthy children (9.6 ± 2.3 U/ml; $p < 0.01$). Levels of total serum IgE were higher than the published mean + 2 SD for age [21] in 70% of the subjects.

Specific serum IgE antibodies were found in 60% of eczematous children and in none of the controls. Among children with AD, 4 were positive to mites, 3 to soy, 3 to egg, 2 to cow's milk, 2 to wheat, 2 to cod and 1 to tomato.

SPT reactions to at least one allergen were positive in 6 children with AD. Four children were positive to egg, 4 to grasses, 3 to cow's milk, 3 to soy, 3 to mites, 2 to wheat, 2 to peanut, 2 to wheat, 1 to tomato, 1 to lamb's meat and 1 to potato.

In children with AD, sCD30 levels did not correlate with the clinical score, serum IgE, circulating eosinophils, sIL-2R, sCD23 or SPT results when examined by the Spearman rank correlation test.

Association of CD30+ Cells with Clinical Score and Laboratory Data

The number of CD30+ cells was not significantly higher in eczematous children with levels of total serum IgE >2 SD for age [21] (11.2 ± 7.3) than in eczematous children with normal levels of total IgE (9 ± 3.5) ($p > 0.05$).

In children with AD and positive specific IgE antibodies, the number of CD30+ cells did not differ from those of children with negative specific IgE antibodies (4.3 ± 3.5 vs. 11.2 ± 7.3 ; $p > 0.05$). In children with AD, CD30 expression by infiltrating T cells was not associated with the inflammatory score (moderate to severe infiltration: 13.2 ± 5.6 vs. mild infiltration: 2.5 ± 0.5 ; $p > 0.05$). The number of CD30+ cells was higher, but not significantly, in eczematous children with hypereosinophilia (>400 cells/mm³) (16.7 ± 8) than in those with levels of circulating eosinophils <400 cells/mm³ (4.3 ± 1.9) ($p > 0.05$). In eczematous children with positive SPT results,

Table 1. Levels of total IgE, eosinophils, sCD30, sIL-2R and sCD23 in the blood of children with AD and of control children

	AD group	Control group	p
Total IgE, UI/ml	$1,336 \pm 573$	21 ± 4	<0.05
Eosinophils, cells/mm ³	$1,065 \pm 381$	164 ± 47	<0.02
sCD30, U/ml	56 ± 7	32 ± 5	<0.03
sIL-2R, pg/ml	$5,024 \pm 704$	$2,412 \pm 619$	<0.02
sCD23, pg/ml	209 ± 39	122 ± 4.4	<0.03

Table 2. Age, clinical score, number of CD30+ cells in lesional skin, and serum levels of total IgE, eosinophils, sCD30, sIL-2R and sCD23 in children with a moderate to severe inflammatory infiltrate and in children with a mild inflammatory infiltrate

	Moderate to severe infiltrate	Mild infiltrate
Age, years	$7 \pm 2^*$	6 ± 1.2
Clinical score	$7.5 \pm 0.8^{**}$	2.7 ± 0.3
Eosinophils, cells/mm ³	$1,485 \pm 608^*$	270 ± 95
CD30+ cells	$13.2 \pm 6.7^*$	2.5 ± 0.5
Total IgE, UI/ml	$1,951 \pm 880^*$	191 ± 113
sCD30, U/ml	$51 \pm 7.9^*$	56 ± 11.5
sIL-2R, pg/ml	$4,936 \pm 987^*$	$4,413 \pm 1,125$
sCD23, pg/ml	$207 \pm 54^*$	156 ± 29

$^{**} p < 0.02$, $^* p > 0.05$; compared to the children with a mild infiltrate.

CD30 expression was not significantly elevated compared with that of children with AD who had negative SPT results (11.2 ± 7.26 vs. 9.33 ± 3.48 ; $p > 0.05$).

CD30+ cells did not correlate with clinical score, serum IgE, circulating eosinophils, sCD30, sIL-2R, sCD23 or SPT results when examined by the Spearman rank correlation test.

Comparison of Cytologic Evaluation with Clinical and Laboratory Data

Neither age, nor total IgE, serum eosinophils, CD30+ cells, sCD30, sIL-2R or sCD23 were associated with the inflammatory score in lesional skin (table 2).

A significant association was found between the clinical score and the presence of a moderate to severe infiltrate in the lesional skin ($p < 0.02$).

Eczematous Children with Circulating IgE

On the basis of IgE findings, children with AD were classified into two groups (table 3). The first group in-

Table 3. Comparison of 6 children with elevated total serum IgE for age and positive specific IgE antibodies (AAD group) with 4 children with normal total serum IgE levels for age and negative specific IgE antibodies (NAAD group)

	AAD	NAAD
Age, years	4.3 ± 1.2*	7 ± 2
Clinical score	5 ± 1.5*	7 ± 1.2
Total serum IgE, UI/ml	2,195 ± 786**	48 ± 38
Eosinophils, cells/mm ³	1,013 ± 249*	1,141 ± 962
CD30+ cells	11.2 ± 7.3*	9.3 ± 3.5
Moderate to severe/mild infiltrate	4/2*	2/1
sCD30, U/ml	58 ± 7.6*	51 ± 14.7
sIL-2R, pg/ml	5,191 ± 787*	4,690 ± 1,665
sCD23, pg/ml	193 ± 36*	242 ± 103

** p < 0.03, * p > 0.05, compared to the NAAD children.

cluded 6 children with elevated total serum IgE for age and positive specific IgE antibodies [allergic AD (AAD)]. The second group consisted of 4 children who had normal total serum IgE levels for age and no specific IgE antibodies [nonallergic AD (NAAD)]. The two groups did not differ in respect of age, clinical score, circulating eosinophils, sCD30, sIL-2R, sCD23, CD30+ cells, or the evaluation of the skin inflammatory infiltrate.

Discussion

These results show that children with AD have significant expression of CD30 antigen in the lesional skin, and elevated serum levels of sCD30.

Other studies have investigated the occurrence of increased CD30 expression by T cells in atopic patients. Esnault et al. [22] showed increased numbers of peripheral mononuclear cells expressing CD30 in atopic patients. Caproni et al. [18] found high proportions of CD30+ cells in the skin of AD adults, but not in allergic contact dermatitis lesional skin. Our results confirm and extend these findings for children with AD. Unfortunately, we could not perform skin biopsies in control children for ethical reasons.

Our findings are also consistent with studies which have shown increased sCD30 levels both in children [16] and adults [18] with AD. However, we did not find any correlation between sCD30 and disease activity. This is in agreement with the results of Bengtsson et al. [23] in adults, but at variance with those of Frezzolini et al. [16]

in children. These conflicting results may perhaps be due to the different characteristics of patients and control subjects (children and adults) or methodological problems (assay sensitivity).

The significance of CD30+ cells in AD needs to be discussed. We observed infiltrates in skin lesions consisting predominantly of CD4+ T cells. On serial sections, the expression of CD30 antigen was predominantly detected on CD4+ T cells. In vitro cloning studies have found that most CD4+ T cells in the lesional skin of patients with AD express a Th2-like phenotype [5–7]. CD30 expression in skin-infiltrating T cell clones of AD patients has been found to be strongly correlated with the production of IL-4 and IL-5, but to be inversely related to IFN-γ [18]. Although we were not able to obtain high-quality staining using antibodies to IL-4 on lesional skin, it can nonetheless be speculated based on findings from the literature and on our own data, that the CD30+ skin-infiltrating cells are CD4+ T cells that actively secrete Th2-type cytokines. The high serum levels of sCD30 observed in children with AD may be due to its release by CD30+ skin-infiltrating T cells. sCD30 may also be released by the CLA+ subset of circulating T cells, in which CD30 expression is increased [17].

Our results confirm that AD patients with high serum IgE have increased levels of sCD23, which is the soluble form of the IgE low-affinity receptor (FcεRII) [24] and which regulates in vitro IgE synthesis [25]. The in vivo activities of sCD23, however, are still unclear. Interestingly, in accordance with previous studies [23, 26], we observed that serum levels of sCD23 did not correlate with serum IgE.

IgE production appears to be favored by the preferential activation of allergen-specific CD4+ Th2 cells [4]. However, we found that neither serum IgE levels nor sCD23 levels were significantly correlated with CD30 expression.

Several hypotheses can be suggested to explain these data. Firstly, the lack of correlation may be due to the small sample size. Secondly, it has been shown that CD30 may be expressed not only by Th2 cells, but also by Th1 cells [13–15]. CD30 has been shown to be expressed by activated T cells producing both IFN-γ and IL-5 [13]. In this context, it is noteworthy that patch test challenges with inhalant allergens may initially induce a predominantly Th2-type infiltrate, and then a late, chronic Th1-dominated response [27]. Therefore, the number of CD30+ cells and sCD30 levels may not exactly reflect that of Th2 cells. Thirdly, it is possible that increased IgE production and CD30 expression are related to distinct pri-

mary dysfunctions of the immune system in AD. In some children with AD, foods and aeroallergens may play a pathogenic role [28, 29]. We did not find any association between positive skin tests or specific IgE antibodies and CD30 expression or serum sCD30 levels. The possibility that the increased expression of CD30 reflects a primary abnormality of the immune system, rather than being the

consequence of contact with allergens, should be considered. The role of food in provoking AD can be definitely ascertained only after an effective elimination diet and reappearance of symptoms after a positive oral food challenge. Therefore, we intend to study the expression of CD30+ cells and sCD30 levels before and after a successful elimination diet or food challenge.

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