**Fcε Receptor II/CD23 Positive Lymphocytes in Atopic Dermatitis: II. Infiltration of FcεR II(+) T cells in the Skin Lesion**

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Cells expressing Fc receptors for IgE (FcεR II) were identified in skin from patients with atopic dermatitis (AD), eczematous dermatitis (ED), and in skin from normal nonatopic subjects, with the use of monoclonal antibodies to human lymphocyte FcεR II, H107, and to lymphoid cell-surface antigens by double immunofluorescence staining. Two to four percent of infiltrating mononuclear cells expressed FcεR II, and more than half of these cells were Tc cells in both acute and chronic AD lesions. FcεR II(+) T cells (Tc cells) bearing CD8 infiltrated preferentially acute lesions, whereas chronic lesions contained either CD8(+) or CD4(+) Tc cells, or both. FcεR II(+) cells rarely were present in ED lesions. There was no significant correlation between % FcεR II(+) peripheral blood mononuclear cells and the proportion of lesional FcεR II(+) cells, extent of skin lesions, or serum IgE levels, implying the selective accumulation of FcεR II(+) cells in the inflammatory infiltrate of AD. These observations suggest that the increased generation of FcεR II(+) cells in skin lesions, including CD8 (+) Tc cells, is involved in the pathogenesis of AD. J Invest Dermatol 95:592–596, 1990

Atopic dermatitis (AD) is a relapsing, chronic cutaneous inflammation, often occurring in individuals with a personal or family history of respiratory allergy [1–3]. AD shows a dense infiltration of mononuclear cells by skin lesions, including activated CD4(+) T-cell and IgE-bearing antigen-presenting cells (APC) such as Langerhans cells, dendritic reticulum cells, and monocytes/macrophages [4–10]. These findings suggest that the interaction between activated T cells and IgE-bearing APC plays an important role in the formation of skin lesions in AD. Although it has been well documented that lymphocytes expressing FcεR II (CD23) are engaged in the isotypic regulation of IgE synthesis [11,12]. FcεR II is also expressed on cells other than lymphocytes such as monocytes and eosinophils, thus exerting functional pleiotropy [13]. FcεR II is considered to be an activation antigen of lymphocytes because mitogens, alloantigens, interleukin 4 (IL-4), and viral infections upregulate its expression on the cell surface [13]. In the present study, double immunofluorescence staining with a monoclonal antibody (MoAb) specific for human lymphocyte FcεR II, H107(14), and a panel of MoAb to lymphoid cell-surface antigens was employed to surface-phenotype and enumerate FcεR II(+) cells in skin lesions from patients with AD. The results show infiltration of FcεR II(+) T cells (Tc cells) in both acute and chronic lesions of AD, suggesting that activated lymphocytes participate in the pathogenesis of AD.

**MATERIALS AND METHODS**

**Subjects** Thirteen patients with AD (ages, 15–40 years; 8 male, 5 female), nine patients with eczematous dermatitis (ED) (ages, 19–80 years; 7 male, 2 female), and two normal nonatopic individuals (ages, 30 and 35, both male) were studied. The diagnosis of AD was based on the following criteria: (i) presence of a focal or generalized maculopapular, lichenified, pruritic skin rash that showed predilection for the flexural areas of the extremities as well as the face and neck; (ii) a chronic or chronically relapsing course of illness; and (iii) a positive family and/or personal history of respiratory atopy or eczema. The patients with ED included four with contact dermatitis, three with generalized exfoliative dermatitis, one with nummular eczema, and one with hand eczema. None of these patients had a personal atopic history, and were chosen as controls because both the clinical and histologic pictures of AD are similar to those of ED [2,4].

The acute lesion was defined as erythematous and occasionally as an exudative maculopapular rash that developed on the normal appearing skin or as a result of the worsening of a pre-existing lesion. The chronic lesion was defined as lichenified, non-exudative skin rash. The extent of skin disease (cutaneous index: CI) was quantified by determining the percentage of body involvement based on Lund and Browder’s law [15]. FcεR II(+) peripheral blood mononuclear cells (PBMC) were enumerated as previously described [16]. Total serum IgE was determined by a Pharmacia PRIST kit. Serum IgE levels in ED patients were variable; some of the patients with ED showed an elevation of serum IgE levels, which occurs indepen-
Table I. Identification of FcR II(+) Cells in Skin Lesions of AD and ED Patients

<table>
<thead>
<tr>
<th>Donor (age/sex)</th>
<th>CI</th>
<th>IgE (IU/ml)</th>
<th>%FcR II(+) PBMC</th>
<th>%FcR II(+) cells</th>
<th>% Tc cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD Acute 1. 20/M</td>
<td>60</td>
<td>7,500</td>
<td>N.D.</td>
<td>4.3</td>
<td>1.9</td>
</tr>
<tr>
<td>2. 27/F</td>
<td>70</td>
<td>490</td>
<td>4.8</td>
<td>4.0</td>
<td>2.4</td>
</tr>
<tr>
<td>3. 15/F</td>
<td>60</td>
<td>180</td>
<td>3.6</td>
<td>5.5</td>
<td>2.4</td>
</tr>
<tr>
<td>4. 16/F</td>
<td>13</td>
<td>1,690</td>
<td>2.0</td>
<td>5.4</td>
<td>1.1</td>
</tr>
<tr>
<td>5. 26/M</td>
<td>45</td>
<td>17,000</td>
<td>5.2</td>
<td>4.1</td>
<td>3.4</td>
</tr>
<tr>
<td>6. 24/F</td>
<td>8</td>
<td>2,000</td>
<td>1.9</td>
<td>5.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Chronic 7. 30/M</td>
<td>90</td>
<td>11,600</td>
<td>7.7</td>
<td>3.4</td>
<td>1.8</td>
</tr>
<tr>
<td>8. 22/M</td>
<td>60</td>
<td>22,000</td>
<td>6.7</td>
<td>2.5</td>
<td>N.D.</td>
</tr>
<tr>
<td>9. 18/M</td>
<td>65</td>
<td>22,700</td>
<td>3.1</td>
<td>0.7</td>
<td>3.0</td>
</tr>
<tr>
<td>10. 40/M</td>
<td>90</td>
<td>82</td>
<td>4.7</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>11. 22/M</td>
<td>60</td>
<td>3,000</td>
<td>4.8</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>12. 20/F</td>
<td>10</td>
<td>N.D.</td>
<td>1.9</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>13. 40/M</td>
<td>10</td>
<td>15,400</td>
<td>3.3</td>
<td>4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>ED Acute 14. 20/M</td>
<td>20</td>
<td>160</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15. 19/M</td>
<td>30</td>
<td>N.D.</td>
<td>2.5</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>16. 28/F</td>
<td>15</td>
<td>100</td>
<td>1.5</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>17. 22/F</td>
<td>10</td>
<td>280</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic 18. 62/M</td>
<td>90</td>
<td>2,200</td>
<td>1.9</td>
<td>0</td>
<td>0.07 ± 0.1</td>
</tr>
<tr>
<td>19. 75/M</td>
<td>85</td>
<td>1,100</td>
<td>2.1</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>20. 80/M</td>
<td>95</td>
<td>760</td>
<td>2.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21. 45/M</td>
<td>15</td>
<td>N.D.</td>
<td>N.D.</td>
<td>3.1</td>
<td>1.9</td>
</tr>
<tr>
<td>22. 32/M</td>
<td>8</td>
<td>1,300</td>
<td>3.1</td>
<td>1.9</td>
<td>0</td>
</tr>
<tr>
<td>mean ± SD</td>
<td></td>
<td>4.8 ± 0.6</td>
<td>2.3 ± 0.9</td>
<td>0.4 ± 0.7</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

* Donors 14–17 had contact dermatitis; donors 18–20 had generalized exfoliative dermatitis; donor 21 had nummular eczema; and donor 22 had hand eczema.

* Values are expressed as cells positive for FcR II and CD5 per 100 infiltrating mononuclear cells.

* N.D., not done.

dent of the atopic diathesis [17]. The clinical and immunologic findings in AD and ED patients are summarized in Table I. None of the patients had received previous oral steroids or any local treatment at the site of biopsy for at least 1 week prior to the study. None of the patients had evident systemic disease.

Reagents A monoclonal anti-human FcR II antibody of the IgG2 class, designated H107(14), was obtained from Nichirei Co, Japan. Anti-Leu 1 (CD5), anti-Leu 2a (CD8), anti-Leu 3a (CD4), and anti-Leu 4 (CD3) were all biotinylated; anti-Leu 16 (CD20), anti-Leu M3 (CD14), and streptavidin were phycoerythrin (PE)-conjugated. (All these reagents were obtained from Beckton-Dickinson, Mountain View, CA). FITC-F(ab)₂ goat anti-mouse IgG(G/M) were from Cappel Laboratories (Malvern, PA). OKT6(CD1) (Ortho Diagnostic System, Raritan, NJ) conjugated with rhodamine isothiocyanate (RITC) was a gift from M. Kashihara [18].

Preparation of Tissue Specimens Specimens were obtained from eczematous lesions, normal-appearing skin of the patients, and normal skin from non-eczematous individuals. One-half of the biopsy tissue was processed for routine histopathology and the other was fixed in formaldehyde-lysine-periodate solution, snap frozen in liquid N₂, and stored at −70°C until used for immunohistochemistry.

Immunofluorescence Staining of Tissues Frozen tissue sections (4 µm thick) were stained with H107(20 µg/ml) plus G/M diluted to 1:40, and further with normal mouse Ig(100 µg/ml) to inhibit the binding of the subsequent MoAb to G/M. The sections were then reacted with either biotinylated anti-Leu series antibody plus PE-streptavidin, PE-anti-Leu series antibody, or RITC-OKT6. Each step was performed in a moist chamber at room temperature for 45 min, followed by washing with phosphate-buffered saline, pH 7.4, 3 times. Control sections were stained with FITC-G/M and either PE-streptavidin or PE- or RITC-conjugated irrelevant antibody.

Tissue Analysis Reactive cells [green fluorescence for FcR II(+) cells and red fluorescence for T and B cells, monocytes/macrophages and Langerhans cells] were enumerated in the epidermal and upper dermal areas of four sections at 500 times in a fluorescence microscope. At least 500 cells with red fluorescence were counted in sections stained with anti-Leu 1, anti-Leu 2a, anti-Leu 3a, and anti-Leu 4 antibodies. The sections were then stained with hematoxylin to count infiltrating mononuclear cells. The data were expressed as reactive cells per total infiltrating mononuclear cells identified in hematoxylin stained sections. In experiments in which anti-Leu 16, anti-Leu M3, and OKT6 were used instead of anti-T-cell marker antibodies, 50–100 cells with red fluorescence were counted. Control sections showed no red fluorescence, whereas the number of cells with green fluorescence in these sections was less than 5% of the value in experimental sections. The actual H107-reactive cells were calculated by subtracting the mean value of the four control sections from that of the four experimental sections.
Statistical Analysis  Student t test was used to determine statistical differences between means. p values < 0.05 were considered significant.

RESULTS

Histologic features of the hematoxylin-eosin stained sections of AD lesions were indistinguishable from those of ED lesions. Acute exacerbated lesions showed acanthotic epidermis with focal spongiosis plus exocytosis and perivascular infiltration of mononuclear cells. Inflammatory infiltration was less intense, and epidermal acanthosis was more prominent in chronic lichenified lesions.

Sixty five to eighty percent of infiltrating mononuclear cells in both acute and chronic lesions of AD and ED were of a T-cell nature, as these cells reacted with anti-Leu 4 and anti-Leu 1 antibodies. The number of CD3(+) or CD5(+) T cells roughly corresponded to the sum of CD4(+) and CD8(+) T cells in each section. The CD4(+) : CD8(+) ratio varied considerably from 7 : 1 to 4 : 1 in AD, and 3 : 1 to 2 : 1 in ED (Fig 1). Most of the remainder reacted with anti-Leu M3 antibody and OKT6, indicating that they were monocytes/macrophages and Langerhans cells, respectively. Infiltration of Leu 16(+) cells was negligible in both AD and ED.

In acute AD lesions, FcεR II(+) cells were found scattered around the vessels in the upper dermis and sparsely distributed in the epidermis. Some of the cells co-expressed FcεR II and the Leu 1 marker, suggesting that these cells were Tc cells (Fig 2). With the use of an anti-Leu 4 antibody instead of anti-Leu 1 antibody, similar results but less superior staining patterns were obtained in the following experiments. Tc cells were enumerated in sections stained with anti-Leu 1 antibody.

As shown in Table I, enumeration of the stained cells in AD lesions revealed that 2%–5% of the infiltrating mononuclear cells bore FcεR II. Acute lesions were infiltrated with significantly higher numbers of FcεR II(+) mononuclear cells than were chronic lesions (0.005 > p > 0.001). Regression analysis revealed that the percentage of FcεR II(+) cells in the skin lesion correlated with neither CI, the percentage of FcεR II(+) PBMC, nor with the serum IgE level. Half or more of the H107-reactive cells were positive for CD5 in both acute and chronic skin lesions.

Means ± SD of CD8(+) and CD4(+) Tc cells among infiltrating mononuclear cells in acute lesions were 0.9 ± 0.6% and 1.0 ± 0.9%, respectively (Fig 3). CD8(+) Tc cells were always found in acute lesions; whereas the infiltration of CD4(+) Tc cells was an occasional finding. On the other hand, chronic lesions contained 0.6 ± 0.8% of CD8(+) Tc cells and 0.3 ± 0.3% of CD4(+) Tc cells.

![Figure 1](image1.png)  
**Figure 1.** CD4(+) : CD8(+) ratio of infiltrating cells in acute (○) and chronic (□) lesions from patients with AD and ED.

![Figure 2](image2.png)  
**Figure 2.** Infiltration of Leu 1(+) cells (A, arrow) bearing FcεR II (B, arrowheads) in the upper dermis of an acute exacerbated lesion of a patient with AD (donor 1 in Table I). A frozen section was double-stained with biotin-anti-Leu 1 plus PE-streptavidin (A) and with H107 plus FITC G/M (B) (magnification X500).

![Figure 3](image3.png)  
**Figure 3.** Surface phenotype of Tc cells in acute (closed symbol) and chronic (open symbol) lesions from AD patients. Closed or open symbols of the same shape represent the same individuals. Values are expressed as Tc cells positive for CD4 or CD8 per 100 infiltrating mononuclear cells. Horizontal bars, mean of each group.
suggesting the lack of a tendency for predominant infiltration of T cells with the particular phenotype.

Most of the remainder of Fe,R II(+) mononuclear cells included monocytes/macrophages (Fig 4). However, the enumeration of these cells was not performed because of very small numbers of cells positively stained with H107. Rarely, CD5(+) cells were in apposition to Fe,R II(+) non-T cells (Fig 5). OKT6(+) cells in both epidermis and dermis were not reactive with H107.

In accordance with the disappearance of inflammatory cells, Fe,R II(+) cells were also not recognized at areas in which the dermatitis was cleared after treatment with topical steroids in AD patients. On the other hand, AD lesions that did not respond to the treatment were infiltrated by numbers of Fe,R II(+) cells comparable to those in untreated areas.

ED lesions did not always contain Fe,R II(+) cells (Table I). When present, most of these cells seemed to be of non-T-cell lineages. H107-reactive cells were absent in normal-appearing skin from AD and ED patients and in the skin of normal nonatopic individuals.

**DISCUSSION**

The results of the present study demonstrated infiltration of Fe,R II(+) T cells and monocytes/macrophages in the skin lesions of AD patients but virtually no infiltration in lesions of ED patients. Because the Fe,R II on lymphocytes is an activation antigen [19,20], subpopulations of T cells might have been activated by a local endogenous or exogenous antigen to express Fe,R II. Alternatively, subsets of peripheral blood T cells bearing Fe,R II preferentially migrated to the AD lesion after antigenic stimulation at extracutaneous sites. High proportions of Fe,R II(+) cells in acute compared with chronic lesions suggested that antigenic stimulation was more extensive in the former.

Using anti-IgE antibody, IgE was not detected on T cells infiltrating skin lesions of AD [8–10]. This indicates that Fe,R II on T cells is not occupied by IgE, or that IgE bound to Fe,R II is rapidly catabolized. On the other hand, IgE-bearing APC have been identified in skin lesions from AD patients. This finding, together with the present demonstration of CD14(+) cells positive for Fe,R II, suggests that the Fe,R II on monocytes/macrophages binds IgE. Because II-4 and interferon-γ induce Fe,R II on Langerhans cells [21], lack of the reactivity of H107 with this cell type indicates that antigenic sites of Fe,R II on Langerhans cells are neither recognized by nor accessible to H107. Close apposition of T cells to Fe,R II(+) non-T cells, presumably monocytes/macrophages, suggested the participation of both T cells and IgE-bearing APC in the inflammatory skin reaction of AD.

The proportion of lesional Fe,R II(+) cells did not correlate with the percentage of Fe,R II(+) PBMC. This finding indicates that preferential accumulation of Fe,R II(+) cells but not mere influx of these cells in association with skin inflammation was responsible for the lesional T cells. Because the sum of skin lesions consisted of a mixture of acute and chronic lesions at various proportions in individual AD patients, CI also did not correlate with the proportion of lesional Fe,R II(+) cells. In patients who showed improvement of AD following topical treatment, infiltrating Fe,R II(+) cells disappeared, and at the same time percentages of Fe,R II(+) PBMC decreased [16]. Because treatment was directed to the dermatitis, this suggested that inflammatory reactions of the skin influenced the expression of Fe,R II in AD.

The surface phenotyping showed that CD8(+) T cells preferentially expressed Fe,R II in the acute lesions of AD patients. Thompson et al [22] detect CD8(+) T cells in the peripheral blood from atopic subjects in remission but not from active atopic patients, postulating that the remission results from the generation of IgE-related suppressor T cells. We have observed the predominance of CD8(+) T cells in acute AD lesions as well as in peripheral blood from patients with severe to moderate AD. The reason for this discrepancy between our results and those of Thompson et al is unknown. Katz and his colleagues [23] have identified CD8(+) T cells that disturb the regulatory function of CD4(+) T cells concerned with IgE synthesis in rodent tissue. Activation of these CD8(+) T cells enhances the in vitro production of IgE. We assume that CD8(+) T cells exert cytotoxic and suppressive effects on regulatory T cells that infiltrate the lesion, thus modulating the inflammation in an adverse direction.

We wish to thank Dr. K. Yasuda at Nichtig Co., Japan, for the continuous supply of H107, and Ms. Rie Matsuo and Keiko Sugaya for their technical assistance.

**REFERENCES**


