

Enhanced expression of the high-affinity receptor for IgE (FcεRI) associated with decreased numbers of Langerhans cells in the lesional epidermis of atopic dermatitis

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Abstract

Epidermal Langerhans cells (LCs) and the high-affinity receptor for IgE (FcεRI) on their surface are considered important in the pathogenesis of atopic dermatitis (AD). We investigated the numbers of epidermal LCs and their FcεRI expression in patients with AD and healthy controls. Biopsy specimens taken from lesional skin from 17 patients with AD, non-lesional skin from five patients with AD and normal skin from five healthy individuals were immunohistochemically stained with a monoclonal antibody against CD1a or with either of two monoclonal antibodies against two different epitopes of FcεRI α chain. Many dendritic cells were positively stained with anti-CD1a antibody in the epidermis of each skin sample, and fewer cells were stained with anti-FcεRI antibodies. The numbers of epidermal LCs positive for FcεRI were significantly increased in both lesional and non-lesional skin from AD patients compared with those in normal skin, suggesting important roles of FcεRI + LCs in the pathogenesis of the disease. In contrast, the numbers of total epidermal LCs (CD1a-positive) were decreased in AD lesional skin compared with those in non-lesional skin from AD patients and in normal skin from healthy subjects. Together with our finding that the numbers of epidermal LCs were negatively correlated with the clinical severity of the AD lesions, we concluded that epidermal LCs may decrease in some conditions of AD, probably in lesions with severe inflammation. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Atopic dermatitis; Langerhans cell, FcεRI

1. Introduction

Atopic dermatitis (AD) is a chronic skin disease occurring in individuals with a genetic back-

ground which facilitates increased IgE synthesis and IgE-mediated allergic reactions. Epidermal Langerhans cells (LCs) are efficient antigen-presenting cells belonging to the family of dendritic cells. In 1986, the importance of IgE molecules on epidermal LCs in the pathogenesis of AD was suggested by Bruynzeel-Koomen et al. [1] who

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reported that IgE-positive cells were present in the epidermis of skin sections from patients with AD, whereas no IgE staining was observed in the epidermis from non-AD control groups. Most of the IgE-bearing cells in the epidermis of AD were found to be LCs [1]. Bieber et al. [2] also demonstrated IgE-positive epidermal LCs in AD, together with the finding that the epidermal IgE staining was stronger in lesional skin than in non-lesional skin. Recent studies revealed that epidermal LCs express high affinity receptors for IgE (FcεRI) [3–5], and that FcεRI is the main structure on epidermal LCs that binds IgE molecules in lesions of AD [6]. Surface expression of FcεRI was increased on LCs from patients with AD when compared with LCs from non-atopic individuals [7], and was correlated with the serum IgE levels of patients with AD [8], however, there have not been any definite studies comparing the numbers of total epidermal LCs, FcεRI expressing LCs and IgE-bearing LCs in lesional and non-lesional skin from AD patients and normal skin from healthy subjects.

In the present study, we immunohistochemically stained epidermal LCs with a monoclonal antibody against CD1a antigen, or with either of the two monoclonal antibodies: one against the IgE binding site of FcεRI and the other against the non-IgE-binding site of FcεRI, and counted the number of cells positive for each antibody in the epidermis of lesional and non-lesional skin from patients with AD and normal skin from healthy individuals.

2. Materials and methods

2.1. Individuals and skin specimens

Seventeen patients with AD (11 men, six women; age: 19–50 years; average: 25.6 ± 7.7 years) participated in the study following informed consent. All patients fulfilled the diagnostic criteria proposed by Hanifin and Rajka [9], and had not received either topical or systemic corticosteroids for more than 1 month before the date of the examinations. Biopsy specimens were taken from lichenified lesions of the 17 AD patients after local anesthesia: seven from the trunk, seven from the upper limb and three from the forehead and neck. The biopsied lesions

were chronic and lichenified, but were associated with some degrees of acute inflammation. Skin biopsies from non-lesional skin were performed in five AD patients who agreed to the biopsy. Five normal skin samples were obtained from normal-looking skin surrounding benign skin tumors excised from otherwise healthy individuals (two men, three women; age, 23–43 years; average, 32.5 ± 9.8 years).

2.2. Severity score

Six clinical features (erythema, pruritus, excoriation or crusting, dryness or scaling, cracking or fissuring and lichenification) of the biopsied lesions were graded on a scale of 0 (none) to 3 (severe), making a maximum of 18.

2.3. Immunohistochemistry

Acetone-fixed cryostat sections (4 μm thick) were incubated with a monoclonal antibody against CD1a antigen (T6, Dako, Denmark) or with either of two monoclonal antibodies against two different epitopes of FcεRI α chain (CRA1 and CRA2) for 1 h at room temperature. The two antibodies were prepared and characterized as described earlier [10]. Briefly, CRA2 recognizes the IgE-binding site of FcεRI α chain and does not bind to IgE-bearing receptors, while CRA1 recognizes the non-IgE-binding site of FcεRI α chain and binds to the receptors regardless of IgE binding. Positive reactions were visualized using the LSAB kit (Dako, Denmark). Positively stained cells in the entire epidermis of the skin sections were counted twice by two observers, and the average was calculated. Epidermal area was estimated by measuring the thickness and length, and the number of positive cells per epidermal area unit (mm²) was calculated.

For double immunofluorescence staining, a frozen section was incubated with CRA1 antibody (1 h, 37 °C) and then with a biotin-labeled anti-mouse IgG antibody (10 min, room temperature). Then the section was incubated with fluorescein isothiocyanate (FITC)-labeled streptavidin (Dako, Denmark) and phycoerythrin (PE)-labeled anti-CD1a antibody (T6-RD1, Dako, Denmark) for 1

h at 37 °C. Sections were observed under a fluorescent microscope (Olympus, Tokyo).

2.4. Data analysis

Statistical analysis was performed using Mann–Whitney's *U* test for comparison of means of positive cell numbers among the three groups of skin samples. Correlation between severity scores and T6-positive or CRA1-positive cell numbers was assessed by Spearman's test.

3. Results

3.1. Immunohistochemical staining of epidermal LCs

Typical staining patterns of AD lesional skin were shown in Fig. 1. Many dendritic cells were positively stained with the T6 (anti-CD1a) antibody in the epidermis of each skin sample, and fewer cells were stained with the CRA1 (anti-FcεRI, non-IgE-binding site) antibody. CRA2 (anti-FcεRI, IgE-binding site)-positive cells were less than CRA1-positive cells. Double immunofluorescence staining of AD lesional skin (Fig. 2) showed that all FcεRI-positive cells were T6-positive, suggesting that most of the FcεRI-expressing cells in the epidermis were LCs. As well as in the epidermis, many CD1a + dendritic cells were seen in the dermis of AD lesions (photographs not shown). No metachromatic epidermal cells were observed in toluidine blue staining.

3.2. Increase in FcεRI-positive LCs and decrease in total LCs in the epidermis of AD lesions

The numbers of positively stained cells are shown in Fig. 3. CRA1 (FcεRI)-positive cells were significantly increased in the epidermis of lesional ($55.8 \pm 51.6/\text{mm}^2$) and non-lesional ($35.9 \pm 16.5/\text{mm}^2$) skin of AD when compared with those in normal skin ($8.3 \pm 7.8/\text{mm}^2$, $P < 0.01$), which is consistent with the earlier reports [7,8]. The differences in numbers of FcεRI-positive cells between the lesional and non-lesional epidermis were not significant. The numbers of CRA2 (FcεRI without IgE)-positive

cells were $25.6 \pm 39.0/\text{mm}^2$ in lesional skin, $16.4 \pm 8.9/\text{mm}^2$ in non-lesional skin and $4.3 \pm 6.6/\text{mm}^2$ in normal skin. The ratios of CRA2 + to CRA1 + were almost consistent (approximately 50%) among the three groups, indicating that about half of FcεRI on LCs was bound to IgE. The most surprising finding, as shown in Fig. 3, was the marked decrease in epidermal T6-positive cells in the lesional skin of AD. T6-positive LCs in the epidermis of lesional skin ($88.8 \pm 44.0/\text{mm}^2$) were significantly fewer ($P < 0.01$) than those in non-lesional skin ($172.1 \pm 74.2/\text{mm}^2$) and normal skin ($164.5 \pm 58.9/\text{mm}^2$). This decrease is a kind of unexpected result, but the differences were quite obvious; the numbers of LCs in the lesional epidermis were almost half of those in the non-lesional and normal epidermis.

3.3. Numbers of epidermal LCs were negatively correlated with the clinical severity of AD lesions

Fig. 4 shows the relationship between the numbers of T6-positive cells in the epidermis of AD lesions and the severity scores of the tested lesions. There was a significant negative correlation between the two parameters ($r = -0.592$, $P < 0.05$), suggesting that the number of epidermal LCs in severe dermatitis may be less than in mild dermatitis. No significant correlation was found between the numbers of CRA1-positive cells and the severity scores, probably due to the relatively small number ($n = 14$) of the tested samples.

4. Discussion

There is accumulating evidence that IgE-bearing LCs play crucial roles in the initiation and the maintenance of allergic inflammation in AD lesions [11–14]. In the epidermis of AD lesions, increase in LCs [15,16], FcεRI-expressing cells [7,8] and IgE-bearing cells [1,2] have been reported.

In earlier studies, different enumeration methods have been used for quantification of LCs. The most satisfactory method of epidermal preparation for LC enumeration was to obtain epidermal sheets [17] until it was possible to study cryosections with specific monoclonal antibodies. Using

vertical cryosections of human skin, Bieber et al. [18] compared various methods for enumeration of LCs, including enumeration of LC per microscopic fields, per mm length of epidermal surface, per mm length of basement membrane, per 200 basal cells, and per mm² epidermal section sur-

face. The results from the different methods varied considerably, but they noted that, with regard to the concept that LCs acted as antigen presenting cells for the whole epidermis volume, enumeration in an epidermal area unit (cells per mm²) seemed to be an appropriate method [18].

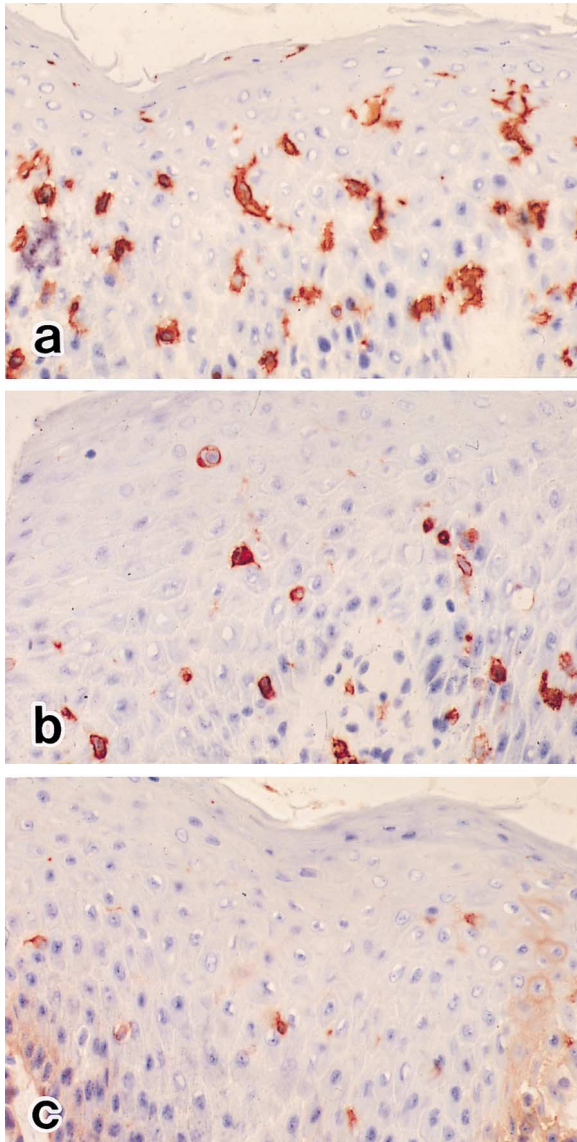


Fig. 1

Fig. 1. Immunohistochemical staining. Skin sections from AD lesional skin were stained with a monoclonal antibody against CD1a (a); the non-IgE-binding site of FcεRI (b); or the IgE-binding site of FcεRI (c).

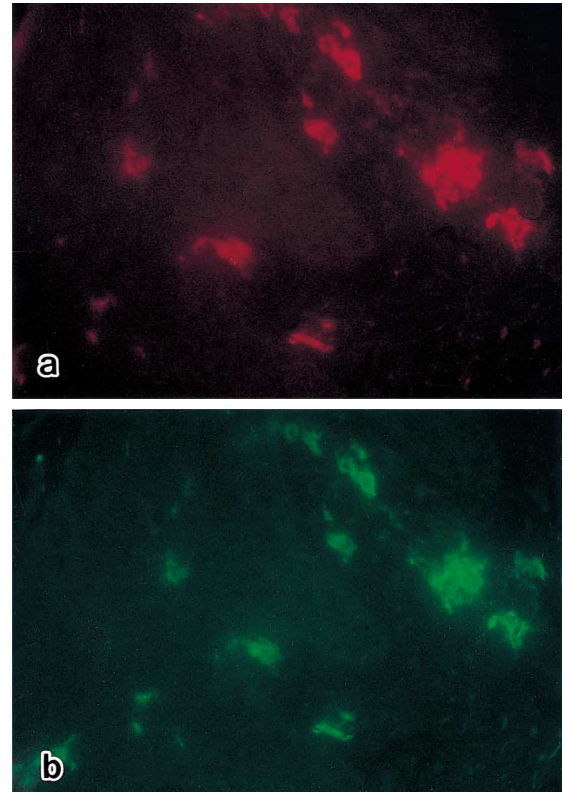


Fig. 2

Fig. 2. Double immunofluorescence staining of a skin section from AD lesional skin. (a) CD1a staining visualized by PE; (b) CRA1 (FcεRI) staining visualized by FITC.

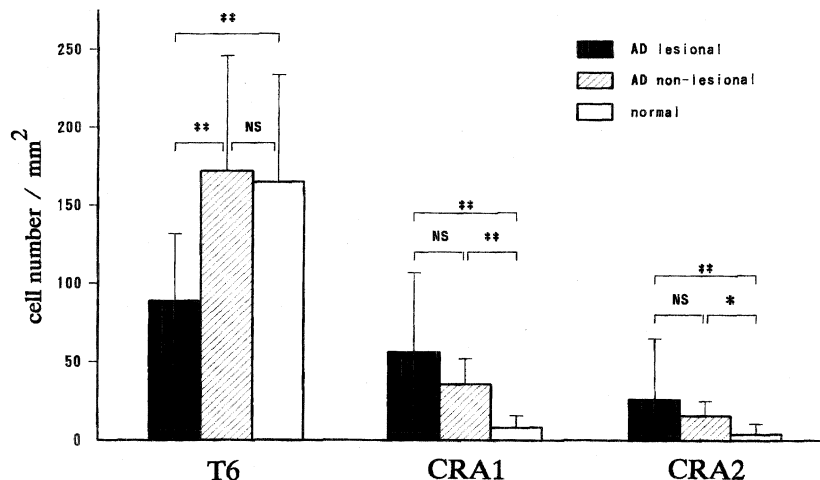


Fig. 3. Positive cell numbers per epidermal area unit (mm²). Skin sections from AD lesional skin ($n=17$), AD non-lesional skin ($n=5$) and normal skin ($n=5$) were immunohistochemically stained with a monoclonal antibody against CD1a (T6), with a monoclonal antibody against non-IgE-binding site of FcεRI (CRA1) or with a monoclonal antibody against IgE-binding site of FcεRI (CRA2). Positively stained cells in the epidermis were counted as described in Section 2. Bars indicate mean $\pm$ S.D. * $P < 0.05$; ** $P < 0.01$.

Since the publication of this report, some important studies on epidermal LC in AD skin [2,6] have been performed by counting LCs per epidermal area unit. Considering these situations, we designed our experiment in which LC numbers were expressed as positively stained cells per epidermal area unit.

In the present study, we also observed elevated expression of FcεRI in the epidermis of AD lesions, but in apparent contradiction to earlier reports [15,16], the numbers of epidermal LCs were significantly decreased in the AD lesional skin compared with those in AD non-lesional and normal skin. Two possible explanations for the decrease are, (i) epidermal proliferation in lichenified lesions led to an apparent decrease in epidermal LCs per epidermal area unit, (ii) migration of LCs into the dermis for antigen presentation [19] resulted in the depletion of epidermal LCs. The latter hypothesis may be supported by our observation that many CD1a⁺ dendritic cells were seen in the dermis of AD lesions. Uno and Hanifin showed increased numbers of LCs in chronic lichenified lesions and fewer LCs in acute erythematous lesions of AD, suggesting a possible relationship between numbers of epidermal LCs and cutaneous inflammatory conditions [15]. The

present study showed that the numbers of epidermal LCs were negatively correlated with the severity scores of the lesions, which represent the degree of inflammation. Taken together, it is conceivable that the number of epidermal LCs may decrease in severely inflamed skin as a result of increased migration of LCs from the epidermis to the dermis for the purpose of antigen presenta-

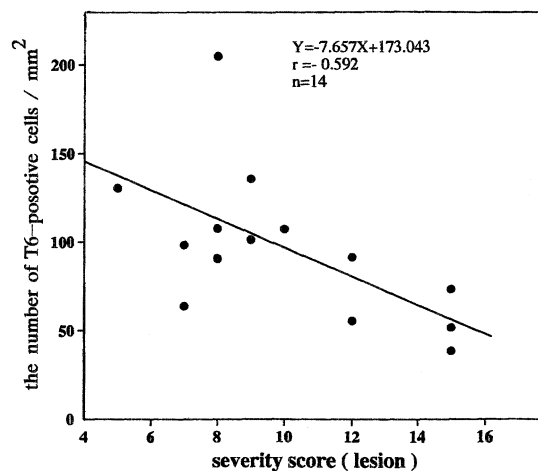


Fig. 4. Negative correlation between the number of epidermal LCs (T6-positive cells) and the severity score of the AD lesion. LC numbers are shown on the ordinate, and severity scores of the lesions are shown on the abscissa.

tion. Anyway, the present study emphasizes that epidermal LCs may decrease in some conditions of AD, probably in lesions with severe inflammation.

There was no difference in the numbers of epidermal LCs between non-lesional skin from patients with AD and normal skin from healthy subjects, however, the expression of FcεRI in epidermal LCs was elevated in non-lesional skin from AD patients compared with that in normal skin, which is consistent with the earlier findings [1,8,20]. Binding of IgE molecules to FcεRI on epidermal LCs is necessary for effective allergen presentation by LCs to T cells in AD patients [21]. Since it is well-known clinically that non-lesional skin of AD patients is susceptible to allergic inflammations, we consider that the up-regulation of FcεRI in non-lesional skin of AD patients may facilitate effective antigen presentation and subsequent allergic reactions.

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