Immunophenotyping of the Cutaneous Infiltrate and of the Mononuclear Cells in the Peripheral Blood in Patients With Atopic Dermatitis

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Fourteen adult patients with chronic atopic dermatitis and active skin lesions had a skin biopsy and venous blood sample taken on the same day. Absolute numbers of circulating lymphocytes were normal in all patients. Fluorescence-activated cell sorter (FACS) analysis revealed normal numbers of total T lymphocytes and T-helper and T-suppressor subsets (helper:suppressor ratio, 2:1) in the atopic patients' peripheral blood, but an increase in circulating B lymphocytes and in HLA-D-related antigen-bearing cells. The skin biopsy showed a dermal infiltrate of predominantly T-helper lymphocytes (helper:suppressor ratio, 7:1). These cells showed strong HLA-DR plasma membrane staining. There was no HLA-DR staining in the membranes of epidermal keratinocytes. Using a monoclonal antihuman IgE, positive staining was observed in the dermis, though none was identified in the epidermis. The dermal anti-IgE staining was concentrated around clusters of T lymphocytes. J Invest Dermatol 89:4-7, 1987

Multiple immunologic abnormalities have been reported in patients with atopic dermatitis. These have been well summarized by Hanifin [1,2]. Some of these abnormalities are seen only transiently when the skin is acutely inflamed. One of the more commonly reported abnormalities is an alteration in the numbers of circulating T lymphocytes. A deficiency in both the total T cells, as measured by E-rosette formation [3-5], and numbers of T-suppressor cells, as measured by either the Fc receptor [6-8] or by using a monoclonal marker such as OKT8, has been reported [9-13]. However, not all studies have recorded similar changes, and some were unable to demonstrate any abnormality in either T-suppressor cell numbers or function [14-16].

Histologic examination of the skin of patients with atopic dermatitis (AD) has revealed a dermal lymphocytic infiltrate [17,18] that was shown to be composed predominantly of T lymphocytes [19]. This infiltrate was later phenotypically identified as T-helper lymphocytes [20,21]. It is difficult to interpret the significance of this cutaneous infiltrate without knowledge of the absolute numbers and relative proportions of T-cell subsets in a concurrent blood sample, and we therefore decided to examine the numbers of T lymphocytes and subsets in both the skin and peripheral blood in the same patients on the same day.

Materials and Methods

Fourteen adults (6 men, 8 women), aged 18-47 years (mean 27.2 years) gave their informed consent. All patients had classic chronically relapsing AD with recurrent severe flares. To enable the necessary coordination of the project, patients were generally studied in a chronically active state requiring moderate-strength topical steroids such as clobetasone butyrate (0.05%) rather than in an acute flare, with 1 exception. Other diagnostic criteria included: onset of dermatitis when the patient was under 5 years old (13 patients, 93%); personal history of other atopic disease (13 patients, 93%); a positive family history of atopy (10 patients, 71%). In addition, 12 patients had either an elevated IgE, positive RAST tests, or positive prick tests. The study was approved by the hospital ethics committee.

An elliptical skin biopsy was taken from the most active area, excluding the face, and a venous blood sample withdrawn from all patients between 9 and 11 A.M. in order to minimize any variation in the lymphocyte subsets due to the circadian rhythm. Four milliliters of the venous sample was taken into a septum bottle for a standard full blood count by Coulter analysis, and the remainder placed in heparinized containers (Evans bottles, Speke, Liverpool, U.K.). A similar blood sample was taken from age- and sex-matched controls with no personal or family history of atopy. The mononuclear cells from both patients and controls were separated using a Ficoll gradient (Lymphoprep, Nyegaard, Oslo, Norway) and were centrifuged at room temperature for 30 min at 400 g. The mononuclear cells (MNC) were then harvested, washed 3 times in RPMI (Gibco, Paisley, Scotland), resuspended in a known volume, and counted. The yield of MNC was checked.
**Table I. Use of Monoclonal Antibodies**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Skin</th>
<th>Peripheral Blood</th>
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<tbody>
<tr>
<td><strong>Pan T cell</strong></td>
<td>Leu-4</td>
<td>Leu-4</td>
</tr>
<tr>
<td><strong>Helper T cell</strong></td>
<td>UCHT1</td>
<td>UCHT1</td>
</tr>
<tr>
<td><strong>Suppressor/cytotoxic T cell</strong></td>
<td>OKT4a</td>
<td>Leu-3a</td>
</tr>
<tr>
<td><strong>Regulatory T cell</strong></td>
<td>Leu-8</td>
<td>Leu-8</td>
</tr>
<tr>
<td><strong>B cell</strong></td>
<td>Leu-12</td>
<td>Leu-12</td>
</tr>
<tr>
<td><strong>HLA-DR antigen</strong></td>
<td>La 231</td>
<td>OKT1</td>
</tr>
<tr>
<td><strong>Natural killer cell</strong></td>
<td>Leu-11b</td>
<td>Leu-11b</td>
</tr>
</tbody>
</table>

- **Used in the Study**
- **Used Exclusively in the Skin**

Most of the antibodies were obtained commercially: OKT series—Ortho, High Wycombe, Bucks, U.K.; Leu series—Becton-Dickinson, Lib Impex, Middlesex, U.K.; UCHT series—Unipath, Bedford, U.K.; La 231 was kindly provided by Dr. V. von Heyningen, Edinburgh, U.K.

**Table II. Full Blood Count—Coulter Counter Results**

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 16)</th>
<th>Atopic Dermatitis (n = 14)</th>
</tr>
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<tbody>
<tr>
<td><strong>Hemoglobin (g/dL)</strong></td>
<td>13.8 ± 0.3</td>
<td>13.8 ± 0.4</td>
</tr>
<tr>
<td><strong>White cell count (x 10^3)/L</strong></td>
<td>6.3 ± 0.5</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td><strong>% Lymphocytes</strong></td>
<td>32.1 ± 2.2</td>
<td>29.1 ± 1.7</td>
</tr>
<tr>
<td><strong>Platelets (x 10^3)/L</strong></td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.2</td>
</tr>
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</table>

Results give the percentage of cells labeled with each antibody. Numbers in parentheses represent the number of patients/controls in each group.

- **p < 0.01**
- **p < 0.001**

**RESULTS**

Total serum IgE levels were elevated in 12 of 14 patients with AD (>1000 IU/ml in 7 and 550–950 IU/ml in 5). All control subjects had total serum IgE levels in the normal range, and 14 of the 15 had values under 30 IU/ml. The results of the Coulter analysis (Table II) showed no abnormality in the peripheral blood of AD patients by comparison with age- and sex-matched control subjects. In particular, there was no evidence of a total lymphopenia in the patients with AD.

The results of the FACS analysis are shown in Table III. There was no difference in total T-lymphocyte numbers, in absolute numbers or ratio of helper and suppressor T cells (helper:suppressor ratio, 2:1) or in regulatory T cells (Leu-8) between the patients with AD and the normal controls. In the patients with AD there was, however, a statistically significant increase in the percentage numbers of circulating B cells (AD patients 6.3%, controls 3.9%; p = 0.01) and in the numbers of HLA-DR antigen-bearing cells (AD patients 9.9%, controls 6.3%; p < 0.001). The numbers of HLA-DR antigen-bearing cells in both patients and controls were higher than numbers of cells stained by B1 or Leu-12, indicating that not all were B lymphocytes. When the antibody Leu-7 was used as a marker of NK cells, the numbers of these cells appeared to be reduced in patients compared with the controls but this difference did not reach statistical significance. In contrast, when Leu-11 was used, no difference was seen between numbers of circulating NK cells in patients with AD and the controls.

The results of the immunoperoxidase staining of the skin are summarized in Table IV. The dermal infiltrate was composed predominantly of T-helper cells (Fig 1) and these were strongly HLA-DR antigen-positive. Relatively few T suppressor/cytotoxic cells were present in the infiltrate and the helper:suppressor ratio was 7:1. In contrast to a number of other chronic inflammatory skin diseases with a lymphoid infiltrate such as allergic...
contact dermatitis, lichen planus, and mycosis fungoides, no HLA-DR antigen-stained epidermal keratinocytes were seen, although Langerhans cells were clearly stained. Numbers of epidermal Langerhans cells were increased, with a range of 25–58 cells (mean 36.5) overlying 200 basal cells (normal range in our laboratory is 20–30) [27]. Very few cells in either the epidermis or dermis showed positive staining with either Leu-12 (B cell) or Leu-11b (NK cell). Cells bearing the interleukin 2 (IL-2) receptor were found in all samples examined although only in small numbers.

Positive IgE staining was seen in the dermis in 13 of the 14 patients. The 1 patient who did not show positive IgE staining had both a normal total serum IgE and a negative RAST screen. Using a double-staining technique, the IgE was mainly seen in association with clusters of T lymphocytes in the dermis. No IgE staining was seen on the epidermal Langerhans cells nor on the dendritic cells in the dermis.

DISCUSSION

It is possible that the observed increase in B-cell numbers in the peripheral blood may be responsible for the increased level of serum IgE that is seen in patients with AD. Our findings with regard to T cells are at variance with a number of reports of a reduction in numbers or function of T suppressor cells. Some of these reports used the Fc receptor as a marker of suppressor cells and, as it is recognized that Fc cells and OKT8 cells do not mark identical populations, our present report is not strictly comparable with such studies.

There are two possible causes of the observed differences between our study and those that also used OKT8 as a suppressor-cell marker. First, in 2 of the studies the low suppressor cell numbers reported were in children. Whereas reduced suppressor cells may be a feature of AD in children, in neither of these 2 studies were the controls age-matched. In the first, patients had a mean age of 9 and control subjects a mean age of 17 [9]. In the second, the mean age of the patients was 5.5 years and the controls 32 years [10]. A second possible explanation may lie in differences in the clinical activity of the disease with reduced numbers of circulating suppressor cells seen only in more acute phases of AD activity. In general, the patients we examined had disease in a chronically active stage rather than in acute flare, but the 1 patient in acute flare had the lowest number of circulating suppressor cells (15.5% vs our mean of 20.6% in this study). Our results for the quantitative analysis of NK cells vary with the antibody used (Leu-7 or Leu-11). Patients with atopic dermatitis are recognized to have reduced NK cell function [28,29]. Leu-11 is said to “better” define NK cells than Leu-7 [24]. If this is accurate, it would imply that the reduction in NK activity in patients with AD is due to a functional defect expressed by a normal number of NK cells.

Our study confirms previous reports that the dermal infiltrate in atopic dermatitis is predominantly composed of T-helper cells [30] and that many of these cells express HLA-DR antigen [21]. The ratio of T-helper to T-suppressor-cytotoxic cells observed in the dermal infiltrate was 7:1, compared with the 2:1 ratio seen in the peripheral blood, implying a selective sequestration of T-helper lymphocytes in the dermal infiltrate. This is in contrast to the findings in acute contact dermatitis wherein the dermal infiltrate also shows the same 2:1 helper:suppressor ratio as that seen in the peripheral blood [31]. In contrast to studies on allergic contact dermatitis, we saw no Ig staining of epidermal keratinocytes in biopsies of atopic skin. An increase in numbers of epidermal Langerhans cells in the skin of patients with AD has been reported previously [20]. A recent study has reported IgE staining on epidermal dendritic cells shown to be Langerhans cells, both by double staining with anti-IgE and OKT6 and by immunogold ultrastructural techniques [32]. We did not observe any epidermal IgE staining in our patients.

Our observation of a spatial relationship between dermal IgE staining and T-helper lymphocytes may be due to the fact they these are T lymphocytes that bear the epsilon receptor [33], which could be involved in modulating the response to IgE. T lymphocytes are known to produce soluble IgE binding factors [34]. No direct relation of dermal dendritic cells was seen in relation to the IgE, although an indirect association may exist with the T lymphocytes relating to both the IgE and the dendritic cells in the dermis. The findings in this study suggest a possible link between the well-recognized but as yet unexplained coexistence of type I IgE-mediated and type IV delayed hypersensitivity abnormalities in patients with AD.

ADDENDUM

This study showed that the dermal infiltrate in atopic dermatitis has the predominant phenotype of Leu 3 + 8 - (inducers of help). Leu 8 stained only 21% of the cells instead of the expected 60–70%. This is in contrast with recent results in allergic contact dermatitis where the infiltrating cell was predominantly Leu 3 + 8 + (inducers of suppression). Wood GS, Volkert SA, Abel EA, Nickoloff BJ, Adams RM: Allergic contact dermatitis: novel immunohistologic features. J Invest Dermatol 87:688-693, 1986.

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