Eotaxin and RANTES enhance 5-oxo-6,8,11,14-eicosatetraenoic acid–induced eosinophil chemotaxis

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Background: The 5-lipoxygenase product 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) is a potent activator of human eosinophils and, among lipid mediators, is the most active chemoattractant for these cells. Studies have demonstrated the importance of 5-lipoxygenase products in allergen-induced pulmonary eosinophilia. Because CC chemokines such as eotaxin and RANTES also play critical roles in this phenomenon, it would seem likely that members of both classes of mediators contribute to this response.

Objective: The study was designed to directly compare the effects of 5-oxo-ETE on eosinophils with those of eotaxin and RANTES and to determine whether these chemokines could enhance the chemotactic response to 5-oxo-ETE.

Methods: Eosinophil chemotaxis was measured with microchemotaxis chambers. CD11b, L-selectin, and actin polymerization were measured by flow cytometry. Calcium mobilization was measured by fluorescence.

Results: 5-Oxo-ETE stimulated eosinophil chemotaxis with a potency between those of eotaxin and RANTES and a maximal response about 50% higher than that of eotaxin. Threshold concentrations of eotaxin and RANTES increased the chemotactic potency of 5-oxo-ETE by more than 4-fold. 5-Oxo-ETE and eotaxin were approximately equipotent in mobilizing cytosolic calcium in eosinophils. Eotaxin was more potent in inducing CD11b expression and actin polymerization, but the maximal responses to 5-oxo-ETE were about 50% higher. 5-Oxo-ETE strongly induced L-selectin shedding, whereas eotaxin elicited only a weak and variable response.

Conclusion: 5-Oxo-ETE is a strong activator of human eosinophils with a chemotactic potency comparable to those of eotaxin and RANTES, both of which enhance 5-oxo-ETE–induced chemotaxis. 5-Oxo-ETE and CC chemokines may combine to induce pulmonary eosinophilia in asthma. (J Allergy Clin Immunol 2001;107:272-8.)

Key words: *Eicosanoids, lipid mediators, 5-oxo-ETE, 5-lipoxygenase products, RANTES, eotaxin, eosinophils, asthma, chemoattractants, adhesion molecules*

Asthma is an inflammatory disease characterized by the infiltration of eosinophils and lymphocytes into the lungs and airways hyperresponsiveness.[1] Although the precise contribution of eosinophils to some of the symptoms of this disease is somewhat controversial, it would seem clear that they make an important contribution to its pathology. Stimulation of eosinophils leads to the release of cytotoxic substances including oxygen radicals and cytotoxic proteins such as major basic protein, eosinophil cationic protein, and eosinophil peroxidase.[2] In addition, eosinophils release a variety of proinflammatory mediators including cytokines, chemokines, and lipid mediators that can further enhance the inflammatory response. In human beings, eosinophils and mast cells are the major sites of production of the potent bronchoconstrictor and proinflammatory cysteinyl leukotrienes (LTs) in the lung.

IL-5 plays an important role in the recruitment of eosinophils from the bone marrow, whereas IL-4 promotes their interaction with endothelial cells by upregulating VCAM-1.[2] Eosinophil recruitment into the tissues is also dependent on the actions of chemoattractants, of which there are 2 main types: chemokines and lipid mediators. Eotaxin appears to be the most important eosinophil chemoattractant among the chemokines,[2] because of both its potency and its selectivity for CC chemokine receptor (CCR) 3, which is localized principally on eosinophils,[3] basophils,[4] and to a lesser extent on T_H2 cells.[5] Eotaxin 2 is also selective for CCR3,[6] whereas RANTES, although a potent eosinophil chemoattractant, also interacts with other CCRs including CCR1 and CCR5.[7]

Among the lipid mediators, 5-oxoeicosanoids,[8,9] in particular 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE),[8,10] appear to be the most active human eosinophil chemoattractants and induce considerably greater responses than platelet-activating factor (PAF). 5-Oxo-ETE is also active in vivo, stimulating pulmonary infiltration of eosinophils after intratracheal administration in Brown Norway rats.[11] LTB_4 is a potent guinea pig eosinophil chemoattractant,[12] but it has only a very modest effect on human eosinophils,[8] in contrast to its potent effect on human neutrophils.[13] Although there is evidence that cysteinyl LTs can induce eosinophil infiltration in vivo,[14] this effect may be indirect[15] because they have relatively little activity in vitro.[8]

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Abbreviations used

5-LO: 5-Lipoxygenase
5-Oxo-ETE: 5-Oxo-6,8,11,14-eicosatetraenoic acid
CCR: CC chemokine receptor
EC_{50}: Median effective concentration
HPF: High-power field
LT: Leukotriene
NBD-phallacidin: N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phallacidin
PAF: Platelet-activating factor
PE: Phycoerythrin
VLA-4: Very late antigen-4

Because both lipid mediators and chemokines are released during pulmonary inflammation, it would seem likely that these 2 classes of chemoattractants contribute to eosinophil infiltration into the lungs in asthma. Furthermore, it is possible that there could be synergy between lipid mediators and chemokines as they act by distinct receptor-mediated mechanisms. In this study we have compared the actions of 5-oxo-ETE on eosinophils with those of the eosinophil-active chemokines eotaxin and RANTES and have investigated possible interactions between these 2 classes of potent eosinophil chemoattractants.

METHODS

Materials

5-Oxo-ETE was synthesized chemically as previously described[16] and purified at regular intervals by normal-phase HPLC to remove decomposition products. Eotaxin and RANTES were purchased from R&D Systems (Minneapolis, Minn) and PeproTech (Rocky Hill, NJ). Indo-1 and N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) phallacidin (NBD-phallacidin) were obtained from Molecular Probes (Eugene, Ore). L-α-lysophosphatidylcholine (type I, egg yolk) was purchased from Sigma Chemical Co (St Louis, Mo). FITC-labeled mouse antihuman CD11b (Bear1) and the corresponding FITC-labeled isotype IgG_1 control antibody were purchased from Immunotech-Coulter (Burlington, Ontario, Canada). FITC-labeled mouse antihuman L-selectin (Leu-8), the corresponding control IgG_2a antibody, and phycoerythrin (PE)-labeled mouse antihuman very late antigen 4 (VLA-4) were purchased from Becton-Dickinson (San Jose, Calif). Mouse plasma was obtained from Cedarlane (Hornby, Ontario, Canada).

Purification of eosinophils

Granulocytes were prepared from whole blood from healthy volunteers. No attempt was made to separate atopic from nonatopic donors, although none had asthma. Red blood cells were removed using Dextran T-500 (Pharmacia, Uppsala, Sweden) and mononuclear cells by centrifugation over Ficoll-Paque (Pharmacia) as described previously.[17] Any remaining red blood cells were then removed by hypotonic lysis. Finally, neutrophils were removed from the resulting granulocyte preparation by treatment with anti-CD16–labeled magnetic microbeads (Miltenyi Biotec Inc, Bergisch-Gladbach, Germany) and passage of the cell suspension through a column containing a steel matrix placed in a permanent magnet (MACS; Miltenyi Biotec Inc). Eosinophils (95% ± 4%, mean ± SD) were obtained in the pass-through fraction, with the major contaminating cells being lymphocytes and monocytes.[18] After centrifugation at 200*g* for 10 minutes, the cells were suspended in Hank's buffered saline solution.

Measurement of chemotactic responses

Cell migration was measured as previously described[8] with 48-well microchemotaxis chambers (Neuro Probe Inc, Cabin John, Md) and Sartorius cellulose nitrate filters (8-μm pore size, 140-μm thick; Neuro Probe Inc). Agonists were added to the bottom well in a volume of 30 μL PBS containing Ca^{++}, Mg^{++}, and 0.3% BSA, whereas eosinophils (150,000 cells in 55 μL RPMI containing 0.4% ovalbumin) were added to each of the top wells. The chambers were incubated for 2 hours at 37°C in 5% CO_2 and humidified air. The filters were then fixed with mercuric chloride and stained with hematoxylin and chromotrope 2R as described in the literature.[19] The numbers of cells on the bottom surfaces of the filters were counted in 5 different fields at a magnification of 400× for each incubation, each of which was performed in duplicate.

Measurement of actin polymerization

The F-actin content of eosinophils was measured with NBD-phallacidin by a procedure similar to that described by Howard and Oresajo.[20] Eosinophils (3×10^5 cells in 260 μL) were incubated with agonists for 20 seconds, after which time the cells were fixed with formaldehyde (30 μL of a 37% solution) at room temperature for 15 minutes. F-actin was then stained by incubation with lysophosphatidylcholine (30 μg in 15 μL) and NBD-phallacidin (49 pmol in 6.2 μL; final concentration, 0.15 μmol/L) overnight in the dark at 4°C. The fluorescence intensity of the stained eosinophils was quantified by flow cytometry with a Becton-Dickinson Facscalibur instrument.

Measurement of intracellular calcium levels

Cytosolic calcium levels were measured in indo-1–labeled eosinophils (10^6 cells in 1 mL) as previously described,[21] by use of a Photon Technology International (Lawrenceville, NJ) Deltascan 4000 spectrofluorometer with a temperature-controlled cuvette holder equipped with a magnetic stirrer. The excitation and emission wavelengths were set at 331 nm and 410 nm, respectively.

Measurement of surface expression of CD11b and L-selectin

Leukocytes were prepared by treatment of whole blood with Dextran T-500. The leukocyte fraction was suspended in Hank's buffered saline solution at a concentration of 10^6 cells/mL. Leukocytes (0.5 mL) were then incubated with agonists for either 10 minutes (L-selectin) or 15 minutes (CD11b). We had previously found that maximal responses to 5-oxo-ETE were obtained within these time periods.[21] The incubations were terminated by the addition of Isoton-II medium (2 mL at 0°C; Coulter Corp, Miami, Fla) and centrifugation for 5 minutes at 400*g* at 4°C. The pellets were incubated for 10 minutes at 4°C with mouse plasma (5 μL) followed by incubation for 30 minutes at 4°C with PE-labeled anti-VLA-4 (5 μL) along with FITC-labeled antibodies (10 μL) to either CD11b or L-selectin or the appropriate isotype-matched control FITC-labeled antibody. The cells were then incubated with Optilyse C (0.25 mL; Beckman Coulter, Fullerton, Calif) for 10 minutes followed by incubation with Ca^{++}/Mg^{++}-free PBS (0.5 mL) for a further 10 minutes. After centrifugation as described above, the cells were resuspended in Ca^{++}/Mg^{++}-free PBS (0.4 mL) containing 1% formaldehyde. The distribution of fluorescence intensities among 60,000 cells was measured by flow cytometry. Eosinophils were gated out on the basis of their granularity (high side scatter) and labeling with VLA-4 (PE fluorescence). CD11b or L-selectin was then measured

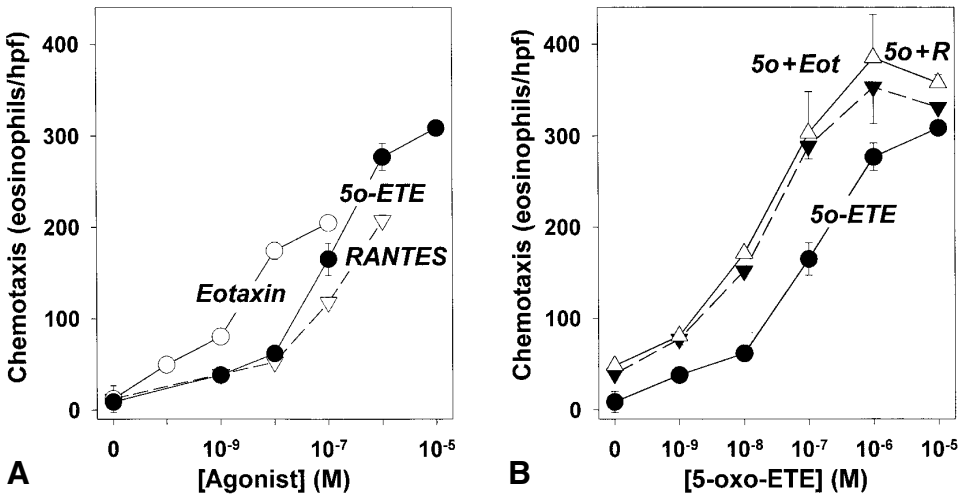

FIG 1. Effects of 5-oxo-ETE, eotaxin, and RANTES on eosinophil migration. **A,** Numbers of cells per HPF (×400) that migrated across nitrocellulose filters in response to different concentrations of 5-oxo-ETE *(5o-ETE),* eotaxin, or RANTES alone. **B,** Numbers of migrated eosinophils per HPF in response to 5-oxo-ETE alone *(5o-ETE)* or 5-oxo-ETE in the presence of either eotaxin *(5o+Eot)* or RANTES *(5o+R).* Values are means ± SE of data from separate experiments on purified eosinophils from 4 different donors, each performed in duplicate. *Error bars* are not shown if they are within the symbol.

in the eosinophil region on the basis of fluorescence due to FITC. All data were corrected for the value obtained for the corresponding isotype control antibody. None of the agonists under investigation affected the binding of the control antibodies or anti-VLA-4.

RESULTS

Chemotactic effects of 5-oxo-ETE, eotaxin, and RANTES for human eosinophils

The chemotactic effects of 5-oxo-ETE, eotaxin, and RANTES for human eosinophils were measured with a modified Boyden chamber assay. As shown in Fig 1, *A,* eotaxin is somewhat more potent than 5-oxo-ETE as an eosinophil chemotaxin, with about 10-fold lower concentrations required to elicit an equivalent chemotactic response. However, the maximal response to 5-oxo-ETE was about 50% higher than that to eotaxin ($P < .001$). Because of this, there was a greater than 10-fold difference between the median effective concentration (EC_{50}) values of these 2 chemoattractants (92 ± 15 nmol/L for 5-oxo-ETE vs 1.8 ± 0.1 nmol/L for eotaxin). Concentrations of RANTES equivalent to those of 5-oxo-ETE induced slightly weaker chemotactic responses than the latter substance. However, the maximal response to RANTES was clearly not achieved at a concentration of 1 μmol/L, and it was not possible to calculate the EC_{50} value and maximal response for this substance. This could possibly be due to the interaction of RANTES with additional chemokine receptors,[3] which may increase its efficacy.

Eotaxin and RANTES enhance 5-oxo-ETE–induced eosinophil chemotaxis

To determine whether CC chemokines could enhance the response of eosinophils to 5-oxo-ETE, we examined the effects of low concentrations of eotaxin (0.1 nmol/L) and RANTES (1 nmol/L) on 5-oxo-ETE–induced chemotaxis. As shown in Fig 1, *B,* both of these chemokines shifted the concentration-response curve for 5-oxo-ETE to the left. Although there were small increases in the basal level of chemotaxis because of the low concentrations of these chemokines, after subtraction of the baseline, the EC_{50} value for 5-oxo-ETE (92 ± 15 nmol/L) was significantly lower in the presence of eotaxin (22 ± 1 nmol/L; $P < .005$) and RANTES (22 ± 3 nmol/L; $P < .02$). The maximal responses observed for 5-oxo-ETE were also significantly greater in the presence of eotaxin (385 ± 23 cells per high-power field [HPF]; $P < .01$) and RANTES (354 ± 23 cells/HPF; $P < .05$) than with 5-oxo-ETE alone (309 ± 3 cells/HPF).

Eotaxin and 5-oxo-ETE have similar effects on calcium mobilization in eosinophils

We compared the effects of eotaxin and 5-oxo-ETE on calcium mobilization in indo-1–loaded eosinophils. As illustrated in Fig 2, these 2 mediators have similar effects on intracellular calcium levels, displaying EC_{50} values of about 2.3 nmol/L (eotaxin) and 7 nmol/L (5-oxo-ETE). The maximal responses were also similar (317 ± 80 nmol/L vs 330 ± 62 nmol/L Ca^{++}).

Effects of 5-oxo-ETE and eotaxin on actin polymerization

The effects of 5-oxo-ETE and eotaxin on actin polymerization in eosinophils were measured with NBD-labeled phallacidin, which binds selectively to polymerized F-actin but not to actin monomers. 5-Oxo-ETE (EC_{50}, 4 nmol/L) was less potent than eotaxin (EC_{50}, 40 pmol/L) in stimulating actin polymerization but induced a greater maximal response (39% ± 6% above control vs 24% ± 4% above control; $P < .02$) (Fig 3).

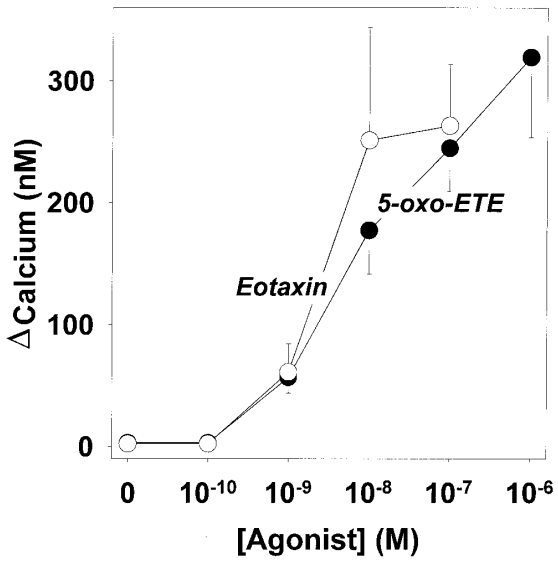

FIG 2. Effects of 5-oxo-ETE and eotaxin on calcium mobilization in purified human eosinophils. Eosinophils were loaded with indo-1, and peak calcium responses were measured by spectrofluorometry. Values are means ± SE of data from separate experiments on purified eosinophils from 4 different donors.

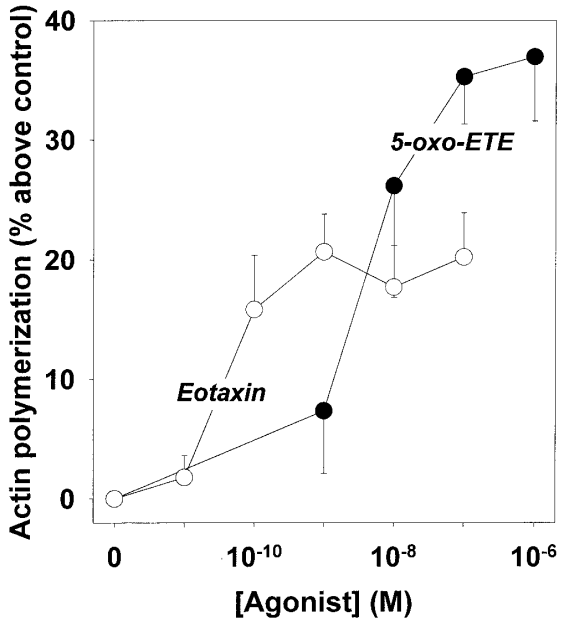

FIG 3. Effects of 5-oxo-ETE and eotaxin on actin polymerization in purified human eosinophils. After incubation with agonists, eosinophils were treated with NBD-phallacidin in the presence of lysophosphatidylcholine, and F-actin was measured by flow cytometry. Values are means ± SE of data from separate experiments on purified eosinophils from 4 different donors.

Stimulation of CD11b expression by 5-oxo-ETE and eotaxin

The effects of 5-oxo-ETE and other lipid mediators on purified eosinophils are rather modest, suggesting that these cells may be partially activated by the lengthy purification procedure.[21] To avoid this problem, we labeled a crude leukocyte fraction prepared by dextran sedimentation of red blood cells with PE-labeled anti-VLA-4, and eosinophils were gated out on the basis of VLA-4 labeling and granularity with flow cytometry. As shown by the dot plot in Fig 4, eosinophils were well separated from neutrophils because of intense labeling with anti-VLA-4 and from mononuclear cells due to their granularity. CD11b expression on these eosinophils was then assessed by labeling with FITC-labeled anti-CD11b. Neither 5-oxo-ETE nor eotaxin affected the expression of VLA-4 by eosinophils under the conditions used for the experiment.

As shown in Fig 5, there was appreciable basal expression of CD11b. However, this was considerably less than that observed for purified eosinophils (5.7 ± 0.6 times isotype control vs 12.2 ± 1 times isotype control). 5-Oxo-ETE and eotaxin at concentrations of 100 nmol/L both increased the surface expression of CD11b by at least 2-fold over basal expression (Fig 5). Although the EC_{50} value for eotaxin (0.13 nmol/L) was less than that for 5-oxo-ETE (10 nmol/L) (Fig 6), the maximal response to 5-oxo-ETE was somewhat greater (134% ± 16% above control vs 94% ± 15% above control; $P < .005$).

5-Oxo-ETE is more active than eotaxin in stimulating L-selectin shedding

The effects of 5-oxo-ETE and eotaxin on the shedding of L-selectin were investigated with anti-VLA-4–labeled

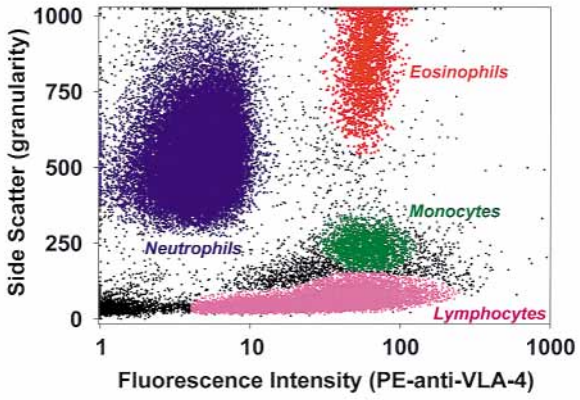

FIG 4. Dot plot showing the flow cytometric analysis of mixed leukocytes stained for VLA-4 and CD11b. Eosinophils were stained with PE-labeled anti-VLA-4 and FITC-labeled anti-CD11b before analysis. The *ordinate* shows the degree of side scatter, indicative of granularity, whereas the *abscissa* shows the fluorescence intensity due to PE, indicative of VLA-4 staining. Eosinophils *(red)* were separated from monocytes *(green)* and lymphocytes *(pink)* on the basis of granularity and from neutrophils *(blue)* on the basis of staining for VLA-4.

eosinophils as described above for CD11b. Although eotaxin induced measurable shedding of L-selectin from eosinophils, the magnitude of this response was much less than that for 5-oxo-ETE (17% ± 3% vs 76% ± 5% of L-selectin lost), and the response was quite variable among experiments (Fig 7). In contrast, 5-oxo-ETE induced a highly reproducible shedding of L-selectin with an EC_{50} of about 30 nmol/L.

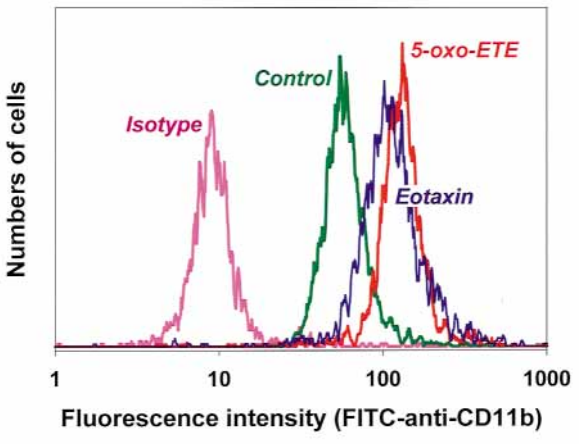

FIG 5. Histograms showing CD11b expression by eosinophils after incubation with eotaxin (100 nmol/L; *blue*), 5-oxo-ETE (100 nmol/L; *red*), or vehicle *(green)*. Cells were incubated with agonists or vehicle for 15 minutes and were then stained with a combination of PE-labeled anti-VLA-4 and either FITC-labeled anti-CD11b (eotaxin, 5-oxo-ETE, and vehicle) or an FITC-labeled isotype control antibody *(pink)*. Fluorescence due to FITC was then measured in the eosinophil region of the dot plot, as shown in Fig 4.

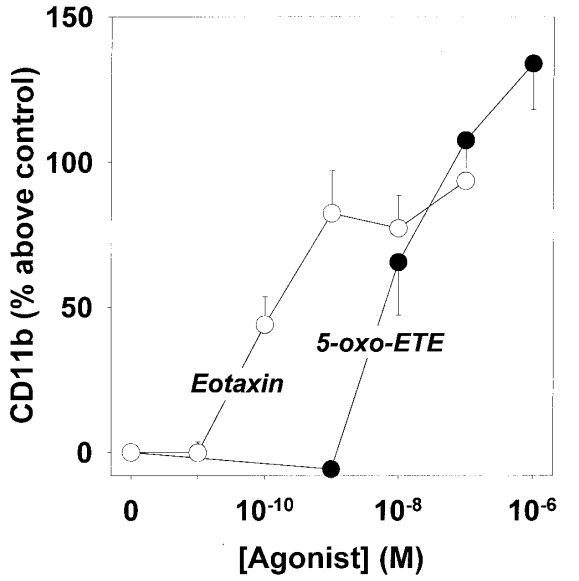

FIG 6. Effects of 5-oxo-ETE and eotaxin on the surface expression of CD11b by eosinophils. After incubation with agonists, crude leukocyte fractions were treated with PE-labeled anti-VLA-4 and FITC-labeled anti-CD11b, and FITC fluorescence was measured by flow cytometry as shown in Fig 5. Values are means ± SE of data from separate experiments on cells from 4 different donors.

DISCUSSION

This study demonstrates that 5-oxo-ETE is an eosinophil chemoattractant with a potency in the range of the peptide CC chemokines eotaxin and RANTES. Although the EC_{50} for 5-oxo-ETE is somewhat higher than that for eotaxin, it induces a greater maximal response. 5-Oxo-ETE appears to be slightly more potent than RANTES, although this is difficult to evaluate because the maximal response to RANTES was not obtained at the highest

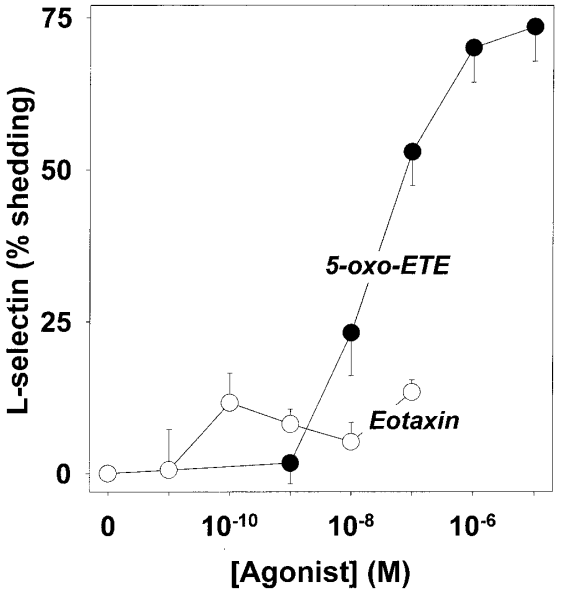

FIG 7. Effects of 5-oxo-ETE and eotaxin on the surface expression of L-selectin by eosinophils. After incubation with agonists, crude leukocyte fractions were treated with PE-labeled anti-VLA-4 and FITC-labeled anti-L-selectin, and FITC fluorescence was measured by flow cytometry as shown in Fig 5. Values are means ± SE of data from separate experiments on cells from 4 different donors.

concentration tested. It is possible that the chemotactic response to 5-oxo-ETE could be somewhat limited by its metabolism by eosinophils because we previously found that this compound has a limited lifetime in the presence of these cells.[8]

Threshold concentrations of eotaxin and RANTES shifted the concentration-response curve for 5-oxo-ETE to the left, increasing its potency. This effect appears to be synergistic rather than additive because the EC_{50} values, which were about 4 times lower in the presence of eotaxin and RANTES, were calculated after subtraction of the baseline, which increased slightly because of the presence of the chemokines. Because the production of both 5-lipoxygenase (5-LO) products and chemokines are increased in inflammation and asthma,[22] it would seem likely that these 2 classes of eosinophil chemoattractants interact with each other to induce eosinophil infiltration in this disease. This interaction could potentially be enhanced by chemokine-induced synthesis of 5-LO products, including 5-oxo-ETE, because CC chemokines, including RANTES, have been shown to induce arachidonic acid release by inflammatory cells.[23]

In addition to chemotaxis, 5-oxo-ETE and eotaxin induce a variety of other responses in eosinophils, including calcium mobilization, actin polymerization, CD11b expression, and L-selectin shedding, although in the case of L-selectin shedding, the response to eotaxin was rather small and variable. Except for calcium mobilization, 5-oxo-ETE tended to induce a greater maximal response than eotaxin but was somewhat less potent. We did not study RANTES further because of the relatively high concentrations required to induce a maximal response.

The effects of 5-oxo-ETE and eotaxin on actin polymerization and CD11b expression paralleled their effects on chemotaxis, in that eotaxin was the more potent of the 2 substances. In contrast, the potencies of these 2 mediators on calcium mobilization were similar. A similar discrepancy exists for the effects of 5-oxo-ETE and PAF on actin polymerization and chemotaxis (5-oxo-ETE > PAF) compared with calcium mobilization (PAF > 5-oxo-ETE).[21] Thus actin polymerization may be more closely related to cell migration than calcium mobilization, although the latter would also appear to play a role.[24-26] Thus calcium mobilization and actin polymerization, which itself is usually considered to be a calcium-independent process,[27] would appear to act in concert to induce cell migration.

The basis for the synergy between 5-oxo-ETE and eotaxin is unclear, but it suggests that there are differences in the signaling mechanisms initiated by activation of the 5-oxo-ETE receptor and CCR3. Although both compounds act through G proteins that are inhibited by pertussis toxin,[28,29] a considerable number of such proteins exist,[30] and there may be differences in the abilities of these chemoattractants to signal through different members of this family. This could also explain the much stronger L-selectin response elicited by 5-oxo-ETE. 5-Oxo-ETE and CC chemokines could thereby act together to induce enhanced chemotactic responses in eosinophils.

5-Oxo-ETE can also act synergistically with the lipid mediator PAF in inducing eosinophil chemotaxis.[8] PAF has also been shown to enhance responses to 5-hydroxy-6,8,11,14-eicosatetraenoic acid,[31] which is a weak agonist at the 5-oxo-ETE receptor.[32] In addition, cytokines, including GM-CSF and TNF-α, strongly enhance responses to 5-oxo-ETE,[33,34] providing further support for the notion that complex interactions between inflammatory mediators may be involved in eosinophil recruitment in asthma.

The potent chemoattractant effects of 5-oxo-ETE, along with its interactions with other inflammatory mediators, suggest that it should be considered, along with eotaxin and other CC chemokines, as an important potential contributor to pulmonary eosinophilia in asthma. Whereas removal of eotaxin through gene disruption[35] or antibody administration[36] caused a marked reduction in antigen-induced eosinophil infiltration, disruption of the gene for 5-LO had a similar effect on eosinophil infiltration into the lung.[37] This could be due, at least in part, to abolition of 5-oxo-ETE synthesis. Considerable pharmacologic evidence also exists for the role of 5-LO products in allergen-induced pulmonary eosinophilia. 5-LO inhibitors block eosinophil infiltration into the airways in human beings[38,39] as well as other species.[40,41] In another study the 5-LO inhibitor PF 5901, but not the LTB_4 antagonist PF 10042, blocked antigen-induced pulmonary eosinophilia in guinea pigs.[42] Sephadex-induced pulmonary eosinophilia was strongly inhibited by another 5-LO inhibitor, Zileuton, in Brown Norway rats.[43] This effect could not be reproduced by either the LTB_4 antagonist CGS-25019c or the cys-LT_1 receptor antagonist montelukast, except at high doses, which also inhibited the formation of LTs.[43] This would be consistent with a role for another 5-LO product such as 5-oxo-ETE in this response.

In conclusion, 5-oxo-ETE is an eosinophil chemoattractant with a potency in the range of the CC chemokines eotaxin and RANTES. Both 5-oxo-ETE and eotaxin elicit a variety of other responses in eosinophils, but there are differences in the relative potencies and maximal responses, with eotaxin being a more potent inducer of CD11b expression and actin polymerization and 5-oxo-ETE being a much better inducer of L-selectin shedding. CC chemokines can act synergistically with 5-oxo-ETE in inducing eosinophil chemotaxis, and such interactions may be important in asthma, where both types of mediators are likely to be present.

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