Eosinophil granular protein deposits have been demonstrated in lesional atopic dermatitis skin. This suggests active tissue infiltration of eosinophils. To find an explanation for the tissue influx of eosinophils, eosinophil migration was studied in vitro by means of a microchemotaxis assay. Eosinophils from the circulation of patients with atopic dermatitis showed an altered capacity to respond to chemotactic stimuli compared with eosinophils from healthy donors. Eosinophils from patients with atopic dermatitis had significantly increased migratory responses toward dose ranges of N-formyl-methionyl-leucyl-phenylalanine, neutrophil-activating factor, and platelet-factor 4. Eosinophils from normal individuals did not respond to N-formyl-methionyl-leucyl-phenylalanine and neutrophil-activating factor and responded only slightly to platelet factor 4. The migratory responses toward tumor necrosis factor-α and complement factor C5a were identical in both groups.

Interleukin-5, an eosinophil-selective cytokine, is a strong modulator of the migratory responses to these chemotaxins in eosinophils from normal donors. A migratory response toward N-formyl-methionyl-leucyl-phenylalanine and neutrophil-activating factor was induced by interleukin-5, whereas the migratory response toward platelet-activating factor and platelet factor 4 was markedly potentiated. In contrast, the response to complement fragment C5a was only slightly influenced. Our findings indicate that the increased migratory responsiveness of eosinophils from patients with atopic dermatitis to various chemotaxins reflects in vivo "priming" of eosinophils, presumably by circulating cytokines such as interleukin-5. This in vivo "priming" is not optimal because it can be further potentiated by renewed contact with interleukin-5. J Invest Dermatol 100:137–142, 1993

In vivo investigations indicate that eosinophils do respond to the following chemotaxins: platelet activating factor (PAF), complement fragment C5a, and leukotriene B4; however, these chemotaxins are not selective for eosinophils because they also attract neutrophils. It was recently shown that interleukin (IL)-5 acts as an eosinophil-selective chemotaxin and promotes the adhesion of eosinophils, but not of neutrophils, to human endothelial monolayers. We were able to show that eosinophils also do respond chemotactically to relatively high concentrations of the cytokines IL-3 and granulocyte/macrophage–colony-stimulating factor (GM-CSF). At relatively low concentrations these cytokines are capable of inducing migratory responsiveness toward N-formyl-methionyl-leucyl-phenylalanine (FMLP) and neutrophil-activating factor.

Eosinophil Migration in Atopic Dermatitis I: Increased Migratory Responses to N-Formyl-Methionyl-Leucyl-Phenylalanine, Neutrophil-Activating Factor, Platelet-Activating Factor, and Platelet Factor 4

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Abbreviations:
- C5a: complement fragment C5a
- FMLP: N-formyl-methionyl-leucyl-phenylalanine
- GM-CSF: granulocyte/macrophage–colony-stimulating factor
- HSA: human serum albumin
- Ig: immunoglobulin
- IL: interleukin
- NAF/IL-8: neutrophil activating-factor/interleukin-8
- PAF: platelet-activating factor
- PF4: platelet factor 4
- TNF: tumor necrosis factor
The circulatory function of eosinophils from normal, healthy individuals [13]. In atopic dermatitis there is a clear dysfunction of T cells [14]. Recently, activated T cells could be demonstrated in the circulation of patients with atopic dermatitis [15]. These activated T cells were capable of spontaneously secreting the cytokines IL-3, IL-5, and GM-CSF [15,16]. These cytokines are important modulators of eosinophil function such as migration [13].

We have investigated whether eosinophils from patients with atopic dermatitis showed a different migratory response to a variety of chemotaxins compared with eosinophils from normal individuals and whether priming by IL-5 could explain the changed responsiveness.

MATERIALS AND METHODS

Subjects All patients (10 women and 16 men, ages between 17 and 48 years) participating in this study had atopic dermatitis as classified according to the criteria of Hanifin and Rajka [17]. They were all allergic to three or more different allergens, had positive skin reactions to these allergens, elevated total immunoglobulin (Ig) E levels, and positive radio allergo sorbtent tests (RASTs) for the relevant allergens. The patients had not taken oral steroids for at least 2 weeks before the study. They were treated only with small amounts of topically applied steroids. All other therapy was stopped at least 2 weeks before the study. At the time of blood collection most patients had mild to moderate eczema; locally active lesions were present. All patients had elevated blood eosinophil levels (>8%). The normal, healthy volunteers were not allergic, had normal blood eosinophil levels, and did not take any medication. All participating individuals gave their informed consent.

Reagents PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was purchased from Bachem Feinchemiealien AG (Bubendorf, Switzerland). Human recombinant C5a, FMLP, and platelet factor (PF) were purchased from Sigma (St. Louis, MO). Tumor necrosis factor (TNF)-α was from Cetus Corp. (Emeryville, CA). FMLP, PAF, and Percoll were obtained from Pharmacia (Uppsala, Sweden). All experiments were carried out in Geys' balanced salt solution supplemented with 1.5 mM CaCl₂, heparin (10 IU/ml), 5 mM glucose, and 1.0% human serum albumin (HSA) (wt/vol).

Cytokines Recombinant human IL-5 (4 × 10⁶ U/ml) was a kind gift of Dr. C.J. Sanderson (National Institute for Medical Research, London, U.K.). The purity of this IL-5 preparation was determined by comparison of its potency with commercially available rhIL-5 (Amersham, Buckinghamshire, U.K.; 10⁶ U/mg). Recombinant human NAF/IL-8 (1.2 μg/ml) was a kind gift from Dr. I. Lindley (Sandoz Forschungsinstitut GmbH, Vienna, Austria). Stock solutions of the cytokines were prepared in phosphate-buffered saline solution supplemented with 0.1% purified HSA and were stored at -70°C until use.

Cell Isolation Blood was obtained from healthy volunteers or from patients with atopic dermatitis. Eosinophils from the blood of normal donors were isolated from the buffy coat ofuffy coat (HSA) by separation of blood over isotonic Ficoll (1.077 g/ml) and the granulocytes were washed and resuspended in RPMI supplemented with HSA (1% wt/vol) and incubated 30 min at 37°C to restore initial densities of the cells. After this incubation period, the cells were washed and resuspended in phosphate-buffered saline supplemented with HSA (1% wt/vol) and trisodium citrate (0.4% wt/vol). After pre-incubation of the cells for 5 min at 37°C, FMLP (10 nM) was added to the cell suspension, and the incubation was continued for 10 min at 37°C before 1 ml of cell suspension was layered on 4 ml of an isotonic Percoll solution (density 1.082 g/ml). To prevent contamination of the cells with cell debris and remaining erythrocytes, 1 ml of Percoll (density 1.100 g/ml) was brought under the Percoll 1.082 g/ml solution. After centrifugation (20 min, 1000 × g maximum, room temperature), the eosinophils were collected from the interface. The eosinophils were washed and resuspended in Gey's buffer and kept at room temperature until use. The FMLP treatment does not influence eosinophil function, as is extensively documented elsewhere [13,18–20]. The purity of the cells was always over 90%, and the recovery ranged from 40% to 70%. For comparison, eosinophils were also isolated using the immunomagnetic bead method, as described by Hansel et al [21]. Briefly, Immunomagnetic beads (Dynal Beads, Dynal A.S., Oslo, Norway) were coated with a monoclonal antibody against CD16 (CLB FeCr gran 1; Central Laboratory of the Red Cross Blood Transfusion, Amsterdam, The Netherlands). These coated beads were coagulated at 4°C for 20 min with the granulocyte preparation (10⁷ cells/ml) in a ratio of 1:4 (cells/beads). The neutrophils were subsequently removed by a magnetic particle concentrator (MPC THI:1; Dynal A.S.).

Migration Assay Migration was measured with a modified Boyden chamber assay using a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, MD). Chemotaxins or Gey's buffer (30 μl) was placed in the lower compartments. Two filters (cell-lense nitrate) were placed between the lower and the upper compartments. The lower chamber had a pore width of 0.45 μm (Millipore type HA; Millipore Corporation, Bedford, MA), and the upper filter had a pore width of 8 μm (Sartorius SM 113; Sartorius AG, Göttingen, Germany). Before use, the filters were soaked in Gey's buffer. Purified eosinophils, pre-incubated with the tested cytokine (IL-5) or with Gey's buffer for 30 min at 37°C, were placed in the upper compartment (25 μl of 5 × 10⁶ cells/ml). The chemotaxis chambers were subsequently incubated for 2.5 h at 37°C unless otherwise stated. Hereafter, the upper filters were removed, fixed in butanol/ethanol (20%/80%, v/v) for 10 min and stained with Weigert solution [1% hematoxylin (v/v) in 95% ethanol (v/v) and an acidic FeCl₃ solution (70 mM) mixed in a volume ratio of 1:1]. The filters were dehydrated with ethanol, made transparent with xylene, and fixed upside down. The number of cells per 10 high-power fields was determined with light microscopy (magnification ×400). In this way, the number of cells that had passed the upper filter was determined.

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

RESULTS

Migratory Responsiveness of Eosinophils from the Circulation of Patients with Atopic Dermatitis and Normal Individuals Toward FMLP, NAF/IL-8, PAF, PF4, TNF-α, and C5α As is depicted in Fig 1, the eosinophils from patients with atopic dermatitis showed significantly increased migratory responses toward dose ranges of FMLP, NAF/IL-8, PAF, and PF4 compared with eosinophils from normal individuals. These data showed that FMLP and NAF/IL-8 were not chemotactic toward eosinophils from normal individuals. PF4 had only weak chemotactic activity on eosinophils from normal individuals. In contrast, eosinophils from patients with atopic dermatitis showed significant migratory responsiveness toward these chemotaxins. In the case of the PAF-induced chemotaxis, a leftward shift in the responsiveness was present. Eosinophils from patients with atopic dermatitis showed a migratory response to PAF at concentrations approximately 10⁻⁵ M. This PAF concentration is not chemotactic for eosinophils from normal individuals. These data are strongly suggestive for “in vivo” priming of the eosinophils from patients with atopic dermatitis [13].

In contrast to the FMLP-, NAF/IL-8-, PAF-, and PF4-induced migratory responses, the TNF-α- and the C5α-induced migratory responses of eosinophils from patients with atopic dermatitis and normal individuals are completely identical.
Table II. Influence of Pre-incubating Eosinophils with Buffer or a Dose Range of IL-5 on the Chemotaxin-induced Migratory Response of Eosinophils (in Cells per 10 High-Power Fields) from Normal Individuals*

<table>
<thead>
<tr>
<th>Chemotaxin</th>
<th>Buffer</th>
<th>11</th>
<th>10</th>
<th>9</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMLP (10 nM)</td>
<td>22 ± 7</td>
<td>70 ± 26</td>
<td>72 ± 37</td>
<td>98 ± 37</td>
<td>87 ± 30</td>
</tr>
<tr>
<td>NAF/IL-8 (10 nM)</td>
<td>22 ± 7</td>
<td>67 ± 30</td>
<td>72 ± 37</td>
<td>74 ± 15</td>
<td>107 ± 50</td>
</tr>
<tr>
<td>PAF (1 nM)</td>
<td>25 ± 8</td>
<td>81 ± 30</td>
<td>ND</td>
<td>130 ± 14</td>
<td>ND</td>
</tr>
<tr>
<td>PF4 (1 nM)</td>
<td>17 ± 5</td>
<td>78 ± 24</td>
<td>ND</td>
<td>128 ± 56</td>
<td>ND</td>
</tr>
<tr>
<td>C5a (10 nM)</td>
<td>25 ± 8</td>
<td>180 ± 34</td>
<td>ND</td>
<td>200 ± 40</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Preincubation was performed for 30 min at 37°C. Mean values ±SD for n = 4 or 5 experiments are presented. All values obtained after preincubation with IL-5 differed significantly from the buffer value (p < 0.05; paired Student t test) (for technical details, see Materials and Methods); ND, not done.

Eosinophils from patients with atopic dermatitis and normal individuals isolated by the two different methods described ([18,21]; see also Materials and Methods) responded completely identically to the chemoattractants used here, as shown in Table I. The results presented in Table I indicate that the isolation of eosinophils from both normal individuals and patients with atopic dermatitis are equally influenced by treatment with FMLP or magnetic beads with respect to the responses studied here.

Influence of IL-5 on the FMLP-, NAF/IL-8-, PAF-, and PF4-Induced Migratory Response of Isolated Eosinophils from Normal Individuals. In Fig 1 it is shown that the FMLP-, NAF/IL-8-, PAF-, and PF4-induced migration of eosinophils from patients with atopic dermatitis is significantly increased compared with responses of eosinophils from normal individuals. We investigated whether priming of eosinophils from normal individuals with IL-5 in vitro resulted in a similar effect. The results are shown in Table II. Pre-incubation of eosinophils from normal individuals with concentration ranges of IL-5 indeed induced significant migratory responsiveness toward FMLP and NAF/IL-8, whereas the responses to PAF and PF4 were considerably enhanced. The kinetics of the migratory responses after this in vitro pre-treatment with IL-5 were almost similar to those observed in eosinophils from patients with atopic dermatitis. IL-5 priming was irreversible because wash-
ing of the cells with cytokine-free buffer did not abrogate priming (data not shown). To show that the above effects could be specifically ascribed to IL-5, these effects were evaluated in the presence of a neutralizing antibody directed against IL-5 (400 U/ml of IL-5 were neutralized by anti-hu-IL-5, 16 μg/ml; the antibody was a kind gift of Dr. J. Tavernier, Roche, Ghent, Belgium). When this antibody was added together with IL-5 to the eosinophils from normal individuals, the “priming” effect for the PAF-induced response ([PAF] = 1 nM) could be blocked for 87% ± 10% (mean ± SD; n = 4).

**Influence of IL-5 on the PAF- and C5a-Induced Migratory Response of Isolated Eosinophils from Patients with Atopic Dermatitis and Normal Individuals**

Because IL-5 is capable of “priming” eosinophils from normal individuals to respond more strongly to certain chemotaxins (e.g., PAF) and to induce migratory responses to others (e.g., FMLP), we investigated whether eosinophils from patients with atopic dermatitis could be further “primed” in their response to PAF and C5a by IL-5 treatment ([IL-5] = 1 nM). Indeed, the responses of these eosinophils were potentiated by IL-5, and the increase was almost similar compared with eosinophils from normal individuals after pre-treatment with IL-5 (Fig 2A). In the case of C5a-induced migration, only a small potentiation was observed (Fig 2B).

**DISCUSSION**

In this report we have shown that eosinophils from the circulation of patients with atopic dermatitis exhibit potentiated migratory responses toward FMLP, NAF/IL-8, PAF, and PF4 compared with eosinophils from normal donors. The migratory responses toward TNF-α and C5a did not differ significantly. Recently, we demonstrated that IL-3 and GM-CSF at picomolar concentrations are capable of increasing the migratory responses of eosinophils from normal individuals toward PAF and leukotriene B_4 but not to C5a [13]. On the other hand, pre-treatment with these cytokines is essential for migratory responses toward NAF/IL-8 and FMLP. Here, we show that IL-5 acts in a similar way. Therefore, our findings indicate that eosinophils from patients with atopic dermatitis are present in a “primed” form. Most likely this priming is caused by cytokines, such as IL-3, IL-5, and GM-CSF. Indeed, these cytokines are produced by activated lymphocytes in the circulation of asthma and patients with atopic dermatitis [15,16]. These findings therefore extend our recent findings on priming mechanisms in allergic asthma [22]. Also, in this allergic disorder the eosinophils are present in a primed state in the circulation, which might be due to previous exposure to the cytokines IL-3, IL-5, and GM-CSF [22]. Taken together, our findings extend existing in vitro evidence that these cytokines may alter a variety of eosinophil responses [23–28] to the in vivo situation (e.g., allergic asthma and atopic dermatitis [22]). Moreover, it has also been suggested that “primed” eosinophils present at an extravascular site may exert pathobiologic effects by responding easily to stimulation by soluble ligands and the release of toxic mediators [29].

Although the eosinophils from patients with atopic dermatitis are already in a “primed” state, they can be further potentiated by in vitro contact with IL-5. This suggests that the in vivo “priming” has not been complete.

The eosinophil is not yet considered an important effector cell in atopic dermatitis; however, eosinophilia is regularly observed in active atopic dermatitis [1]. Furthermore, deposits of eosinophil-derived mediators such as major basic protein have been reported in lesional atopic dermatitis skin [3]. Patients with atopic dermatitis with elevated circulating IgE levels and positive intracutaneous skin reactions to Aeroallergens frequently show a positive patch-test reaction to these allergens [4]. During such a patch-test reaction, which shows a macroscopic and microscopic resemblance to lesional atopic dermatitis skin [5,6], eosinophils not only infiltrate but are also activated, causing release of mediators. In earlier reports, we considered the above-mentioned patch-test reaction to Aeroallergens in patients with atopic dermatitis an in vivo model to study the pathogenesis of atopic dermatitis [5]. In this model, IgE-bearing Langerhans cells, present in the epidermis and dermis, are considered to present allergen to T cells [30]. These T cells are activated, leading to T-cell proliferation and concomitant cytokine release. Recently, T cells have been cloned from these patch-test reactions, and it has been found that these cells are of the Th₂ subtype [31], producing, among others, IL-4 and IL-5 but not IL-2 or interferon-γ [32]. Therefore, T cells can, by the release of IL-5, contribute to selective eosinophil mobilization; however, other cells present in allergic lesions (e.g., mast cells and endothelial cells) may be activated as well and can therefore also contribute to eosinophil recruitment. Of note, a similar reaction sequence seems to take place in the allergen-induced late-phase skin reaction and the classical delayed-type hypersensitivity reaction [33]. Also, in the allergen-induced late-phase reaction in the skin, activated eosinophils are present in the tissue.
By the release of the above-mentioned cytokines themselves. In this way, the eosinophil could amplify the inflammatory response [35].

Taken together, our findings indicate that eosinophils from patients with atopic dermatitis are present in a pre-activated state in the circulation. This is likely to be caused by contact with the cytokines IL-3, IL-5, and GM-CSF from activated T cells. IL-5 is selective for eosinophils and may therefore be important in modulating/inducing the migratory response of eosinophils toward a variety of chemotaxins formerly thought to be mainly important for neutrophil mobilization.

We thank the Dutch Asthma Centre, Davos, Switzerland, for financial support. The Medical Biological Laboratory TNO is acknowledged for general support.

REFERENCES


12. Warringa RAJ, Koenderman L, Kok PTM, Kreukniet J, Bruijnzeel PLB: Modulation and induction of eosinophil chemotaxis by granulo­


ROTHMAN CLUB MEETING REPORT

The annual meeting of the Stephen Rothman Club was held in the Faculty Club at the University of California at San Francisco on Tuesday evening, December 8, 1992. The 50 attendees enjoyed an evening of collegial camaraderie, a fine meal, and a stimulating presentation by Dr. June K. Robinson, Professor of Dermatology at Northwestern University, entitled "A Surgeon’s Approach to Clinical Research: Predicting the Biology of Basal Cell Carcinoma."

The Rothman Club is a non-profit organization whose activities are supported predominately by unrestricted financial donations from industry. The current Councilors of this organization (Drs. David Woodley, Kim Yancey, and Rick Sontheimer) would like to take this opportunity to thank the following corporations for their unconditional generosity in this regard.

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Submitted by R.D. Sontheimer, M.D.  
Secretary-Treasurer