Recombinant Human Eotaxin Induces Oxygen Radical Production, Ca\(^{2+}\) Mobilization, Actin Reorganization, and CD11b Upregulation in Human Eosinophils Via a Pertussis Toxin-Sensitive Heterotrimeric Guanine Nucleotide-Binding Protein

By Kirsten Tenscher, Beatrix Metzner, Erwin Schöpf, Johannes Norgauer, and Wolfgang Czech

The novel human CC-chemokine Eotaxin is a potent and selective chemotaxin for eosinophils. Here, the biological activities and the activation profile of Eotaxin were further characterized and compared with those of other eosinophil chemotaxins such as complement fragment C5a (C5a), platelet-activating factor (PAF), and RANTES in human eosinophils. Eotaxin stimulated the production of reactive oxygen metabolites as shown by lucigenin-dependent chemiluminescence and superoxide dismutase-inhibitable cytochrome C reduction. Furthermore, Eotaxin induced upregulation of the integrin CD11b. In addition, fluorescence measurements with Fura-2-labeled eosinophils in the presence of EGTA indicated \(\text{Ca}^{2+}\)-mobilization from intracellular stores by Eotaxin. Flow cytometric studies showed rapid and transient actin polymerization on stimulation with Eotaxin. At optimal concentrations, the changes induced by Eotaxin were comparable with those obtained by C5a, PAF, and RANTES. Cell responses elicited by Eotaxin were inhibited by pertussis toxin, indicating coupling of its putative receptor to heterotrimeric guanine nucleotide-binding proteins. These results indicate that Eotaxin is a strong activator of eosinophils with biological activity comparable with those of the eosinophil chemokains C5a, PAF, and RANTES. These findings point to a role of Eotaxin in the pathogenesis of eosinophilic inflammation as a chemotaxin as well as an activator of proinflammatory effector functions.

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Eotaxin and actin response. The influence of Eotaxin on the actin network in eosinophils was analyzed by flow cytometry. This novel CC chemokine caused a rapid and transient polymerization of actin molecules (Fig 1). There was a transient increase of the f-actin content of about 50% within 30 seconds. Half-maximal and maximal effects were observed at 10 ng/mL and 100 ng/mL, respectively. The time for subsequent recovery to initial values depended on the added concentration of the stimulus.

Mobilization of intracellular Ca2+ by Eotaxin. Intracellular Ca2+ transients were followed in Fura-2-labeled eosinophils by fluorospectrometry. Eotaxin induced a rapid and concentration-dependent intracellular response (Fig 2). To analyze whether Eotaxin stimulates mobilization of Ca2+ from intracellular stores or influx across the plasma membrane from extracellular medium, measurements were performed in the presence of added EGTA. Because this Ca2+ chelator had no effect on the magnitude or time course of induced Ca2+ transients, Eotaxin stimulated mobilization of Ca2+ from intracellular stores.

Eotaxin activated the respiratory burst: and stimulated CD11b upregulation. Next, the activation of the respiratory burst by Eotaxin was studied by lucigenin-dependent chemiluminescence. These experiments showed production of reactive oxygen species in a concentration-dependent manner; half-maximal and maximal effects were observed at 10 ng/mL and 100 ng/mL, respectively (Fig 3). At optimal concentrations continuous measurements indicated a rapid induction of the response with maximum values after 5 minutes.
EOTAXIN AND CELL ACTIVATION IN HUMAN EOSINOPHILS

Fig 3. Eotaxin activates the respiratory burst in eosinophils. The time courses of the lucigenin-dependent chemiluminescence response in eosinophils on stimulation with 10 ng/mL (line 1), 100 ng/mL (line 2), and 1,000 ng/mL Eotaxin (line 3) are shown. Representative data of one experiment are shown. The experiment was repeated five times with identical results.

Superoxide anion production was analyzed by cytochrome C reduction (data not shown). Optimal doses of Eotaxin generated about 15 nmol/L superoxide anion per 10^6 eosinophils within 30 minutes. Similar amounts of superoxide anion were also found after longer stimulation periods (up to 60 minutes; data not shown).

The influence of Eotaxin on the expression of the integrin CD11b was measured by flow cytometry. Again, Eotaxin induced a concentration-dependent response. Half-maximal and maximal effects were observed at 10 ng/mL and 100 ng/mL, respectively (Fig 4).

Comparison of the activation profiles of different eosinophil stimuli. The activation profile of Eotaxin was compared with the responses provoked by other well-defined eosinophil activators such as C5a, PAF, and RANTES. Similar to Eotaxin, the other eosinophil activators induced intracellular Ca^{2+} transients, stimulated actin reorganization, triggered the respiratory burst, and stimulated expression of CD11b in a concentration-dependent manner (data not shown). At optimal concentrations, all tested activators induced similar responses (Table 1).

Pertussis toxin inhibition of Eotaxin-induced cell response. Pertussis toxin blocks cell activation induced by G_{i}-protein–coupled receptors. Pretreatment of eosinophils with pertussis toxin completely inhibited the Eotaxin-induced chemiluminescence response (Fig 5), the transient increase of actin polymerization and Ca^{2+} mobilization (data not shown). To prove the metabolic activity of eosinophils after pertussis toxin treatment, the chemiluminescence response with phorbol 12-myristate 13-acetate was followed. Toxin treatment did not influence the phorbol-ester–triggered response (data not shown).

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Table 1. Influence of Different Eosinophil Activators on Actin Response, Ca^{2+} Transients, Lucigenin-Dependent Chemiluminescence, and CD11b Expression

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>f-Actin</th>
<th>Ca^{2+} Transients</th>
<th>Chemiluminescence</th>
<th>CD11b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00 (±0.00)</td>
<td>0.87 (±0.04)</td>
<td>16 (±4)</td>
<td>10.48 (±1.76)</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>1.47 (±0.07)</td>
<td>2.15 (±0.21)</td>
<td>305 (±41)</td>
<td>41.20 (±2.56)</td>
</tr>
<tr>
<td>RANTES</td>
<td>1.52 (±0.04)</td>
<td>2.07 (±0.14)</td>
<td>352 (±53)</td>
<td>37.68 (±0.06)</td>
</tr>
<tr>
<td>C5a</td>
<td>1.53 (±0.06)</td>
<td>2.42 (±0.09)</td>
<td>342 (±58)</td>
<td>38.89 (±1.17)</td>
</tr>
<tr>
<td>PAF</td>
<td>1.47 (±0.04)</td>
<td>2.29 (±0.36)</td>
<td>454 (±49)</td>
<td>42.25 (±2.16)</td>
</tr>
</tbody>
</table>

Eosinophils were stimulated without or with 100 ng/mL Eotaxin, 100 ng/mL RANTES, 100 nmol/L C5a, and 100 nmol/L PAF. The relative f-actin content and the ratio of the intracellular Ca^{2+} measurements were taken after 10 seconds. The chemiluminescence response is given as integral (counts x 10^6) after 60 minutes. The mean channel number of CD11b expression was analyzed after 30 minutes. Data are means ± SEM (n = 3).

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Fig 4. Dose-dependency of the Eotaxin-induced CD11b expression in eosinophils. Eosinophils were activated without or with 10 ng/mL, 100 ng/mL, and 1000 ng/mL Eotaxin for 30 minutes at 37°C. Expression of CD11b was analyzed by flow cytometry with phycoerythrin-conjugated anti-CD11b antibodies. Data are means ± SEM (n = 5).

Fig 5. Pertussis toxin inhibits Eotaxin-induced lucigenin-dependent chemiluminescence in eosinophils. Cells were preincubated without or with 1 µg/mL pertussis toxin for 90 minutes at 37°C and were further incubated without or with 100 ng/mL Eotaxin for 60 minutes at 37°C. Data are means ± SEM (n = 5).
DISCUSSION

Well-defined chemotaxins for eosinophils are C5a, PAF, and RANTES.\(^7\) Besides eosinophils, all these chemotaxins act on various types of leukocytes. Recently, a novel potent CC-chemokine with selectivity for eosinophils has been identified.\(^8\) This peptide has been purified from bronchoalveolar lavage fluid of allergen-challenged guinea pigs.\(^9\) Enhanced expression of human Eotaxin has been reported during eosinophilic infiltration within the mucosa and submucosa of nasal polyps. Expression of Eotaxin at other sites as well as in diseases has not yet been analyzed.

To improve our understanding of the biological activities of Eotaxin, we analyzed various intracellular signal mechanisms and cell effector functions in eosinophils. As could be expected for a chemotactic agent, we have shown here that Eotaxin induced a transient reorganization of the actin network. The precise regulation mechanisms for the actin response are not fully understood; however, it is believed to involve interaction of phospholipids with actin-binding proteins.\(^10\) In addition, we here showed mobilization of Ca\(^{2+}\) from intracellular stores by Eotaxin. Similar responses have been reported after stimulation of eosinophils with the chemotaxins C5a, PAF, and RANTES.\(^10\)\(^12\)\(^13\) Mobilization of Ca\(^{2+}\) from intracellular stores is presumably the consequence of phospholipase-C activation and generation of soluble inositol triphosphate.\(^14\) Intracellular Ca\(^{2+}\) transients and actin reorganization have been implicated in many biological regulatory mechanisms including the activation of the NADPH oxidase.\(^11\)\(^15\) Production of reactive oxygen metabolites by Eotaxin was shown here with different methods. Moreover, Eotaxin induced upregulation of the CD11b integrin.

Cell biology studies and cloning of the cDNA of the C5a- and PAF-receptors showed interaction of these chemotaxin receptors with G-proteins.\(^16\)\(^17\) Here, we have shown that all cell responses induced by Eotaxin were inhibited by pertussis toxin, which inactivates heterotrimeric G-proteins by adenine diphosphate ribosylation.\(^14\) This finding suggests that the putative receptor belongs to the superfAMILY of serpentine receptors. The receptors for C5a, PAF, and RANTES are well-established members of this family.\(^16\)\(^17\) In addition to migration, the chemotaxins C5a, PAF, and RANTES also induce proinflammatory activation such as production of reactive metabolites. The direct comparison of Eotaxin with these activators showed an identical stimulation pattern. Therefore, one can assume that Eotaxin, similarly to other chemotaxins, not only recruits eosinophils to the site of inflammation, but also possesses proinflammatory activity.

These results indicate that Eotaxin in eosinophils induces actin polymerization and intracellular Ca\(^{2+}\) mobilization via pertussis toxin-sensitive G-proteins. In addition, it is a strong activator of the respiratory burst as well as upregulation of CD11b. These findings implicate the role of Eotaxin in the pathogenesis of eosinophilic inflammation to be that of a chemotaxin as well as an activator of proinflammatory activities.

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