Increased number of IgE positive Langerhans cells in the conjunctiva of patients with atopic dermatitis

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Abstract
Aim—To determine the role of Langerhans cells (LCs) found to bear IgE in patients with atopic dermatitis (AD) by evaluating the surface distribution of these cells in the conjunctival epithelium and epidermis of skin lesions in patients with AD.

Methods—The double labelling method was used to evaluate IgE positive cells that were positive for anti-CD1a or anti-CD23 antibody in an epithelial sheet of the conjunctival limbus. Specimens of conjunctiva were obtained from 12 men, six of whom had AD and ocular complications. Five patients without atopic disease served as controls, plus one additional patient with asthma but no AD. A similar study was conducted using epidermal sheets obtained from two patients with AD and from one without AD.

Results—The number of CD1a+ cells present in the conjunctival epithelium of the patients with AD significantly exceeded that of the patients without AD. Most CD1a+ cells in the conjunctival epithelium and epidermis from the patients with AD bore IgE on their surfaces. Few such cells from patients without AD bore IgE. No CD23+ cells were found in the patients with or without AD.

Conclusions—The presence of an increased number of LCs bearing IgE on their surfaces in the conjunctival epithelium of patients with AD suggests that these cells may be involved in eliciting the hypersensitivity reaction and participate in ocular inflammation.

Materials and methods
PATIENTS

The study protocol was approved by the board of clinical research of Kyushu University. We evaluated specimens of conjunctiva obtained from 12 subjects, six patients with AD and six control patients without AD. Six Japanese men, age range 14–32 years (mean 22 years) had AD and ocular complications consisting of conjunctivitis (in five), cataract (in six), and retinal detachment (in six). The conjunctivitis was severe. Five of these patients exhibited oral dilies or tears in the peripheral retina. Biopsy samples (1 × 6 mm) had been taken from the limbal conjunctiva at the initial operation for retinal detachment. The diagnosis of AD was based on the clinical features together with an elevated level of serum IgE. Skin lesions had been treated for years with corticosteroid ointments. Of the six control patients, five were men aged 16–25 years (mean 20 years) without AD, and who were operated on for retinal detachment or exotropia. These five patients lacked any evidence of allergic conjunctivitis or other allergic disease. They were a source of
the control specimens of the conjunctiva. The sixth control patient was a 12-year-old boy with bronchial asthma but no AD, and who was operated on for retinal detachment. All patients gave their informed consent to participate.

Specimens of skin measuring 3 × 6 mm were obtained at biopsy of skin lesions of two of the six patients with AD and ocular complications, and from the normal skin of the abdomen of one control patient without AD.

IMMUNOLABELLING

For immunolabelling, we used monoclonal antibody IOT6a (IgG1; Immunotech, Marseilles, France) which reacts with CD1a that is expressed exclusively on LCs in the skin and conjunctiva. We also used anti-FcεRII/CD23 monoclonal antibody, which is specific for the low affinity receptor for IgE. Negative controls were obtained by omitting the primary monoclonal antibodies. Samples of the conjunctiva and skin were subdivided into portions for use in double staining for CD1a and IgE and for CD23 and IgE. Specimens were treated with dispase (Godo Shusei Co, Tokyo, Japan) in minimum essential medium (Gibco, Grand Island, NY, USA) for 2–4 hours at 37°C to separate the epithelium from the underlying tissue. The epithelial sheets thus obtained were fixed briefly with 1% paraformaldehyde (30 minutes at 4°C). Sheets were then soaked in phosphate buffered saline (PBS) plus 0.4% human AB serum (Cosmo Bio Co, Tokyo, Japan) and 0.6% bovine serum albumin (Sigma Chemical Co, St Louis, MO, USA) for 30 minutes to reduce the number of nonspecific immunoreactions. One of the two sheets was incubated with monoclonal antibody IOT6a (diluted 1:40 in PBS; Immunotech), and the other with anti-CD23 monoclonal antibody (diluted 1:40 in PBS; Becton Dickinson, San Jose, CA, USA) for 60 minutes at 37°C. The sheets were incubated with biotin labelled rabbit anti-mouse immunoglobulin (Nichirei, Tokyo, Japan) for 60 minutes, and then with rhodamine (diluted 1:40 in PBS; Vector Laboratories Inc, Burlingame, CA, USA) for 60 minutes. They were then incubated for 60 minutes with fluorescein isothiocyanate labelled goat anti-human IgE (diluted 1:40 in PBS; Cappel, Durham, NC, USA). Specimens were rinsed three times with PBS after each step. The sheets thus prepared were observed under a Zeiss (Oberkochen, Germany) fluorescent microscope.

Cells with a brightly fluorescent cytoplasm were considered to be positive for the test substance. The number of cells that were positive for CD1a, IgE, or CD23 was counted in each sheet, and the number of stained cells per mm² of epithelium was calculated.

STATISTICAL ANALYSIS

Data were reported as mean (SD). The Kruskal–Wallis test was used to evaluate the significance of the differences between the means. Spearman’s rank correlation coefficient (rs) was used to evaluate correlations. A level of p<0.05 was accepted as statistically significant.

Figure 1 Epithelial sheet of conjunctival limbus from patient no 4 with AD and ocular complications. Cells were double stained for IgE (A) and CD1a (B). Numerous IgE positive cells (396 cells/mm²) (A) were found in the same area as the CD1a positive cells (B). Some IgE positive cells were CD1a negative. Bars = 10 µm.

Figure 2 Epithelial sheet of conjunctival limbus from patient no 11 without AD. Cells were stained for IgE (A) and CD1a (B). Although no IgE positive cells (A) were observed, CD1a positive cells (B) were present (294 cells/mm²). Bars = 10 µm.
Results
Numerous IgE positive cells (mean 337 (62.5) cells/mm²) and CD1a positive cells (mean 370 (72.1) cells/mm²), many of which were dendritic, were present in the epithelial sheets from the six patients with AD. Double labelling with anti-IgE and anti-CD1a showed that the IgE positive dendritic cells (Fig 1A) were consistently positive for CD1a (Fig 1B), while the IgE positive round cells were negative for CD1a. The epithelium from the patients without AD exhibited few IgE positive cells (mean 2.2 (4.4) cells/mm²) (Fig 2A), but numerous CD1a positive cells (mean 144 (86.7) cells/mm²) (Fig 2B). The sheet of conjunctival epithelium from the patient with bronchial asthma and no AD exhibited 287 cells/mm² that were positive for CD1a and IgE antibodies.

The specimens of epidermis obtained from the patients with AD exhibited numerous IgE positive (Fig 3A) and CD1a positive cells (Fig 3B) (mean 583 cells/mm²). However, no IgE positive cells were observed in the epidermis of the patient without AD (Fig 4A), although numerous CD1a positive cells (913 cells/mm²) were observed (Fig 4B).

No cells in the epidermal or the conjunctival epithelial sheets from any patients were stained with anti-CD23 antibody (data not shown).

Table 1 shows the total serum level of IgE, the number of CD1a positive cells, and the incidence of IgE/CD1a positivity in the conjunctival epithelium of all patients. The number of CD1a positive cells in the conjunctival epithelium of the six patients with AD was significantly higher (370 (72) cells/mm²) than in the five patients without AD (144 (87) cells/mm²) (Fig 5, p = 0.0106). The number of CD1a positive cells was related to the total serum level of IgE (r = 0.72). The mean incidence of IgE/CD1a positivity in the six patients with AD was 0.90 (0.13) v 0.02 (0.05) in the five patients without AD (p = 0.0047). In patient no 8, who presented with inflammation of the anterior segment of the right eye after trauma, the incidence of IgE/CD1a positivity was only 11%, lower than that of the patients with AD. The incidence of IgE/CD1a positivity was correlated with the total serum level of IgE (r = 0.65). The incidence of IgE/CD1a positivity in the epidermis of patients with AD and ocular complications was 100%.

Discussion
The present study showed two key findings: (1) patients with AD and ocular complications exhibited significantly more LCs in their conjunctival epithelium than did the patients without AD, and (2) most of the LCs from patients with AD and ocular complications bore IgE on their surfaces (incidence of IgE/CD1a positivity was 100% in three of the six patients). Findings indicated that the patients with AD and ocular complications exhibited a large number of LCs with a high affinity for IgE in their conjunctiva and skin. In contrast, patients without AD rarely showed such LCs in their conjunctiva and skin.

Regarding the patients with AD but without ocular problems, it has been reported that only occasional IgE positive LCs are found in the uninvolved epidermis, and those LCs bear a
AD = atopic dermatitis; LC = Langerhans cells.

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Serum IgE (U/ml)</th>
<th>Number of LCs (cells/mm²)</th>
<th>IgE/LC (%)</th>
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<td>AD and ocular complications</td>
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</tr>
<tr>
<td>1</td>
<td>53 000</td>
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<td>2</td>
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<td>3</td>
<td>4 000</td>
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<td>4</td>
<td>3 800</td>
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<td>5</td>
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<td>Mean (SD)</td>
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<td>370 (72.1)</td>
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<tr>
<td>11</td>
<td>17</td>
<td>294</td>
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<tr>
<td>Mean (SD)</td>
<td>160 (240)</td>
<td>144 (86.7)</td>
<td>2.2 (4.9)</td>
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<tr>
<td>12</td>
<td>420</td>
<td>287</td>
<td>100</td>
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</table>

IgE positive Langerhans cells in conjunctiva in atopic dermatitis

**Figure 5** Number of CD1a positive cells in the conjunctival epithelium of patients with and without AD. The number of such cells in the patients with AD and ocular complications significantly exceeded that in the patients without AD (p<0.05).

Barker et al. reported that 35% of IgE positive cells in the skin of patients with AD were positive for CD1a. We also observed that some IgE positive cells in the conjunctival epithelium, mainly round cells, were negative for CD1a. We therefore suggest that the IgE bearing cells in the conjunctival epithelium are not limited to the LCs.

Human LCs express high affinity receptors for IgE (FceRII), low affinity receptors for IgE (FceRII/CD23), and IgE binding protein (eBP). FceRI is the major IgE binding molecule on the surfaces of LCs in the epidermis. Antibodies against FceRI, but not against FceRII, abrogate the binding of IgE to LCs on cryostat skin sections. The receptors of FceRII and FceRI on LCs are thought to be linked to the pathogenesis of IgE mediated allergic skin diseases such as AD. The FceRII/CD23 positive LCs appeared to be more dense in the areas of positive patch test reactions to aeroallergens in skin lesions of patients with AD. However, Schmitt et al. failed to detect the expression of FceRII in LCs that were freshly isolated from patients with AD. We found no CD23 positive cells in the epithelium of patients with or without AD. A possible explanation is that the FceRII/CD23 were fully occupied with IgE, and thus competitively inhibited the binding of anti-CD23 antibody. However, it is also possible that the LCs expressed little or no FceRII.

Conjunctivitis (in five of six), cataract (in six of six), and retinal detachment (in six of six) were present in the patients with AD. The conjunctivitis was sometimes severe. In five of the six patients, oral dials or tears adjacent to the ora serrata were detected. The frequent occurrence of oral dials and tears of the non-pigmented epithelium of the ciliary body in patients with AD has been attributed to an abnormality of the vitreous gel at its base. An increase in pigmentation on the anterior chamber angle is a characteristic and predictive sign of the retinal detachment associated with AD. The pigment appears to migrate from latent breaks resulting from inflammation of the peripheral retina and ciliary body. These findings suggest that inflammation tends to occur in the anterior segment of the eyes of patients with AD. It is likely that IgE bearing LCs can present certain specific antigens to T cells, since antigen presentation by LCs is mediated by IgE in patients with AD. CD1a positive cells in the nasal mucosa of allergic patients are also positive for IgE, and such cells are more dense in the nasal mucosa of allergic than non-allergic patients. Since the conjunctiva and nasal mucosa are directly exposed to airborne antigens, we suggest that the numerous IgE bearing LCs present in the conjunctival limbus may contribute to the allergic reaction to antigens in patients with AD and participate in ocular inflammation.

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