Interleukin-2 production of lymphocytes in food sensitive atopic dermatitis

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Abstract

The proliferative responses of peripheral blood mononuclear cells (PBMC) to food antigens in 22 patients with food sensitive atopic dermatitis were significantly higher than the responses of healthy children and food sensitive children with immediate symptoms. Moreover, the activity of interleukin-2 (IL-2) in supernatants of food antigen stimulated PBMC cultures from patients with atopic dermatitis was significantly higher than that in healthy children and food sensitive children with immediate symptoms. The activity of IL-2 in culture supernatants of separated cell populations stimulated with food antigens from patients with atopic dermatitis and healthy children was investigated. The activity of IL-2 in supernatants of food antigen stimulated T cell cultures could be detected in patients with atopic dermatitis but not in healthy children. These results suggest that the increased IL-2 production after food antigen stimulation is due to increased T cell activity in food sensitive atopic dermatitis.

Food allergy clearly seems to play an aggravating part in some patients with atopic dermatitis, and the significance of IgE in the pathogenesis had been discussed. Patients with atopic dermatitis caused by food allergens do not always have food specific IgE antibodies. Wraith et al have reported that 36% of foods causing 'non-immediate' symptoms gave a negative radioallergosorbent test (RAST). There is no clear correlation between the quantities of specific IgE antibodies and the nature and the severity of allergic symptoms, and patients may retain IgE antibodies for many years after they have ceased to suffer symptoms. Moreover, it is certain that atopic patients have abnormal responses in cell mediated immunity. There have been some reports on proliferative responses of PBMC and interleukin-2 (IL-2) production of inhaled antigen stimulated PBMC from allergic patients. However, little has been reported on IL-2 production of food antigen stimulated PBMC from food sensitive patients. The aim of the present study was to find the proliferative responses of PBMC and T cells to food antigens and the IL-2 production of PBMC and T cells stimulated with food antigens in patients with atopic dermatitis who were sensitive to food allergens.

Subjects and methods

Subjects

Twenty two patients with atopic dermatitis were studied. Twelve were selected on the basis of clinical history and food challenges as being sensitive to hens' egg and 10 were selected as being sensitive to cows' milk. The 12 patients with atopic dermatitis who were sensitive to hens' egg ranged in age from 4 months–11 years (mean 2.5 years) and the 10 patients with atopic dermatitis were sensitive to cows' milk ranged in age from 4 months–10 years (mean 4.9 years). Food challenges were performed generally in the double blind, placebo controlled manner of Bock et al,12 and in an open manner if there was a clear cut history of major allergic skin symptoms after ingestion of a specific food or if there was a chance of systemic anaphylaxis. Diagnosis of atopic dermatitis was defined by the criteria of Hanifin.13 Cutaneous manifestations of most patients with atopic dermatitis appeared after two hours or more (usually within 48 hours) after hens' egg or cows' milk ingestion. The control group included 11 non-atopic healthy children without hens' egg or cows' milk sensitivity, ranging in age from 1–10 years (mean 4.2 years) (control group 1), and seven hens' egg sensitive and nine cows' milk sensitive patients ranging in age from 11 months–13 years (mean 3.7 years) with urticaria, angio-oedema, acute gastroenteritis, and asthma (immediate symptoms) that appeared within one hour (usually within 15 minutes) after hens' egg or cows' milk ingestion (control group II).

The RAST scores for hens' egg or cows' milk, proliferative responses of PBMC to food antigens, and IL-2 production of PBMC stimulated with food antigens were examined when diseases were diagnosed.

RAST

The RAST was performed as recommended by the Phadebas RAST test kit (Pharmacia). Hens' egg and cows' milk discs were supplied by Pharmacia. RAST results were scored 0 to 4 + by comparison with serially diluted reference sera (Pharmacia) graded A to D (that is, a serum <D=0, between D and C=1+, and >A=4+). RAST scores of 2+, 3+, and 4+ were recorded as positive.

Cell separation and culture

Blood was taken into preservative free heparin and was separated by gradient centrifugation with the use of Ficoll-Paque (Pharmacia). PBMC were harvested from the interface and washed three times in phosphate buffered saline. To remove adherent cells, 2–5×10^7 PBMC were incubated in 20 ml of culture medium consisting of Roswell Park Memorial Institute (RPMI)
medium 1640 supplemented with 15% pooled human AB positive serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and l-glutamine (2 mM) in 75 cm² tissue culture flasks (Falcon) for 2–3 hours at 37°C. Non-adherent cells were recovered and were separated into a T cell enriched population and a B cell enriched population by the rosetting method with neuraminidase treated sheep red blood cells (SRBC), after which they were subjected to centrifugation over Ficoll-Paque and a nylon wool column. The rosetted cells were collected from the bottom, recovered by lysis of the SRBC in an isotonic ammonium chloride buffer for 10 minutes at 4°C, passed through the column, and washed three times with phosphate buffered saline. These cells were mainly T cells (CD3+ >98%). Non-rosetted cells were collected from the interface and passed through the column. They were used as B cells (72% CD20+, 2% CD3+, and 2% CD14+). The adherent cells were harvested by a rubber policeman and washed three times with phosphate buffered saline. PBMC, T cells with adherent cells, and B cells with adherent cells were separately cultured at 1×10⁶/ml in RPMI 1640 culture medium supplemented with 15% pooled human AB positive serum. Cultures were performed in triplicate at 0.2 ml per well in round bottomed microtiter plates or culture test tubes with or without food antigens (A/S Nunc) at 37°C in a humidified atmosphere containing 5% carbon dioxide for 3–5 days. Food antigens, ovalbumin (Wako Junyaku), and bovine serum albumin (Wako Junyaku) were separately diluted in culture medium and were added in final concentrations of 0.25 μg/ml, 2.5 μg/ml, and 25 μg/ml, respectively.

DNA synthesis was measured by adding 0.5 μCi tritiated [³H]-thymidine per well four hours before harvesting onto glass fibre filters. [³H]-thymidine incorporation was measured by liquid scintillation counting as counts per minute (cpm) and the results were expressed as the mean of triplicate. To compare responses between individuals, we expressed the results as a stimulation index (SI) using the following formula:

\[
\text{SI} = \frac{\text{cpm in cultures stimulated with food antigens}}{\text{cpm in cultures stimulated with medium} \times 100}
\]

On the other hand, culture supernatants in the culture test tubes were spun to remove cells after three days and stored frozen at −40°C until measurement of the activity of IL-2.

**MEASUREMENT OF THE ACTIVITY OF IL-2**

The biological activity of IL-2 was determined by proliferation of cells from a mouse T cell line, NKC-3, which was highly dependent on IL-2 activity for growth. NKC-3 was a gift from Takeda Chemical Industries Co Ltd. Cells were cultured at 6×10⁵/ml in RPMI 1640 culture medium with 20% heat inactivated fetal calf serum in round bottomed microtiter plates in triplicate at 50 μl per well. Cultures were performed with 50 μl culture supernatant and 50 μl RPMI 1640 culture medium in a humidified atmosphere with 5% carbon dioxide for 24 hours, and were then pulsed with 0.5 μCi [³H]-thymidine per well four hours before harvesting. To compare responses between individuals we expressed the results as a SI using the following formula:

\[
\text{SI} = \frac{\text{cpm in cultures with supernatants of food antigen stimulated PBMC}}{\text{cpm in cultures with supernatants of unstimulated PBMC}} \times 100
\]

**STATISTICS**

Student's t test was used for the comparison of SI in different groups. The comparison of RAST scores was assessed by χ² analysis.

**Results**

**RAST scores**

The results of RAST scores, proliferative responses of PBMC and the activity of IL-2 for control group I, control group II, and patients with atopic dermatitis are presented in tables 1 and 2. The RAST values for hens’ egg of patients with atopic dermatitis with hens’ eggs sensitivity were significantly (p<0.01) lower than the RAST values of control group II. Although the RAST values for cows’ milk of patients with atopic dermatitis with cows’ milk sensitivity tended to be lower than the RAST

**Table 1** Results of RAST scores for hens’ egg sensitivity, proliferative responses of PBMC to ovalbumin, and the activity of IL-2 in culture supernatants of PBMC stimulated with ovalbumin for control group I, control group II, and patients with atopic dermatitis

<table>
<thead>
<tr>
<th>Group</th>
<th>No of subjects</th>
<th>Mean (SD) age (years)</th>
<th>Mean (SD) proliferative responses of PBMC to ovalbumin (SI)</th>
<th>Mean (SD) IL-2 activity (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group I (healthy children)</td>
<td>7</td>
<td>4.3 (2.8)</td>
<td>0.1 (0.6)</td>
<td>0.99 (0.44)</td>
</tr>
<tr>
<td>Control group II (immediate allergic symptoms to hens’ egg)</td>
<td>7</td>
<td>6.2 (6.4)</td>
<td>1.2 (0.6)</td>
<td>1.14 (0.33)</td>
</tr>
<tr>
<td>Atopic dermatitis (sensitivity to hens’ egg)</td>
<td>12</td>
<td>2.5 (3.5)</td>
<td>3.2 (1.9)</td>
<td>2.53 (0.87)</td>
</tr>
</tbody>
</table>

Cases with positive RAST score/totals cases.

†The proliferative responses of PBMC to ovalbumin in patients with atopic dermatitis sensitive to hens’ egg were significantly higher than control group I (p<0.005) and II (p<0.01).

‡Activity of IL-2 in supernatants of ovalbumin or stimulated PBMC cultures from patients with atopic dermatitis sensitive to hens’ egg were significantly higher than control group I and II (p<0.005).
Table 2  Results of RAST scores for cows' milk sensitivity, proliferative responses of PBMC to bovine serum albumin, and the activity of IL-2 in culture supernatants of PBMC stimulated with bovine serum albumin for control group I, control group II, and patients with atopic dermatitis.

<table>
<thead>
<tr>
<th>Group</th>
<th>No of subjects</th>
<th>Mean (SD) age (years)</th>
<th>RAST scores for cows' milk (%)</th>
<th>Mean (SD) proliferative responses of PBMC to bovine serum albumin (SI)</th>
<th>Mean (SD) IL-2 activity (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group I (healthy children)</td>
<td>4</td>
<td>4.0 (4.2)</td>
<td>2</td>
<td>1-11 (0-30)</td>
<td>0-83 (0-81)</td>
</tr>
<tr>
<td>Control group II (immediate allergic symptoms to cows' milk)</td>
<td>9</td>
<td>1-7 (0-9)</td>
<td>0</td>
<td>1-12 (0-42)</td>
<td>1-25 (0-59)</td>
</tr>
<tr>
<td>Atopic dermatitis (sensitivity to cows' milk)</td>
<td>10</td>
<td>4-9 (4-0)</td>
<td>7</td>
<td>2-42 (1-24)†</td>
<td>3-23 (1-49)‡</td>
</tr>
</tbody>
</table>

*Cases with positive RAST score/total cases.

1 The proliferative responses of PBMC to bovine serum albumin in patients with atopic dermatitis sensitive to cows' milk were significantly higher than control group I and II (p<0.005).

2 Activity of IL-2 in supernatants of bovine serum albumin stimulated PBMC cultures from patients with atopic dermatitis sensitive to cows' milk were significantly higher than control group I and II (p<0.005).

values of control group II, there was no significant difference.

**PROLIFERATIVE RESPONSES OF PBMC TO OVAALBUMIN OR BOVINE SERUM ALBUMIN**

The proliferative responses of PBMC to ovalbumin in patients with atopic dermatitis who were sensitive to hens' egg were significantly higher than those of either control group I or control group II (p<0.005, p<0.01, respectively; table 1). Similar results were seen in cows' milk allergy, as shown in table 2. The proliferative responses of PBMC to bovine serum albumin in patients with atopic dermatitis who were sensitive to cows' milk were significantly higher than those of either control group I or control group II (p<0.005, respectively). These results suggest that the proliferative responses of PBMC to ovalbumin or bovine serum albumin have a significant value in patients with atopic dermatitis who are sensitive to hens' egg or cows' milk. We have previously reported such findings.³

IL-2 PRODUCTION IN OVALUMIN OR BOVINE SERUM ALBUMIN STIMULATED PBMC CULTURES

The activity of IL-2 in supernatants of ovalbumin stimulated PBMC cultures from patients with atopic dermatitis who were sensitive to hens' egg was significantly higher than that in either control group I or control group II (p<0.005, respectively; table 1). Similarly, the activity of IL-2 in supernatants of bovine serum albumin stimulated PBMC cultures from patients with atopic dermatitis who were sensitive to cows' milk was significantly higher than that in either control group I or control group II (p<0.005, respectively; table 2).

IL-2 PRODUCTION IN OVALUMIN OR BOVINE SERUM ALBUMIN STIMULATED T CELL OR B CELL CULTURES

We investigated the proliferative responses of PBMC, T cells with adherent cells, and B cells with adherent cells to ovalbumin or bovine serum albumin and IL-2 activity of culture supernatants in two randomly selected patients whose clinical manifestations of atopic dermatitis were severe and in two age matched healthy children. One of the two patients with atopic dermatitis was sensitive to hens' egg and the other was sensitive to cows' milk. The proliferative responses of T cells with adherent cells and B cells with adherent cells to bovine serum albumin and IL-2 activity of culture supernatants from a healthy child who is not sensitive to cows' milk are shown in fig 1. Both T cells with adherent cells and B cells with adherent cells did not respond to bovine serum albumin. Furthermore, IL-2 activity could not be detected in culture supernatants of bovine serum albumin stimulated T cells with adherent cells or B cells with adherent cells. The results for a patient with atopic dermatitis who is sensitive to cows' milk are shown in fig 2. T cells with adherent cells responded to bovine serum albumin, whereas B cells with adherent cells did not respond. IL-2 activity could be detected in culture supernatants of bovine serum albumin stimulated T cells with adherent cells; however, it could not be detected in culture supernatants of bovine serum albumin stimulated B cells with...
another report suggests that, when stimulated with concanavalin A, OKT+8 cells may also produce IL-2.24 Rawle et al have reported that IL-2 production was detected in supernatants of antigen P1 stimulated T cell cultures from Dermatophagoides pteronyssinus sensitive patients.10 In other studies, 'lymphocyte mitogenic factor' was reported in three day supernatants of PBMC cultures of allergic patients proliferating in response to grass pollen.11 Mosmann et al have reported that mouse helper T cell clones fall into two main groups (TH1 and TH2), defined primarily by differences in the pattern of lymphokines synthesised.25 26 TH1 clones synthesise IL-2, interferon γ, and lymphotoxin, whereas these lymphokines are not detectably expressed in TH2 clones. Conversely, only TH2 clones synthesise detectable amounts of IL-5. IL-5, TH1 clones also cause effective delayed type hypersensitivity reactions.25 26

In this study, proliferative responses of both PBMC and T cells to food antigens were recognised. Moreover, IL-2 production of PBMC and T cell cultures stimulated with food antigens was detected in patients with atopic dermatitis who were sensitive to food allergens. These results suggest that the increased IL-2 production after food antigen stimulation is due to increased T cell activity in patients with atopic dermatitis who are sensitive to food allergens.

We are grateful to Central Research Division, Takeda Chemical Industries Ltd for supplying, NKC-3, and to Mines Yukiko Hirano, Ryo Takenaka, and Tsukasa Kameyama for the culture assistance.

Table 3 Results of proliferative responses and the activity of IL-2 in culture supernatants from separated cell populations stimulated with ovalbumin in a patient with atopic dermatitis and a healthy child

<table>
<thead>
<tr>
<th>Serum IgE (U/ml)</th>
<th>RAST scores for hens' egg</th>
<th>Proliferative responses to ovalbumin (SI)</th>
<th>IL-2 activity in culture supernatants (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unstimulated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBMC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T+Ad+</td>
<td></td>
</tr>
<tr>
<td>Healthy child</td>
<td></td>
<td>1-63</td>
<td></td>
</tr>
<tr>
<td>Patient with atopic dermatitis (sensitive to hens' egg)</td>
<td>38</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Healthy child</td>
<td></td>
<td>1-63</td>
<td></td>
</tr>
<tr>
<td>Patient with atopic dermatitis (sensitive to hens' egg)</td>
<td>150</td>
<td>2</td>
<td>1-06</td>
</tr>
<tr>
<td>ND, not done.</td>
<td></td>
<td>1-21</td>
<td></td>
</tr>
<tr>
<td>Healthy child</td>
<td></td>
<td>2-68</td>
<td></td>
</tr>
<tr>
<td>Patient with atopic dermatitis (sensitive to hens' egg)</td>
<td>ND</td>
<td>0-81</td>
<td></td>
</tr>
</tbody>
</table>


1 T cells with adherent cells.
2 B+Ad- cells with adherent cells.
3 B+Ad+ cells with adherent cells.
4 ND, not done.
10 Rawle FC, Mitchell EB, Platts-Mills TAE. T cell responses to the major allergen from the house dust mite Dermatophagoides pteronyssinus, antigen-P; comparison of patients with asthma, atopic dermatitis and perennial rhinitis. J Immunol 1984;133:195-201.
24 Luger TA, Smolen JS, Chused TM, Steinberg AD, Oppenheim JJ. Human lymphocytes with either the OKT4 or OKT8 phenotype produce interleukin 2 in culture. J Clin Invest 1982;70:470-3.