IL-4 Induces Chemotaxis of Blood Eosinophils from Atopic Dermatitis Patients, but Not from Normal Individuals

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T lymphocytes present in allergically inflamed tissue synthesize and secrete the cytokines interleukin (IL)-3, IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor (GM-CSF). IL-3, IL-5, and GM-CSF, but also IL-4, may act as a chemotaxin on eosinophils. In contrast to the former cytokines, IL-4 is only chemotactic for eosinophils from the peripheral blood of patients with atopic dermatitis and not for eosinophils from normal individuals. IL-4 has the same chemotactic potency as the other cytokines. The optimal chemotactic potency is reached at a concentration of 10 nM. In contrast, neutrophils do not respond chemotactically to IL-4. Checkerboard analysis, inhibition studies with monoclonal anti-IL-4 antibodies, and desensitization experiments indicated specific interaction of IL-4 with eosinophils. In eosinophils from normal individuals, IL-4 responsiveness could be induced by pretreatment of the cells with IL-5 and GM-CSF. In addition to the fact that IL-4 may be responsible for selective eosinophil transendothelial migration, IL-4 may exert an important modulatory mode of action on eosinophil migration and function within allergically inflamed tissue. Our findings suggest the presence of a functional IL-4R on eosinophils from atopic dermatitis patients. Key words: atopic dermatitis/cytokines/eosinophils/migration/interleukin 4. J Invest Dermatol 102:843–846, 1994

Atopic dermatitis is considered a T-cell–mediated disorder [1]. Both T cells in the circulation and within the tissue are in an activated stage [2,3]. Although hardly any intact eosinophils are present in lesional atopic dermatitis skin, deposits of eosinophil-derived granules can be observed [4,5]. In non-lesional atopic dermatitis skin, an eczematous skin reaction may be induced after patch testing the skin with allergen [6]. The patch test reaction shows macroscopic and microscopic resemblance with active lesional atopic dermatitis skin [5,6]. Six hours after patch testing, eosinophils and T cells already start to infiltrate the skin. Macroscopically, an eczematous reaction takes place 24–48 h after patch testing. At this time point, T cells and eosinophils appear in an activated stage, with some of the T cells being CD4 positive, exactly as in lesional atopic dermatitis skin. T-cell clones derived from biopsies from active lesional skin and from biopsies taken 24 h after patch testing non-lesional atopic dermatitis skin have indicated that these T cells synthesize and secrete various cytokines [7]. The most important cytokines in this respect are interleukin (IL)-3, IL-4, IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF). Because these T cells produce both IL-4 and IL-5, they are considered to be of the Th2 phenotype [8]. IL-4 has many biologic activities and cellular targets. It was originally described as a costimulant of B cells [9]. Other effects on B lymphocytes include induction of class II major histocompatibility antigens [10] and induction of FceRII [11,12]. Additionally, IL-4 induces the proliferation of human thymocytes and primary mast cells [13]. It also enhances the immunoglobulin (Ig)E and IgG1 production [14] and influences tumor-infiltrating lymphocytes and lymphokine-activated killer cells [15]. IL-4 has also been reported to act as a chemotaxin for fibroblasts [16].

Here we report that IL-4 acts chemotactically on eosinophils from atopic dermatitis patients but not on those from normal individuals. IL-4 possesses almost the same chemotactic potency as IL-3, IL-5, and GM-CSF. These findings therefore further extend the importance of IL-4 for the pathogenesis of allergic inflammation.

Subjects All patients (17 women and 23 men between 21 and 42 years of age) participating in this study had atopic dermatitis as classified according to the criteria of Hanifin and Rajka [17]. They all were allergic, i.e., showed positive intracutaneous skin reactions to three or more different allergens, having elevated total IgE levels and positive radio-allergo-sorbent tests for the relevant allergens. The patients had not taken oral steroids for at least 2 weeks before the study. All patients had increased blood eosinophilia. They had received only small amounts of topicalally applied steroids. All other therapy was abandoned at least 2 weeks before the study. At the time of blood collection most patients had moderate to mild eczema; active lesions were present on predilection sites. All patients had elevated blood eosinophil levels (more than 4%). The normal healthy volunteers were not allergic, did not have increased blood eosinophilia, and did not take any kind of medication. All participating individuals gave their informed consent.

Reagents Ficoll-Paque and Percoll were obtained from Pharmacia (Uppsala, Sweden). All experiments were carried out in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer containing 132 mM NaCl, 6 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 1.2 mM potassium phosphate, 20 mM HEPES, 5 mM glucose, and 1.0% HSA (wt/vol), pH 7.4.

Manuscript received September 16, 1993; accepted for publication January 14, 1994.
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Abbreviations: C5a, complement factor 5a; fMLP, N-formyl-methionyl-leucyl-phenylalanine; LTB₄, leukotriene B₄; PAF, platelet-activating factor.
Cytokines Recombinant human GM-CSF (11.5 x 10^4 U/mg) and IL-3 (3 x 10^4 U/mg) were a kind gift from Dr. G. Zenke (Sandoz Ltd., Basel, Switzerland). Recombinant human IL-4 was a kind gift of Dr. A. Stutz (Sandoz Forschungsinstitut, Vienna, Austria). Recombinant human IL-5 (4 x 10^6 U/ml) was a kind gift of Dr. C. J. Sanderson (National Institute for Medical Research, London, UK). The purity of this IL-5 preparation was determined by comparison of its potency with commercially available rhIL-5 (Amersham, Buckinghamshire, UK; 10^8 U/mg). Stock solutions of the cytokines were prepared in phosphate-buffered salt solution supplemented with 0.1% purified human albumin, and were stored at -70°C until use.

Monoclonal Antibodies Anti-human GM-CSF monoclonal antibody (MoAb) (mouse IgG1) was a kind gift from Dr. G. Zenke and GM-CSF, GM-CSF, GM-CSF.

Cell Isolation Blood was obtained from healthy volunteers or from atopic dermatitis patients. Eosinophils from the blood of normal donors were isolated from the buffy coat of 500 ml of blood and eosinophils from the patients were isolated from 50 ml of blood anticoagulated with 0.4% (wt/vol) trisodium citrate (pH 7.4) as described before [18]. Eosinophil purity was always > 95% and the viability was over 98%.

Migration Assay For the microchemotaxis assay, migration was measured with a modified Boyden chamber assay using a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, MD), exactly as described before [18]. The number of cells per 10 high-power fields (hpf) was determined with light microscopy (magnification 200X). In this way the number of cells that had passed the upper filter was determined.

Statistical Analysis All data are presented as mean ± SEM. The Student t test for paired or unpaired data was applied. p < 0.05 was considered significant.

RESULTS

The Migratory Responsiveness of Eosinophils from the Circulation of Normal Individuals and Atopic Dermatitis Patients Toward IL-4 We examined the ability of IL-4 to mobilize eosinophils using a modified Boyden chamber assay. As depicted in Fig 1A, eosinophils from atopic dermatis patients showed a dose-dependent migratory response toward IL-4. At the highest IL-4 concentration used (100 nM), the magnitude of the observed migratory response almost equaled that of the platelet-activating factor (PAF)-induced migratory response at 10 nM [18]. In contrast, eosinophils from normal individuals showed no significant migratory response toward IL-4.

Having observed that IL-4 induces migration of eosinophils from atopic dermatitis patients and not from normal individuals, we also investigated the cell specificity of the action of IL-4 on migration. As depicted in Fig 1B, IL-4 does not induce a migratory response of neutrophils from both atopic dermatitis patients and normal individuals. Therefore, IL-4 seems to act specifically on eosinophils from atopic dermatitis patients.

Checkerboard Analysis To investigate whether the IL-4-induced migratory response was due to chemotaxis or chemokinesis, checkerboard analysis was performed. The checkerboard analysis revealed that IL-4 acts as a chemotaxin on eosinophils from atopic dermatis patients and that the effect on the chemokinesis of eosinophils can be neglected (see Table I).

Specificity of the IL-4-Induced Chemotaxis The IL-4-induced chemotactic response of eosinophils from the circulation of atopic dermatis patients could be strongly inhibited by a specific monoclonal antibody (Fig 2). The inhibition was cytokine specific, because the antibody could not inhibit the chemotactic response towards other cytokines (data not shown). Desensitization experiments with rhIL-4 also showed a specific interaction between IL-4 and the eosinophils (Fig 3).

Comparison of Chemotactic Potency of IL-4 with Other Chemotaxins Table II shows a comparison of the chemotactic potency of IL-4 with other chemotaxins for eosinophils from the circulation of normal individuals and atopic dermatis patients. As can be seen, IL-4 possesses a chemotactic potency similar to that of IL-3, IL-5, IL-8, and GM-CSF for eosinophils from atopic dermatis patients.

Induction of IL-4 Chemotactic Response by GM-CSF and IL-5 To see whether the difference between the migratory response of eosinophils from normal individuals and atopic dermatis patients was due to previous in vivo contact of the eosinophils from atopic dermatis patients with GM-CSF or IL-5, experiments were performed with eosinophils from normal individuals that had been incubated overnight with 10 µM GM-CSF or IL-5. Indeed, eosinophils that had been pretreated with these cytokines and washed

Table I. Checkerboard Analysis of IL-4-Induced Chemotaxis of Eosinophils from Atopic Dermatitis Patients

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Upper Compartment IL-4</th>
<th>Lower Compartment IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.25 x 10^-9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 ± 10</td>
</tr>
<tr>
<td>1.25 x 10^-9</td>
<td>22 ± 5</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>2.5 x 10^-9</td>
<td>21 ± 4</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>5.0 x 10^-9</td>
<td>23 ± 7</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>10 x 10^-9</td>
<td>24 ± 4</td>
<td>26 ± 6</td>
</tr>
</tbody>
</table>

* Different IL-4 preparations were used in the upper and lower compartment of the modified Boyden chamber. The results are expressed as the number of eosinophils/10 high-power fields (hpf). Mean values ± SEM for four different experiments are presented.
shown). This priming could be inhibited with neutralizing antibodies for about 80% (mean of n = 4 different experiments). Preincubation of eosinophils from atopic dermatitis patients overnight with GM-CSF or IL-5 did not result in a significant increase of the IL-4 responsiveness (data not shown). The responsiveness towards the lower IL-4 concentrations (1–10 pM) slightly increased, but the maximal observed migratory response did not significantly increase. The fact that the overall increase was not significant was due mainly to an increase in the random migration (chemokinesis).

**DISCUSSION**

The eosinophil is recognized as an important effector cell capable of contributing to the pathogenesis of allergic inflammation. However, the recruitment and function of eosinophils is strongly dependent upon the production and secretion of cytokines by other cell types, e.g., T cells. Here we show that IL-4 is chemotactic for eosinophils from patients with atopic dermatitis but not for eosinophils from normal individuals. In recent years we have concentrated on the question of how eosinophils can selectively migrate into the tissue, in particular, in case of allergic inflammatory reactions [18,19]. So far, these investigations have indicated that eosinophils in the circulation from atopic dermatitis patients are in a “primed” state. Other studies showed that the migratory response of eosinophils from normal individuals could be increased by pretreatment of those cells with IL-3, IL-5, or GM-CSF. Pretreatment with these cytokines resulted in the induction of a migratory response towards neutrophil activating factor (NAP)/IL-8 and N-formyl-methionyl-leucyl-phenylalanine (fMLP) [20]. Here we show that GM-CSF and IL-5 are capable of inducing a migratory response of eosinophils from normal individuals toward IL-4. Therefore, these findings extend the evidence that eosinophils from the circulation of patients with allergic disorders are “primed” in vivo, most likely due to previous contact with the cytokines IL-3, IL-5, or GM-CSF.

**Table II. Comparison of IL-4–Induced Chemotaxis of Eosinophils from the Circulation of Normal Individuals and Atopic Dermatitis Patients with Other Chemotaxis**

<table>
<thead>
<tr>
<th>Chemotaxin</th>
<th>Optimal Chemotactic Concentration</th>
<th>Normals</th>
<th>Atopic Dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-3</td>
<td>10 nM</td>
<td>24 ± 5</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>IL-5</td>
<td>10 nM</td>
<td>50 ± 8</td>
<td>90 ± 15</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>10 nM</td>
<td>70 ± 15</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>IL-8</td>
<td>10 nM</td>
<td>80 ± 11</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>TNFα</td>
<td>10 nM</td>
<td>50 ± 10</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>IL-4</td>
<td>10 nM</td>
<td>21 ± 4</td>
<td>71 ± 15</td>
</tr>
<tr>
<td>TNFα</td>
<td>10 nM</td>
<td>35 ± 7</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>IL-4</td>
<td>10 nM</td>
<td>29 ± 6</td>
<td>83 ± 8</td>
</tr>
</tbody>
</table>

* Optimal concentration for eosinophils from atopic dermatitis patients.

**The presence of activated T cells in the circulation and tissue of atopic dermatitis patients, which, according to their phenotype, will secrete IL-4 in addition to the priming cytokines IL-3, IL-5, and GM-CSF, allows the suggestion that IL-4 may contribute to tissue recruitment of eosinophils. Skin biopsies from atopic dermatitis patients showed increased levels of mRNA for both IL-4 and IL-5 [Kilgus O et al: (abs) Invest Dermatol 100:489, 1993]. This is in contrast to the skin of normal individuals, where hardly any mRNA for these cytokines can be detected. In addition, the presence of IL-4 in lesional atopic dermatitis skin was directly demonstrated by immunocytotoxic staining (Dr. T. Thepen, personal communication).**

Transendothelial migration of eosinophils into the tissue involves a cumulative series of interactions in which eosinophil adhesions, adhesion molecules on endothelial cells, and the activity of many chemotactants play a crucial role. Most of these chemotactants lack singular specificity for eosinophils and therefore it is questionable to what extent they contribute in vivo to the accumulation of eosinophils in the tissue. In this respect, IL-4 can be an important cytokine. IL-4 can induce the expression of vascular cell adhesion molecule-1 on endothelial cells [21]. Through the expression of this adhesion structure it allows eosinophils to attach to and migrate through the endothelium [22]. Eosinophils, but not neutrophils, do express the counterpart structure of vascular cell adhesion molecule-1, i.e., very late antigen-4 [23].

Taken together, this study shows that IL-4 not only plays an
important role in the selective eosinophil extravasation but could also contribute to the directed migration of eosinophils within the tissue. Furthermore, our findings suggest the presence of a functional IL-4R on eosinophils from atopic dermatitis patients. This is currently under investigation.

REFERENCES