Allergic diseases such as asthma are characterized by tissue eosinophilia induced by the combined effects of chemoattractants and cytokines. Lipid mediators are a major class of endogenous chemoattractants, among which 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) is the most potent for human eosinophils. In this study, we investigated the effects of 5-oxo-ETE on eosinophil survival by flow cytometry. We found that this compound could promote eosinophil survival in the presence of small numbers of contaminating monocytes, but not in their absence. The conditioned medium from monocytes treated for 24 h with 5-oxo-ETE also strongly promoted eosinophil survival, whereas the medium from vehicle-treated monocytes had no effect. An antibody against the granulocyte/macrophage colony-stimulating factor (GM-CSF) completely blocked the response of eosinophils to the conditioned medium from 5-oxo-ETE-treated monocytes, whereas an antibody against interleukin-5 had no effect. Furthermore, 5-oxo-ETE stimulated the release of GM-CSF from cultured monocytes in amounts compatible with eosinophil survival activity, with a maximal effect being observed after 24 h. This effect was concentration-dependent and could be observed at concentrations in the picomolar range. 5-Oxo-ETE and leukotriene B4 had similar effects on GM-CSF release at low concentrations, but 5-oxo-ETE induced a much stronger response at concentrations of 10 nM or higher. This is the first report that 5-oxo-ETE can induce the release of any cytokine, suggesting that it could be an important mediator in allergic and other inflammatory diseases due to its chemoattractant properties and to its potent effects on the synthesis of the survival factor GM-CSF.

Pronounced tissue eosinophilia is a hallmark of a number of diseases, including allergic disorders such as asthma and rhinitis and parasitic infections (1). In asthmatic subjects, increased numbers of eosinophils are found in the airways following exposure to allergens (2). Pulmonary eosinophils are believed to contribute to the pathophysiology of asthma through the release of cationic granule proteins, reactive oxygen metabolites, lipid mediators, and pro-inflammatory cytokines (3). In addition to eliciting tissue damage, eosinophil-derived mediators can perpetuate the inflammatory reaction and lead to chronic changes in airway function (3).

Accumulation of eosinophils in inflammatory sites is thought to be mediated by a number of factors. Following mobilization from bone marrow in response to cytokines, eosinophils migrate into the lung and other tissues in response to the release of locally produced chemoattractants. Certain lipid mediators, most notably products of the 5-lipoxygenase pathway and platelet-activating factor, are potent stimulators of this process (4). Antigen-induced pulmonary eosinophilia is dramatically reduced in mice lacking the 5-lipoxygenase gene (5) as well as in humans (6) and other species (7, 8) treated with inhibitors of this enzyme. Among lipid mediators, the 5-lipoxygenase product 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) is the most potent and has been shown to induce eosinophil migration both in vitro (9, 10) and in vivo in rats (11) and humans (12).

5-Oxo-ETE is produced by a variety of human inflammatory cells (9, 13, 14) as well as by mouse macrophages (15). In addition to chemotaxis, it induces a variety of other responses in eosinophils, including calcium mobilization, degranulation, superoxide production, actin polymerization, CD11b expression, and L-selectin shedding (16–18). 5-Oxo-ETE acts via a highly selective G protein-coupled receptor (19, 20), which has recently been cloned (21, 22). The accumulation of eosinophils in tissues is dependent not only on their migration from the blood, but also on their survival within the tissue, as these cells are normally quite short-lived and rapidly undergo apoptosis (23). Because of the potent effects of 5-oxo-ETE on eosinophils, the aim of this study was to determine whether it can also promote the survival of these cells.

EXPERIMENTAL PROCEDURES

Reagents—5-Oxo-ETE was prepared by total chemical synthesis (24). Leukotriene B4 (LTB4) was a gift from Merck Frosst. Recombinant

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The abbreviations used are: 5-oxo-ETE, 5-oxo-6,8,11,14-eicosatetraenoic acid; LT, leukotriene; IL-5, interleukin-5; GM-CSF, granulocyte/macrophage colony-stimulating factor; LPS, lipopolysaccharide; FITC, fluorescein isothiocyanate; CRTH2, chemoattractant receptor-homologous molecule expressed on TH2 cells.
human interleukin-5 (IL-5), granulocyte/macrophage colony-stimulating factor (GM-CSF), eotaxin, and RANTES (regulated on activation normal T cell expressed and secreted) were purchased from Peprotech Inc. (Rocky Hill, NJ). The neutralizing monoclonal antibodies against recombinant human IL-5 and recombinant human GM-CSF were purchased from R&D Systems (Minneapolis, MN). Lipopolysaccharide (LPS) and polymyxin B were purchased from Sigma. The polyclonal antibody against human LPS-binding protein was obtained from Cedarlane (Hornby, Ontario, Canada).

Preparation of Eosinophils—Granulocytes were prepared from heparinized whole blood collected from healthy volunteers in Vacutainer blood collection tubes (BD Biosciences). No attempt was made to separate atopic from non-atopic donors, although none had asthma. Red blood cells were removed using dextran T-500 (Amersham Biosciences, Uppsala, Sweden) and mononuclear cells by centrifugation over Ficoll-Paque (Amersham Biosciences) as described previously (25). Any remaining red blood cells were then removed by hypotonic lysis. Finally, neutrophils and monocytes were removed from the resulting granulocyte preparation by treatment with a mixture of anti-CD16 (26) and anti-CD14 (unless otherwise indicated) antibodies coupled to paramagnetic microbeads (Miltenyi Biotec Inc., Bergisch-Gladbach, Germany), followed by passage through a column containing a steel matrix placed in a permanent magnet (MACS, Miltenyi Biotec Inc.). Eosinophils, contained in the passthrough fraction, were washed by centrifugation at 200 x g for 10 min. The purity of eosinophils, as determined using a laser-based flow cytometer (CELL-DYN 3700 system), was 94 ± 1%, the contaminating cells being neutrophils (4.6 ± 0.9%) and lymphocytes (1.4 ± 0.6%). No monocytes were detected in these preparations. Eosinophil viability was >99% as determined by trypan blue dye exclusion.

Preparation of Monocytes—Monocytes were purified from normal donors using a monocyte isolation kit from Miltenyi Biotec Inc., which permits negative selection of monocytes from peripheral blood mononuclear cells by immunomagnetic depletion of T cells, natural killer cells, B cells, dendritic cells, and basophils. Briefly, mononuclear cells were isolated from diluted heparinized peripheral blood by density gradient centrifugation over Ficoll-Paque (d 1.077). The mononuclear cells were then incubated with a mixture of hapten-conjugated antibodies against CD3, CD7, CD19, CD45RA, CD56, and IgE, followed by incubation with anti-hapten microbeads (Miltenyi Biotec Inc.) as described by the supplier. Monocytes were obtained in the passthrough fraction following immunomagnetic cell sorting using the MACS column as described above. The purity of monocytes (88 ± 2%) was determined using the CELL-DYN 3700 system. Viability was >99% as determined by trypan blue exclusion.

Conditioned Medium from Monocytes—Monocytes (1 x 10⁶ cells/ml) were incubated in macrophage serum-free medium (Inovitrogen) containing 2 µg/ml L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin in the presence or absence of 5-oxo-ETE (1 µM), unless otherwise indicated, for 24 h at 37°C. The conditioned medium was collected following centrifugation of the cells and was stored at -20°C until used. In some experiments, the conditioned medium was incubated for 1 h at 37°C with saturating concentrations of neutralizing monoclonal antibody against IL-5 (20 µg/ml) or GM-CSF (20 µg/ml) prior to addition to monocyte-depleted eosinophils.

Assessment of Eosinophil Survival—Purified eosinophils were suspended in RPMI 1640 medium (Inovitrogen) containing 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Eosinophils (2 x 10⁶ cells/200 µl) were incubated in 96-well flat-bottomed tissue culture plates (Corning Inc., Corning, NY) at 37°C for 48 h in the presence or absence of agonists or the conditioned medium from monocytes in the presence of humidified air containing 5% CO₂. Eosinophil survival was analyzed by flow cytometry using a fluorescein isothiocyanate (FITC)-labeled annexin V/propiodium iodide staining kit (R&D Systems). In brief, cells were removed from each well by gentle pipetting. To confirm that all the cells were removed, the wells were examined using an inverted microscope. After washing with phosphate-buffered saline by centrifugation, the cells were gently resuspended and incubated with FITC-labeled annexin V (0.25 µg) and propidium iodide (0.5 µg) in the dark for 15 min at room temperature. Samples were analyzed within 1 h by flow cytometry using a FACS-Calibur instrument with CellQuest software (BD Biosciences). Cell viability is expressed as the percentage of total cells that were not stained with either FITC-labeled annexin V or propidium iodide.

Quantitation of GM-CSF in Conditioned Medium—GM-CSF concentrations in the conditioned medium from monocytes were determined using a solid-phase enzyme-linked immunosorbent assay (BIO-SOURCE International, Camarillo, CA), which uses the multiple sandwich technique. GM-CSF was detectable in the linear portion of the binding curve (7-250 pg/ml), which was determined using recombinant human GM-CSF.

Statistics—The results are presented as the means ± S.E. The statistical significance of the differences between various treatments was assessed using one- or two-way analysis of variance, followed by the Tukey test for post-hoc analysis. A p value of <0.05 was considered significant.

RESULTS

5-Oxo-ETE Induces Monocyte-dependent Survival of Eosinophils—Eosinophils were isolated from peripheral blood by centrifugation over Ficoll-Paque, followed by immunomagnetic cell sorting of the granulocyte fraction using anti-CD16 antibody coupled to paramagnetic microbeads to remove neutrophils. These cells were treated with either vehicle or 5-oxo-ETE (1 µM) for 48 h in the presence of 10% fetal calf serum, and the numbers of live cells were determined by flow cytometry following labeling with annexin V and propidium iodide. As shown in Fig. 1A (lower left quadrant, unstained cells), only 23% of the vehicle-treated cells remained alive, with the remainder of the cells being either apoptotic (lower right quadrant, annexin V+/propidium iodide⁻) or apoptotic/necrotic (upper right quadrant, annexin V+/propidium iodide⁺). In contrast, 71% of the 5-oxo-ETE-treated cells remained alive after 48 h (Fig. 1B, lower left quadrant).

Examination of a larger number of subjects revealed that 5-oxo-ETE significantly increased eosinophil survival from 27 ± 5 in vehicle-treated eosinophils to 50 ± 10% (p < 0.01).
compared with 83 ± 5% in cells treated with the eosinophil survival factor IL-5 (Fig. 2, left). However, there was considerable variability in the responses of different eosinophil preparations to 5-oxo-ETE, with some showing virtually no response. In contrast, all preparations tested responded well to IL-5. This raised the possibility that the effect of 5-oxo-ETE was mediated by the release of an eosinophil survival factor from small numbers of contaminating cells. In support of this hypothesis, we found that the two cell preparations that displayed virtually no response to 5-oxo-ETE contained no detectable monocytes, whereas each of the five eosinophil preparations that responded to 5-oxo-ETE was contaminated with at least 1% monocytes (3.2 ± 1.6%). We confirmed that monocytes were responsible for the survival-enhancing effects of 5-oxo-ETE by removing these cells from our eosinophil preparations using anti-CD14 antibody coupled to paramagnetic beads. This resulted in a nearly complete loss of the effect of 5-oxo-ETE on eosinophil survival without any reduction in the response to IL-5, which acted directly on these cells (Fig. 2, right).

Monocytes Secrete a Soluble Eosinophil Survival Factor in Response to 5-Oxo-ETE—Monocytes purified immunomagnetically by negative selection were incubated with either vehicle or 5-oxo-ETE (1 μM) for 24 h. Aliquots of the conditioned medium from monocytes from a single donor were then incubated for 48 h with monocyte-depleted eosinophils from four different donors, and cell survival was assessed by flow cytometry. As described above, 5-oxo-ETE added directly to the four eosinophil preparations had only a small, statistically non-significant effect on cell survival, whereas IL-5 dramatically increased survival (Fig. 3A, open bars). In contrast, the conditioned medium from 5-oxo-ETE-treated monocytes strongly stimulated eosinophil survival (67 ± 10%; p < 0.001) to almost the same extent as the direct addition of IL-5, whereas the conditioned medium from vehicle-treated monocytes had no detectable effect (11 ± 4%). This experiment clearly demonstrates that different eosinophil preparations respond consistently to a 5-oxo-ETE-inducible factor released by monocytes. Therefore, for statistical purposes, for all of the experiments described below, n was taken to be the number of different monocyte preparations tested against one or more eosinophil preparations.

To determine the potency of 5-oxo-ETE in inducing the release of the monocyte-derived survival factor, four different preparations of monocytes were incubated separately with different concentrations of 5-oxo-ETE. Aliquots (10 μl) of the conditioned medium from each of the monocyte preparations were then incubated for 48 h at 37 °C in the presence or absence of different concentrations of 5-oxo-ETE. Aliquots (10 μl) of the conditioned medium from each of the four monocyte preparations were then incubated for 48 h at 37 °C with eosinophils (2 × 10^6 cells/200 μl) from two different donors, and cell survival was assessed by flow cytometry. For the purposes of statistical analysis, the average percent survival for the two eosinophil preparations was calculated for each of the monocyte preparations, and n was taken to be equal to the number of monocyte donors (i.e., n = 4). **, p < 0.01; ***, p < 0.001; NS, not significant.

As LPS has also been shown to induce the monocyte-dependant survival of eosinophils (27), we wanted to ensure that the response we observed was not an artifact due to contamination with this substance. We therefore conducted experiments in which either polymyxin B, which binds to the lipid A portion of LPS (28), or an antibody to LPS-binding protein was added to the conditioned medium from monocytes. Neither of these substances had any effect on either basal eosinophil survival or the survival-enhancing effect of 5-oxo-ETE (Fig. 4). In contrast, both inhibitors nearly completely blocked the increase in eosinophil survival elicited by incubation with the conditioned medium from monocytes that had been treated with LPS for 24 h (p < 0.001).

The Monocyte-derived Eosinophil Survival Factor Induced by 5-Oxo-ETE Is Identical to GM-CSF—Because both IL-5 and

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**Fig. 2.** 5-Oxo-ETE-induced eosinophil survival is dependent on the presence of monocytes. Eosinophils (Eos) purified using either anti-CD16 antibody alone (left, closed symbols) or a combination of anti-CD16 and anti-CD14 antibodies (right, open symbols) were incubated for 48 h at 37 °C with control medium (Con), 1 μM 5-oxo-ETE (5o), or 1 nM IL-5, and survival was assessed following staining with FITC-labeled annexin V and propidium iodide. Data for individual eosinophil preparations along with the means ± S.E. are shown for incubations with vehicle and 5-oxo-ETE, whereas only the means ± S.E. are shown for IL-5. **, p < 0.01; ***, p < 0.001; NS, not significant.

**Fig. 3.** Effect of the conditioned medium from 5-oxo-ETE-treated monocytes on eosinophil survival. A, eosinophils (Eo) from four different donors were purified using a combination of anti-CD16 and anti-CD14 antibodies and incubated for 48 h at 37 °C with vehicle (control (Con)), 1 μM 5-oxo-ETE (5oETE), or 1 nM IL-5 (left, open bars). Alternatively, eosinophils were incubated with the conditioned medium obtained from monocytes (MC) treated for 24 h at 37 °C with either vehicle alone (MCM) or 1 μM 5-oxo-ETE (MCM/5oETE) (right, closed bars) as described under “Experimental Procedures.” Eosinophil survival was assessed by flow cytometry using FITC-labeled annexin V/propidium iodide staining. B, shown is the concentration-response curve for the production of an eosinophil survival factor by monocytes in response to 5-oxo-ETE. Monocytes (10^6 cells/ml) from four different donors were incubated for 24 h at 37 °C in the presence or absence of different concentrations of 5-oxo-ETE. Aliquots (10 μl) of the conditioned medium from each of the four monocyte preparations were then incubated for 48 h at 37 °C with eosinophils (2 × 10^6 cells/200 μl) from two different donors, and cell survival was assessed by flow cytometry. For the purposes of statistical analysis, the average percent survival for the two eosinophil preparations was calculated for each of the monocyte preparations, and n was taken to be equal to the number of monocyte donors (i.e., n = 4). **, p < 0.01; ***, p < 0.001; NS, not significant.
GM-CSF are known to be potent eosinophil survival factors (1), we determined whether the effects of 5-oxo-ETE could be prevented by blocking antibodies to these two cytokines. To test the efficacy of the antibodies, monocyte-depleted eosinophils were incubated with IL-5 or GM-CSF in the presence or absence of anti-IL-5 or anti-GM-CSF antibody, respectively. As shown in Fig. 5 (left), these antibodies nearly completely abolished the responses mediated by their respective antigens \( p < 0.001 \). In contrast, a control isotype-matched antibody had no effect on the survival of either vehicle- or cytokine-treated monocytes (data not shown).

To determine whether these antibodies could block the effects of 5-oxo-ETE, the conditioned medium from 5-oxo-ETE-treated monocytes was treated with either anti-IL-5 or anti-GM-CSF antibody. As described above, the conditioned medium from 5-oxo-ETE-treated monocytes strongly induced eosinophil survival \( p < 0.001 \). This effect was completely abrogated by the addition of anti-GM-CSF antibody \( p < 0.001 \), but was unaffected by anti-IL-5 antibody (Fig. 5, right), providing strong evidence that the effect of 5-oxo-ETE is mediated by GM-CSF.

**5-Oxo-ETE Stimulates Monocytes to Produce GM-CSF.**—To provide direct evidence that 5-oxo-ETE can induce GM-CSF release from monocytes, we measured its concentration following incubation of these cells with 5-oxo-ETE (1 \( \mu \)M) for different times (Fig. 6A). 5-Oxo-ETE strongly stimulated GM-CSF release from monocytes, with the maximal level of this cytokine being observed after 24 h \( p < 0.01 \). The effects of concentrations of 5-oxo-ETE between 1 pm and 100 nM on GM-CSF release from monocytes are shown in Fig. 6B. The effect of 5-oxo-ETE was concentration-dependent, with significant increases occurring at subnanomolar concentrations. However, the maximal response was not reached within this concentration range. The effects of higher concentrations of 5-oxo-ETE are shown in Fig. 6B (inset). The greatest response \((15.3 \pm 2.9 \text{ versus } 0.36 \pm 0.14 \text{ pm CSF in vehicle-treated cells})\) was observed at 10 \( \mu \)M 5-oxo-ETE, but it was not clear whether a plateau had been reached even at this concentration. The effects of LTB\(_4\) on GM-CSF release were also investigated, but only a limited number of concentrations could be tested because of the small numbers of monocytes available. Concentrations of LTB\(_4\) and 5-oxo-ETE in the picomolar range had similar effects on GM-CSF production, whereas 5-oxo-ETE induced a much greater response at higher concentrations (Fig. 6B).

**DISCUSSION**

Our preliminary results suggested that 5-oxo-ETE is capable of prolonging the survival of eosinophils. However, the variability of this response led us to suspect that it may be indirect, possibly mediated by small numbers of contaminating cells. The complete absence of monocytes in the two eosinophil preparations that failed to respond to 5-oxo-ETE suggested that these cells might be required for its survival-enhancing effect. This was confirmed when we found that 5-oxo-ETE was unable to promote survival in preparations of eosinophils from which all of the monocytes had been removed by treatment with anti-CD14 antibody. The medium from purified monocytes incubated with 5-oxo-ETE for 24 h strongly stimulated eosinophil survival to nearly the same extent as IL-5, a well known eosinophil survival factor, indicating that this effect was mediated by the release of a soluble factor from monocytes. This effect was quite reproducible, as it was consistently observed using both eosinophils and monocytes from many different donors. As the most likely candidates for the soluble factor released by 5-oxo-ETE appeared to be GM-CSF and IL-5, we tested the effects of monoclonal antibodies against these two cytokines. Anti-GM-CSF antibody completely blocked the survival-enhancing effect of the conditioned medium from 5-oxo-
ETE-treated monocytes, whereas anti-IL-5 antibody was without effect. Furthermore, 5-oxo-ETE was a potent stimulator of GM-CSF release from monocytes. Incubation of monocytes with 1 μM 5-oxo-ETE induced the release of GM-CSF in concentrations equivalent to its EC₅₀ value after dilution in the medium used to culture eosinophils in survival studies. The base-line levels of GM-CSF in the medium from vehicle-treated monocytes were >10 times lower and would be expected to elicit only a small increase in eosinophil survival. These results provide compelling evidence that GM-CSF can account for most, if not all, of the eosinophil survival-promoting activity released from monocytes in response to 5-oxo-ETE.

The concentration-response curve for 5-oxo-ETE-induced GM-CSF production did not reach a plateau, and it was not clear whether the maximal response had been achieved, even at a concentration as high as 10 μM. This may be due to the metabolism of 5-oxo-ETE over the relatively long 24-h incubation period, which could have limited the response to lower concentrations of this substance. When present at higher concentrations, biologically effective levels of 5-oxo-ETE could have persisted for a much longer period of time, thus enhancing its effect on GM-CSF production and skewing the concentration-response curve. Alternatively, we cannot rule out the possibility that the effects of 5-oxo-ETE are mediated by its conversion to an active metabolite during the 24-h incubation period with monocytes. Murine macrophages convert 5-oxo-ETE to both ω-oxidation products (15) and 5-oxo-7-glutathionyl-8,11,14-eicosatrienoic acid, a glutathionyl conjugate, the formation of which is catalyzed by LTC₄ synthase (29). 5-Oxo-7-glutathionyl-8,11,14-eicosatrienoic acid is known to stimulate migration and actin polymerization in human eosinophils, and it is not known whether it also has other effects on these cells. However, unlike murine macrophages, human monocytes do not appear to have high levels of ω-oxidation activity (14); and at least in short-term incubations (30 min), we have not detected significant amounts of any 5-oxo-ETE metabolites formed by these cells.

This is the first report that 5-oxo-ETE can stimulate the release of a cytokine from any target cell, as until now, it had only been shown to elicit fairly rapid responses such as actin polymerization, calcium mobilization, and adhesion molecule expression (16–18), which are associated with functional responses such as cell adhesion and migration (9). We also found that LTB₄ can induce GM-CSF release from monocytes, but the maximal response was much lower than that elicited by 5-oxo-ETE. LTB₄ has also been shown to stimulate monocytes to release interleukin-6 (31), but the present study appears to be the first direct evidence that a 5-lipoxygenase product can induce the release of GM-CSF. Antagonists of the cys-LT₁ receptor have been reported to inhibit GM-CSF release from antigen-stimulated mononuclear cells from asthmatics (32, 33). However, the effect of LTD₄ itself on these cells was not reported, and the precise mechanism of action of cys-LT₁ antagonists in this mixed cell population is not well understood.

5-Oxo-ETE has weak chemotactic effects on monocytes and enhances their response to MCP-1 (monocyte chemotactant protein) and MCP-3 (34). It also induces actin polymerization in these cells, but, in contrast to eosinophils, does not elicit calcium mobilization (34). Macrophages have been shown to contain mRNA for the recently cloned 5-oxo-ETE receptor, although at a lower level than eosinophils and neutrophils (22), suggesting that this receptor may also be present in monocytes and may mediate the effects of 5-oxo-ETE on GM-CSF release from these cells.

5-Oxo-ETE occupies a distinct position among eosinophil chemotactants in that it is a potent stimulus for cell migration and, through its effects on monocyte GM-CSF release, can also promote eosinophil survival. In contrast, the potent eosinophil chemotactants eotaxin and RANTES do not prolong eosinophil survival (35), even in the presence of contaminating monocytes (data not shown). The lipid mediator platelet-activating factor, another eosinophil chemoattractant, also fails to promote eosinophil survival (36). Another eicosanoid with eosinophil chemotactant activity is prostaglandin D₂, which acts through the DP₂ receptor/CRTTH2 (37, 38). However, this substance does not promote eosinophil survival, despite the
fact that the selective \(\delta_1\) receptor agonist BW245C does have this effect (39), presumably due to stimulation of adenyl cyclase, an effect shared by prostaglandin \(E_2\) (40).

The present findings have significant implications regarding the potential role of 5-oxo-ETE in asthma, as GM-CSF is known to be very important for the survival of eosinophils once they have reached the lung (41). 5-Oxo-ETE is synthesized by both monocytes (14) and macrophages (15), which are prominent cells in the lung, and it could act on these cells to release GM-CSF. In this way, 5-oxo-ETE could both elicit the infiltration of eosinophils into the lung in concert with other chemotactants such as eotaxin, with which it has a synergistic effect (42), and increase the lifetime of these eosinophils once they have entered the tissue. It is also possible that 5-oxo-ETE could promote the survival of other inflammatory cells by this mechanism because of the potent effects of GM-CSF on neutrophils and monocytes (43).

Another intriguing possibility is that GM-CSF, produced in response to 5-oxo-ETE, could act in a feed-forward loop to enhance both the production of and cellular responses to 5-oxo-ETE. GM-CSF has been shown to increase the production of 5-lipoxygenase products at multiple levels in neutrophils (44–46) and would be expected to stimulate 5-oxo-ETE synthesis in these cells in the lung, and it could act on these cells to release GM-CSF. In conclusion, 5-oxo-ETE can prolong the survival of eosinophils (47) to 5-oxo-ETE.

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