Background: The pathogenesis of atopic dermatitis (AD) is still not completely understood. AD is characterized by the presence of clinical symptoms of both IgE antibody-mediated immediate hypersensitivity and specific T lymphocyte-mediated delayed hypersensitivity.

Objective: To evaluate the immunological mechanisms involved in children with acute AD lesions.

Material and methods: Ten children with acute AD lesions and 10 non-atopic controls were studied. Total IgE was measured by immunoassay. T cell marker expression (CD3, CD4, CD8, cutaneous lymphocyte-associated antigen [CLA]) and cytokine production (interferon [IFN]-γ, interleukin [IL]-13) were analyzed in peripheral blood mononuclear cells by flow cytometry.

Results: In children with AD the percentage of CD3+ cells (p = 0.015) increased while that of CD8+ cells (p = 0.023) decreased, with no differences in CLA expression. We found increased IL-13 production in CD3+ cells (p = 0.01) and CD3+CD4+ (p = 0.001) cells with no difference in IFN-γ. Total IgE was significantly higher in patients with AD (p = 0.01). Comparison of IL-13 production in CD4+ cells categorized by total IgE level showed that IL-13 production was significantly increased in subjects with a higher IgE level.

Conclusion: Peripheral blood from children with AD showed an increase in IgE levels and a Th2 pattern. There was a correlation between IL-13 production and total IgE levels.

Key words: Atopic dermatitis. Children. IgE. T cell markers. Cytokines.

RESUMEN

Introducción: La patogenia de la dermatitis atópica (DA) es compleja y en algunos aspectos difícil de entender. Se caracteriza por la presencia de síntomas relacionados tanto con mecanismos de hiper sensibilidad inmediata, mediada por anticuerpos IgE, como de hiper sensibilidad tardía media da por linfocitos T.

Objetivo: Evaluar los mecanismos inmunológicos implicados en las lesiones agudas de la DA en población infantil.

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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. AD usually presents in infancy and childhood and may persist into or even start in adulthood. AD usually presents in infancy and childhood and may persist into or even start in adulthood. AD is characterized by the presence of clinical symptoms of both IgE antibody-mediated immediate hypersensitivity and specific T lymphocyte-mediated delayed hypersensitivity. One typical characteristic is an elevated serum total and specific IgE level, occurring in 80% of AD patients and which is called "extrinsic AD". Despite multiple studies the pathogenesis of AD is still not completely understood, though T cells are known to play a critical role. Most T cells located in the skin in AD are of the CD45RO + memory/effector phenotype and express the selective skin-homing receptor cutaneous-lymphocyte-associated antigen (CLA). These cells are known to produce higher levels of Th2-type cytokines (IL-4, IL-5, IL-13), which are thought to play a key role in the pathogenesis of AD, and lower levels of Th1-type cytokines (IFN-γ). IL-4 and IL-13 are produced primarily by activated T cells and mast cells, and the genes for both these cytokines are located in the same region on chromosome 5. Both IL-4 and IL-13 can induce switching of IgG to IgE. Moreover, the activation of T lymphocytes is followed by the activation of other types of cells, keratinocytes and mainly endothelial cells, which results in the production of cytokines and chemokines, enabling recruitment of inflammatory cells from the blood to the skin.

The aim of this study was to assess the immunological mechanisms involved in AD children with acute lesions. We compared T lymphocyte markers and the cytokine production pattern, including Th1 (IFN-γ) and Th2 (IL-13), in peripheral blood mononuclear cells (PBMC) from these AD children and from non-atopic controls.

**METHODS**

**Patients**

Twenty children from the paediatric allergy Unit of Carlos Haya Hospital were selected and classified into two groups:

- Ten children diagnosed with AD according to Hanifin and Rajka and with acute lesions were selected (lesion onset less than three days before study), with no respiratory problems or other skin diseases. No systematic steroids, tacrolimus or psoralen-ultraviolet A had been used for at least six months prior to the study.

- Ten non-atopic children with no skin or respiratory diseases, included as controls.

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

**Total serum IgE was quantified by fluoroenzymoimmunoassay (UniCAP, Pharmacia Diagnostic AB, Uppsala, Sweden) according to the manufacturer’s instructions.**
Phenotypic immunofluorescence analysis

PBMC from each patient were isolated by density gradient centrifugation (Nycoromed, Oslo, Norway) from 6 ml of heparinized venous blood for immunofluorescence staining, as previously described[16]. Briefly, 5 x 10^5 cells were sequentially stained with different MoAbs at 4 °C. Stained cells were fixed in 1 % paraformaldehyde in PBS. CD3+ T cells were assessed with CD3-PerCP, CD4-APC and CD8-APC and the homing receptor expression with CLA-FITC (Becton-Dickinson, San Jose, CA).

Cytokine production was determined in cells stimulated for 4 hours at 37 °C with phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO,) and ionomycin (Sigma at a final concentration of 25 ng/ml and 1 μg/ml of cell suspension, respectively, in which the intracellular protein transport of cytokines was disrupted with monensin (Sigma) at 10 μg/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) and permeabilized with the same kit and stained intracellularly with monoclonal antibodies to cytokines (IFN-γ-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 downregulation of certain surface proteins, including CD4. Therefore, as most CD4+ T cells are contained within the CD3+/CD8- population, when we refer to the CD4+ population in the analysis of the cytokine production we in fact measured the CD3+/CD8- population. Six-parameters were analysed on a FacsCalsibur flow cytometer using Cell Quest software. Negative isotype controls were used to verify the staining specificity of the antibodies used.

Table I

<table>
<thead>
<tr>
<th>Surface markers</th>
<th>Atopic dermatitis Median (75-25)</th>
<th>Controls Median (75-25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+</td>
<td>76.60</td>
<td>81.07-71.97</td>
</tr>
<tr>
<td>CD3+/CD4+</td>
<td>56.72</td>
<td>62.13-50.62</td>
</tr>
<tr>
<td>CLA+/CD3+</td>
<td>6.53</td>
<td>6.89-3.78</td>
</tr>
<tr>
<td>CLA+/CD3+/CD4+</td>
<td>4.36</td>
<td>5.57-3.00</td>
</tr>
<tr>
<td>CLA+/CD3+/CD8+</td>
<td>1.22</td>
<td>1.96-0.81</td>
</tr>
</tbody>
</table>

Statistical study

Data are presented as box-plots displaying medians and interquartile ranges (IR). As the variables evaluated were not distributed normally, the mean comparisons were done by non parametric analysis (Kruskall-Wallis and, if significant, Mann-Whitney tests). All reported P values represented two-tailed tests, with Bonferroni adjustment applied. A difference was considered statistically significant. The statistical analysis was performed using the SPSS program version 11.5.

RESULTS

Comparisons between AD patients and controls showed that total IgE was significantly higher in AD patients (median: 388 IKU/L; IR: 2626-299 IKU/L) compared to controls (median: 53.3 IKU/L; IR: 103-26 IKU/L; p = 0.01).

The percentages of the total CD4+ and CD8+ subpopulations in CD3+ and in CLA- cells are presented in table I. We detected an increase in the percentage of CD3+ cells in AD children compared to controls (p = 0.015), and a decrease in the CD8+ subpopulations in AD patients (p = 0.023). The CD4+/CD8+ ratio was significantly increased in AD patients compared to controls (p = 0.04). There were no differences in the CLA expression in peripheral blood T cells between both groups.

The median and IR of the percentage of CD4+ and CD8+ subpopulations expressing IL-13 and IFN-γ are shown in figure 1. Comparisons of cytokine production showed an increase in the IL-13 production in CD3+ cells (p = 0.01), and only detect in the CD3+/CD4+ cells (p = 0.001). No difference in the IFN-γ production between AD children and controls was detected. Figure 2 shows flow cytometry examples of IL-13 and IFN-γ production in the CD4+ and CD8+ subpopulations from one child in each group.

We evaluated the relationship between IL-13 production and total IgE levels. As IL-13 production was only shown in the CD4+ subsets we compared the production of IL-13 in CD4+ cells in all subjects (AD patients and controls) categorized by the total IgE level (fig. 3). The categories were IgE < 100 IKU/L, from 100 to 1.000 IKU/L and > 1.000 IKU/L. Results showed that IL-13 production was significantly increased in subjects with higher IgE levels, with a p = 0.03 between the first two categories and p = 0.017 between the first and the third categories. Although the difference between the second and the third category was not significant there was a tendency to increase.
Antúnez C, et al.—DIFFERENT LYMPHOCYTE MARKERS AND CYTOKINE EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN CHILDREN WITH ACUTE ATOPIC DERMATITIS

Figure 1.—Box plot of the percentage of IL-13 (A) and IFN-γ (B) production in CD3⁺, CD4⁺ and CD8⁺ subpopulations. Comparisons have been made between AD children and controls. Statistical differences (*) are considered significant with a p < 0.05.
DISCUSSION

Many studies indicate that AD is the cutaneous manifestation of a systemic immunological disorder named atopy, and this term describes the genetic predisposition to IgE-mediated allergies. B cell production of IgE requires the T cell derived cytokines IL-4 and/or IL-13, whereas IFN-\(\gamma\) inhibits this IgE synthesis\(^{16}\). In order to understand the mechanisms involved in AD we evaluated several lymphocyte markers and the cytokine production, including Th1 (IFN-\(\gamma\)) and Th2 (IL-13), in PBMC from a group of AD children with acute lesions and a group of non-atopic children as controls. We chose IL-13 instead of IL-4 for defining a Th2 pattern because IL-13 is produced in rapid response to activation of peripheral blood T cells and maintained over a longer period of time whereas IL-4 secretion is more transient\(^{17}\).

As has been described in skin biopsies\(^{18}\), in our patients we found an increase in CD3\(^+\) cells and an increase in the CD4 \(+\)/CD8 \(+\) ratio. Although T cells specific for skin related allergens are known to be confined to the CLA\(^+\) T cell population\(^{19}\), we found no differences in the expression of T cell skin homing receptors in these AD children with acute lesions compared with the controls. These findings, which agree with previous results from our group\(^{20}\), may be explained by the fact that in the initial stage of the reaction the CLA lymphocytes are located in the skin, with lower levels in peripheral blood.

We used flow cytometry to analyse the frequencies of CD3\(^+\), CD4\(^+\) and CD8\(^+\) cells expressing IL-13 and IFN-\(\gamma\). The flow cytometer has the advantage of being able to study cytokine-producing cells at the single cell level. Thus, we were able to analyse which CD4 or CD8 subpopulation was producing these cytokines. In the AD children we detected a typical Th2 cytokine pattern with increased IL-13 production in T lymphocytes. Classification of these T lymphocytes into CD4\(^+\) or CD8\(^+\) showed that this increase only occurred in the CD4\(^+\) subpopulation. This agrees with other authors who have also found this increased in PBMC upon stimulation with superantigen\(^{21}\), with antiCD3\(^\text{mAb}\) or in skin lesions provoked by epicutaneous application.
of allergens. Moreover, this IL-13 expression has been mainly detected in skin biopsies obtained from acute lesions, which is in agreement with our results. Although a decrease could be expected, we detected no significant changes in the production of IFN-γ in either the CD4+ or the CD8+ subsets. As increased levels of IFN-γ had been detected in chronic AD skin lesions and it is thought that this cytokine may be involved in the pathogenesis of AD, our results are not so surprising.

As previously reported by others, we found that the subjects with higher total IgE levels were those who were producing higher levels of IL-13. This is in consonance with the role of IL-13 in the induction of IgE synthesis. In conclusion, in children with acute atopic dermatitis lesions there is an increase in CD4+ T-cells expressing the IL-13 cytokine that acts on monocytes and B cells, but not on T cells. This is in agreement with our results.

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tive) CD8+ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. J Immunol 1999;163:466-75.