

5-Oxo-6,8,11,14-eicosatetraenoic acid is a potent chemoattractant for human basophils

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Background: 5-Oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-EETE) is a chemoattractant for eosinophils and neutrophils, and the messenger RNA for its receptor, the oxo-eicosatetraenoic acid receptor (OXE), has been detected in several tissues.

Objectives: This study aimed at clarifying the role of 5-oxo-EETE in the regulation of basophil function.

Methods: Basophil responses were determined in assays of flow-cytometric shape change, Ca^{2+} flux, chemotaxis, and histamine release. Messenger RNA for OXE was detected by real-time PCR.

Results: We observed that human eosinophils were 3 to 10 times more sensitive to 5-oxo-EETE than neutrophils in flow-cytometric shape change and Ca^{2+} flux assays, as estimated from the half-maximal responses of the cells. Basophils responded to 5-oxo-EETE in the shape change assay with a sensitivity similar to that of eosinophils. 5-Oxo-EETE was a weak inducer of Ca^{2+} flux in basophils and did not cause histamine release but was a highly effective chemoattractant for basophils in the low nanomolar concentration range in a pertussis toxin-sensitive manner. In agreement with these functional studies, the messenger RNA for the 5-oxo-EETE receptor, OXE, was detectable in basophils as in monocytes, eosinophils, and neutrophils, but not in fibroblasts. Specimens from sinus mucosa, tonsils, and adenoids also contained detectable levels of messenger RNA for OXE.

Conclusion: Our data suggest that 5-oxo-EETE is potentially involved in the regulation of basophil recruitment and might hence be a useful therapeutic target in atopic disease. (*J Allergy Clin Immunol* 2005;116:1014-9.)

Key words: Basophils, chemoattractants, eicosanoids, allergy, chemotaxis, Ca^{2+} flux

The eicosanoid 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-EETE) arises from the 5-lipoxygenase pathway and is finally formed from 5-hydroxy-eicosatetraenoic acid by a highly specific dehydrogenase expressed in several

Abbreviations used

5-oxo-EETE: 5-Oxo-6,8,11,14-eicosatetraenoic acid

FITC: Fluorescein isothiocyanate

G3PDH: Glyceraldehyde-3 phosphate dehydrogenase

MCP: Monocyte chemotactic protein

perCP: Peridinin-chlorophyll-protein complex

inflammatory cells.^{1,2} 5-oxo-EETE is a potent chemoattractant for eosinophils, neutrophils, and monocytes,^{1,3-5} but its effects also include Ca^{2+} mobilization, degranulation, CD11b expression, actin polymerization, and activation of phospholipase A_2 and mitogen-activated protein kinase.^{1,5,6}

Basophils are prominent in late-phase cutaneous allergic reactions⁷ and constituents of the cellular infiltrate in the asthmatic lung.⁸ Activated by allergen or other stimuli, basophils are able to release an array of proinflammatory mediators, including histamine and leukotriene C_4 , and also IL-4 and IL-13,⁹ which are capable of amplifying the recruitment of inflammatory cells to the tissue by upregulating adhesion molecules¹⁰ and by inducing the expression of chemokines.¹¹ Basophil-derived IL-4 and IL-13 can induce the switch to the IgE isotype in B cells independently of T cells, because basophils can also provide the B cell with CD40L.¹² Although basophils express several chemokine receptors (CC chemokine receptors 1, 2, and 3, and CXC chemokine receptors 1 and 2),¹³ basophil numbers do not correlate with expression of the corresponding ligands, such as eotaxin, eotaxin-2, RANTES, or monocyte chemotactic protein (MCP)-3,¹⁴ which suggests that the chemoattractants governing the recruitment of basophils are still unknown. In the current study, we have observed that 5-oxo-EETE is a highly effective and potent chemoattractant for human basophils, suggesting that this eicosanoid might be an important mediator in allergic inflammation and asthma.

METHODS

Reagents

All laboratory reagents were from Sigma (Vienna, Austria) unless specified. Assay buffer was made from Dulbecco modified PBS (with 0.9 mmol/L Ca^{2+} and 0.5 mmol/L Mg^{2+} ; Invitrogen, Vienna, Austria); 0.1% BSA; 10 mmol/L HEPES; and 10 mmol/L glucose, pH 7.4. Eotaxin, MCP-1, IL-3, IL-4, and IL-8 were from Peprotech (London, United Kingdom), 5-oxo-EETE from Cayman (Ann Arbor, Mich), and CellFix and FACSFlow from Becton Dickinson (Vienna, Austria). Fixative solution was prepared by adding 9 mL

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distilled water and 30 mL FACS-Flow to 1 mL CellFix. Antibodies to HLA-DR conjugated with fluorescein isothiocyanate (FITC) or Quantum Red were from Sigma, and antibodies to CD123 conjugated with phycoerythrin, CD14 (peridinin-chlorophyll-protein complex [perCP]), and CD16 (FITC or phycoerythrin-Cy5) were from Becton Dickinson.

Preparation of human leukocytes

The study was approved by the Ethics Committee of the University of Graz. Peripheral blood leukocytes were obtained by dextran sedimentation of citrated whole blood from healthy volunteers. Preparations of polymorphonuclear leukocytes (containing eosinophils and neutrophils) and PBMCs (including basophils, monocytes, and lymphocytes) were prepared by Histopaque gradients (Sigma) as described.^{13,15,16} In some experiments, eosinophils, monocytes, and basophils were further purified by negative magnetic selection using antibody cocktails from StemCell Technologies (Vancouver, Canada), with resulting purities and viabilities of >95%. The purity of basophil preparations was $94.6 \pm 0.4\%$ ($n = 13$), with the contaminating cells being lymphocytes.

Leukocyte shape change assay

Ninety-microliter aliquots of whole blood were mixed with CD16 FITC, CD14 PerCP, CD123 phycoerythrin, and/or HLA-DR FITC antibodies and stimulated with 10 μ L agonists for 4 minutes at 37°C. The samples were then transferred to ice and fixed with 250 μ L fixative solution followed by NH₄Cl-induced lysis of red blood cells.¹⁷ Cells were then washed and resuspended in 250 μ L fixative solution. Samples were immediately analyzed on a FACSCalibur flow cytometer (Becton Dickinson). Eosinophils were identified according to their autofluorescence in fluorescence channels (FL) 1 and 2, whereas monocytes and neutrophils were identified by staining positive for CD14 and CD16, respectively. Basophils were identified as CD123⁺HLA-DR⁻ cells.^{13,18}

Ca²⁺ flux

To study eosinophil and neutrophil Ca²⁺ flux by flow cytometry,¹⁵ polymorphonuclear leukocytes (10⁷ cells/mL) were labeled with CD16 phycoerythrin-Cy5 antibodies for 10 minutes at room temperature and treated with 2 μ mol/L of the acetoxymethyl ester of Fluo-3 in the presence of 0.02% pluronic F-127 for 60 minutes at room temperature. To record monocyte and basophil Ca²⁺ flux,¹⁹ mononuclear cell preparations (10⁷ cells/mL) were labeled with CD14 PerCP antibodies, or CD123 phycoerythrin and HLA-DR Quantum Red antibodies, respectively, for 10 minutes at room temperature. The cells were then treated with 2 μ mol/L of the acetoxymethyl ester of Fluo-3 in the presence of 0.02% pluronic F-127 and 2.5 μ mol/L probenecid for 20 minutes at room temperature. The leukocytes were washed and resuspended in assay buffer with Ca²⁺/Mg²⁺ for a concentration of 3×10^6 leukocytes/mL. Changes in intracellular free Ca²⁺ levels were detected at room temperature as increase in fluorescence intensity of the Ca²⁺-sensitive dye Fluo-3 in the FL1 channel for eosinophils (CD16⁻/high side scatter), neutrophils (CD16⁺/low side scatter), monocytes (CD14⁺), and basophils (CD123⁺HLA-DR⁻).

Histamine release

Aliquots of basophil-containing PBMCs were mixed with IL-3 (300 pmol/L) and then stimulated with C5a, 5-oxo-ETE, or vehicle for 1 hour, and the release of histamine into the supernatant was quantified by RP-HPLC after perchloric acid extraction followed by condensation with o-phthalaldehyde. Fluorescence was monitored at 360-nm excitation and 450-nm emission wavelengths after elution with mixtures of 0.1 N acetic acid containing 0.1% pentanesulfonic

acid and acetonitrile.²⁰ A nonstimulated sample was boiled and centrifuged, and the supernatant was used to determine the total histamine contents of basophils.

Chemotaxis

Twenty thousand purified basophils or 4×10^5 PBMCs containing basophils were suspended in assay buffer and placed into Transwell inserts with 5- μ m pore size polyvinyl pyrrolidone-free polycarbonate filters (Corning, Acton, Mass). The cells were allowed to migrate toward agonists in the bottom wells of 24-well tissue culture plates for 1 hour at 37°C in a humidified CO₂ incubator. The bottom wells were then gently flushed to recover the migrated cells until virtually no cells were left in the wells as verified by microscopy. The recovered cells were stained with CD123 phycoerythrin and HLA-DR FITC antibodies for 10 minutes at room temperature, washed, and counted by flow cytometry, as previously described.²¹ Basophils were distinguished from contaminating cells as CD123⁺HLA-DR⁻ cells. Eosinophil and neutrophil chemotaxis was determined by using polymorphonuclear cell preparations (4×10^5 cells/well) as described. Cells were allowed to migrate for 30 minutes, stained with CD16 FITC antibodies for 10 minutes at room temperature, and enumerated by flow cytometry. Neutrophils were detected as CD16⁺ cells in FL1, whereas eosinophils were distinguished from neutrophils as CD16⁻ cells with high side scatter.

Real-time RT-PCR

Total RNA was extracted by using Trizol (Invitrogen) and after DNase treatment by using DNasefreeTM (Ambion, Huntington, United Kingdom) to remove contaminating DNA. Reverse transcription of 100 ng total RNA of monocytes, eosinophils, and neutrophils and 50 ng total RNA of basophils and fibroblasts was performed with avian Omniscript RT-kit (Qiagen, Hilden, Germany), and oligo (dT)15 primer (Promega, Mannheim, Germany). Poly-A RNA was extracted from tissue total RNA preparations by using Dynal beads (Dynal, Oslo, Norway), and reverse transcription of 20 ng poly-A RNA was performed by using Sensiscript RT-kit (Qiagen) and oligo (dT)15 primer (Promega). Real-time PCR (using the LightCycler Instrument, Roche, Mannheim, Germany) was performed with fluorescence resonance energy transfer detection as described previously.²² Amplification was performed in duplicate by using LightCycler-FastStart DNA Master Hybridization Probes (Roche) mix containing 4 mmol/L MgCl₂, 1 μ L and 1 μ L of a 1:10 dilution of the reverse transcribed product, 10 pmol of the specific primers for the 5-oxo-ETE receptor, OXE (Gen Bank accession number: AY158687; forward: 5'-CTC CAT GAG ACC TGG CG-3' and reverse 5'-TAG CGG TTG AGT GCG ATG-3'), and 4 pmol of specific hybridization probes, one labeled with fluorescein on the 3' end and the second with LC-Red640 on the 5' end and hybridization probes (5'-TGG TGG ACA GCA TGA AGA GGT TGA-3'-fluoresceine and LC-Red 640-5'-TTT GCA GGC AGC AGC CCC-3'). Primers and hybridization probes were designed and synthesized by TibMolbiol (Berlin, Germany). For hot start, the samples were kept at 95°C for 6 minutes; cycling parameters were 95°C for 3 seconds, 64°C for 10 seconds, and 72°C for 10 seconds. This primer pair combination was predicted to give a PCR product of 115 base pairs, which was verified by electrophoresis on agarose gel. In all samples, glyceraldehyde-3 phosphate dehydrogenase (G3PDH) was amplified to verify intact mRNA. Reagent control (PCR-grade water instead of cDNA) was included in each amplification run. The levels of mRNA were quantified by the crossing point, which was determined by the Second Derivate Maximum Method using the LightCycler Software 3.5 (Roche). For relative quantification of the target gene, standard curves were constructed from a stock of cDNA obtained from reverse-transcribed RNA isolated from tissues in 1:10 dilution

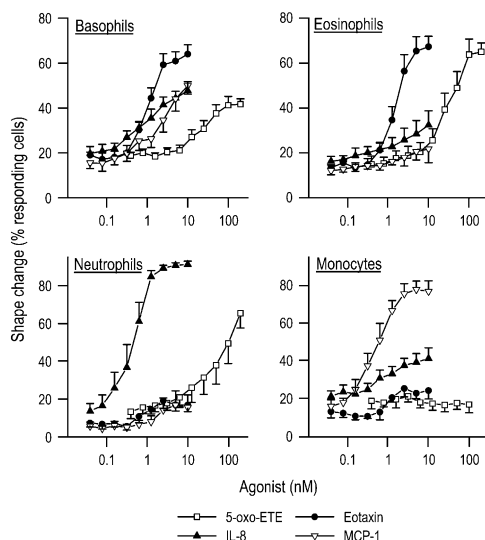


FIG 1. 5-Oxo-ETE induces shape change in basophils, eosinophils, and neutrophils, but not monocytes, in whole blood. Shape change was analyzed by flow cytometry as increase in forward scatter and expressed as percent of cells responding; $n = 6$.

steps. The same stock cDNA was used for all amplifications to determine the relative quantities across multiple runs. For each sample, the expression level of OXE mRNA was determined from the appropriate standard curve and normalized to G3PDH.

Statistics

Data are shown as means $\pm$ SEMs for n observations. Comparisons of groups were performed by using the Mann-Whitney U test, and probability values of $P < .05$ were considered statistically significant.

RESULTS

Stimulation of leukocytes by chemoattractants or chemokinetic agonists results in changes in the cell shape, which is reflected by increase of light scattering in flow cytometry.^{13,15} By staining whole blood with antibodies against cell type-specific markers, we quantified the shape change responses of basophils, eosinophils, neutrophils, and monocytes as the percentage of cells moving into a higher forward scatter region. 5-Oxo-ETE was an effective inducer of basophil shape change, showing a potency similar to that in eosinophils (Fig 1). As estimated from the half-maximally effective concentrations, eosinophils and basophils responded with 3-fold to 10-fold higher sensitivity than neutrophils. In contrast, 5-oxo-ETE had no effect on monocyte shape change, although these cells were highly sensitive to the chemokine MCP-1 (Fig 1).

Agonist-induced elevation of intracellular free Ca^{2+} concentration is a key event in many cellular responses, and chemoattractants are potent inducers of Ca^{2+} flux in basophils.¹⁹ Basophils responded only to high concentrations (>100 nmol/L) of 5-oxo-ETE with Ca^{2+} flux, and the magnitude of these responses reached only 50% of

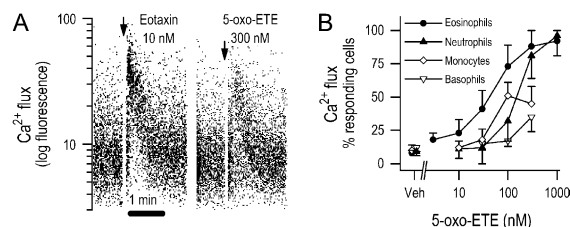


FIG 2. Effects of 5-oxo-ETE or vehicle (Veh) on Ca^{2+} flux in leukocytes. **A**, A representative tracing of 5-oxo-ETE- and eotaxin-induced Ca^{2+} flux in basophils is shown out of 5 experiments. **B**, Effects of 5-oxo-ETE were expressed as percent of cells responding; $n = 4-6$.

those to eotaxin (Fig 2, A). Comparison of leukocyte responses showed that the rank order of cell responsiveness to 5-oxo-ETE in the Ca^{2+} flux assay was eosinophils $>$ monocytes $>$ neutrophils $\gg$ basophils (Fig 2, B). Because of their hydrophobicity, lipid mediators can accumulate in the cell membrane and hence cause perturbations of ion channel and receptor function. The presence of 5-oxo-ETE (300 nmol/L), however, did not alter the eotaxin-induced Ca^{2+} flux in basophils ($n = 4$; data not shown). Elevation of intracellular free Ca^{2+} concentrations is also an indispensable signal for mediator release, such as histamine.²³ C5a (10 nM) is a very effective inducer of Ca^{2+} mobilization¹⁹ and released $60.4 \pm 4.3\%$ of the total histamine content of basophils primed with 300 pmol/L of IL-3 ($n = 6$). In contrast, the 5-oxo-ETE-induced histamine release (12 ± 1.6 and $10 \pm 1.2\%$ at 30 and 300 nmol/L of 5-oxo-ETE, respectively) did not exceed spontaneous histamine release ($12 \pm 1.8\%$; $n = 6$), which corresponded to the limited efficacy of 5-oxo-ETE at mobilizing Ca^{2+} .

Basophil chemotaxis to 5-oxo-ETE was investigated by using mononuclear cell preparations, which contain 1% to 3% basophils. The cells were collected from the lower wells, and basophils were enumerated by flow cytometry as $\text{CD123}^+ \text{HLA-DR}^-$ cells¹⁸ (Fig 3, A). 5-Oxo-ETE increased the number of basophils in the bottom wells, reaching a maximum at 30 nmol/L (Fig 3, B), whereas higher concentrations had smaller effects. The magnitude of the response to 5-oxo-ETE was similar to that of MCP-1 or eotaxin. Basophils did not migrate if 5-oxo-ETE was present in the top wells, which suggests that 5-oxo-ETE is chemotactic rather than chemokinetic for basophils (Fig 3, B). To compare the sensitivity to 5-oxo-ETE of basophils with that of eosinophils and neutrophils, we performed analogous chemotaxis assays with polymorphonuclear cell preparations, which contained mainly neutrophils and 2% to 8% eosinophils. To allow the direct comparison of basophil, eosinophil, and neutrophil responsiveness, we expressed their responses to 5-oxo-ETE relative to their maximal responses to adequate control stimuli, eotaxin (3-30 nmol/L) for basophils and eosinophils, and IL-8 (3-30 nmol/L) for neutrophils. Although 5-oxo-ETE induced effective migration of all 3 cell types, the rank order of cell sensitivity to 5-oxo-ETE was basophils $\geq$ eosinophils $>$ neutrophils (Fig 3, C). To rule out

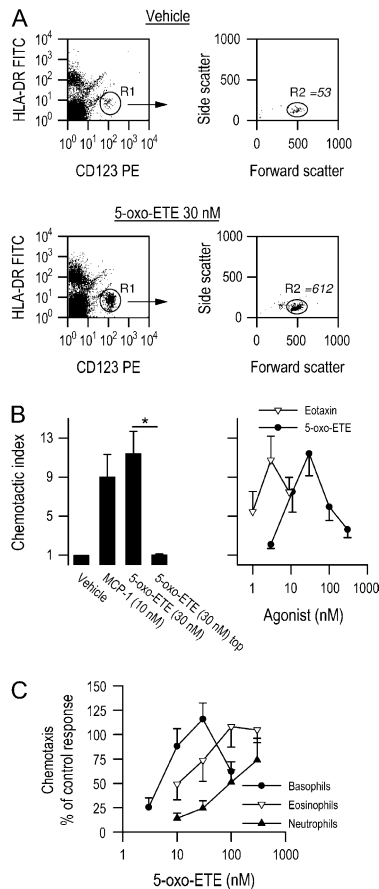


FIG 3. Effects of 5-oxo-EETE on basophil chemotaxis. Aliquots of basophil-containing mononuclear cells were loaded into Transwell inserts and were allowed to migrate toward chemoattractants. Basophils were enumerated by flow cytometry as CD123⁺ and HLA-DR⁺ cells (R1) and by forward scatter/side scatter gating (R2). **A**, A representative plot is shown. **B**, 5-Oxo-EETE-induced migration of basophils was compared with MCP-1 and eotaxin. Responses were expressed relative to spontaneous migration (chemotactic index); $n = 6-10$; $*P < .05$. **C**, Chemotactic responses of basophils, eosinophils, and neutrophils to 5-oxo-EETE were expressed as percent of the maximum response to eotaxin (basophils and eosinophils) or IL-8 (neutrophils); $n = 5-10$. PE, Phycoerythrin.

the possibility that the basophil chemotactic response to 5-oxo-EETE was modulated by other cell types present in the mononuclear preparations, we purified the basophils to near homogeneity. Purified basophils migrated to 5-oxo-EETE with the same concentration response-relationship as in the mixed cell preparation (Fig 4, A). Incubation with pertussis toxin (10 μ g/mL) for 60 minutes at 37°C enhanced the spontaneous migration of basophils but abolished their chemotactic response to 5-oxo-EETE (Fig 4, A) and also to eotaxin (3 nmol/L; $n = 5$; data not shown). These data suggest that 5-oxo-EETE is a potent chemoattractant for basophils acting through a pertussis toxin-sensitive G protein-coupled receptor.

We next investigated whether basophils expressed mRNA for OXE, the 5-oxo-EETE receptor,^{24,25} using real-time PCR. In fact, we could detect OXE mRNA in all 5 samples of isolated basophils investigated (Fig 4,

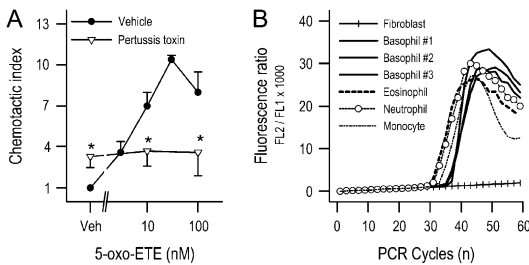


FIG 4. Basophils express mRNA for OXE, the 5-oxo-EETE receptor, which is sensitive to pertussis toxin. **A**, Chemotactic responses of purified basophils was expressed relative to spontaneous migration (chemotactic index). $*P < .05$ pertussis toxin vs vehicle (Veh); $n = 6-10$. **B**, Representative real-time PCR tracings for OXE mRNA in leukocytes are shown as fluorescence ratio of the FL2 and FL1 channels vs cycle number, with fibroblasts as negative control.

TABLE I. Expression of the mRNA for the 5-oxo-EETE receptor, OXE, in human tissues of the upper respiratory tract*

Tissue	Patient number	OXE mRNA
Palatine tonsil	1	47.1
	2	6.7
	3	6.0
	4	4.2
	5	ND
Nasal mucosa (sinusitis)	6	61.5
	7	8.5
	8	ND
Adenoid	1	8.3
	9	11.0

ND, Not detectable.

*Expression levels of 5-oxo-EETE receptor mRNA were quantified as described in Methods and expressed relative to G3PDH mRNA. Units shown are OXE $\times 1000$ /G3PDH.

B), and also in monocytes ($n = 3$), eosinophils ($n = 6$), and neutrophils ($n = 2$). The specificity of the primers and the PCR reaction was demonstrated by the fact that no increases in fluorescence were observed in the presence of cDNA from 2 different preparations of human fibroblasts (Fig 4, B) or in the absence of cDNA (data not shown). Moreover, we found that OXE mRNA was expressed in tissues of the upper respiratory tract. In detail, we could detect OXE mRNA in 4 out of 5 specimens of palatine tonsil, in 2 out of 3 adenoid specimens, and in both specimens of sinus mucosa investigated (Table I).

DISCUSSION

We observed that 5-oxo-EETE is a highly effective and potent chemotactic factor for human basophils, and the mRNA for the receptor of 5-oxo-EETE is expressed by basophils. Cytoskeletal rearrangement is one of the first events after stimulation of leukocytes by chemoattractants.^{13,15} 5-Oxo-EETE potentially induced shape change of basophils, and as estimated from the half-maximally

effective concentrations, the rank order of cell sensitivity to 5-oxo-EETE was basophils = eosinophils > neutrophils >> monocytes. The capacity of agonists to induce elevations of intracellular free Ca^{2+} levels corresponds with their efficacy as secretagogues: although cross-linking of IgE or C5a elicit profound Ca^{2+} flux and histamine release in basophils, eotaxin is of lower potency at inducing Ca^{2+} flux and does not cause histamine release.^{19,26} Similarly, 5-oxo-EETE elicited Ca^{2+} flux only at very high concentrations (300 nmol/L) and did not induce histamine release in basophils, which is in agreement with a recent study showing that the eicosanoid did not cause the upregulation of the granule-associated marker CD63 and was a weak inducer of CD11b and CD203c in basophils.²⁷ The rank order of cell sensitivity to 5-oxo-EETE in Ca^{2+} flux assays was eosinophils > monocytes > neutrophils >> basophils. In contrast with our data, Sozzani et al³ did not observe appreciable Ca^{2+} responses in monocytes, although monocytes showed actin polymerization and migrated toward 5-oxo-EETE. Ca^{2+} signals are indispensable for degranulation, but it is less the initial Ca^{2+} mobilization from intracellular sources than the sustained second phase of Ca^{2+} influx from the extracellular space, which is important for granule exocytosis.²⁸ In contrast, chemotaxis is Ca^{2+} -independent.²⁹ Consequently, 5-oxo-EETE induced the migration of basophils, and this was a result of chemotaxis rather than chemokinesis, because 5-oxo-EETE did not induce migration when added directly to the cells. The effect of 5-oxo-EETE on basophil migration was not mediated by the release of a chemotactic factor from other cells such as monocytes, because it was also observed in purified basophils. 5-Oxo-EETE was as effective as eotaxin, which is the most potent basophil chemoattractant known so far,³⁰ although 3-fold to 10-fold higher concentrations of 5-oxo-EETE were required. In chemotaxis, basophils and eosinophils showed similar sensitivity toward 5-oxo-EETE, whereas neutrophils were less sensitive. These data hence suggest that the primary role of 5-oxo-EETE in the regulation of basophil function is their recruitment to, rather than their activation at, the site of inflammation.

The receptor for 5-oxo-EETE, termed OXE, belongs to the family of 7-transmembrane domain G protein-coupled receptors.^{24,25} In keeping with this, we found that pertussis toxin abrogated the chemotactic response of basophils to 5-oxo-EETE. By using real-time PCR, we could detect the expression of OXE mRNA in all of the 5 samples of isolated basophils investigated, similar to results with monocytes, eosinophils, and neutrophils. In healthy human tissue, OXE mRNA has been detected in kidney and liver but not in any of the other tissues examined.²⁵ We have investigated whether OXE mRNA is expressed in tissues of the upper respiratory tract under pathological conditions, that is, tissues removed because of chronic inflammation. OXE mRNA was detectable in 4 out of 5 specimens of palatine tonsil, in 2 out of 3 adenoid specimens, and in both specimens of sinus mucosa investigated. The fact that not all specimens contained detectable amounts of OXE mRNA suggests that it is the infiltrating

leukocytes rather than tissue resident cells that express the 5-oxo-EETE receptor. This in turn might hint toward a role of OXE in the tissue recruitment of inflammatory cells.

Sparse information is available to date on the *in vivo* effects of 5-oxo-EETE. Intratracheal administration of 5-oxo-EETE induces pulmonary eosinophilia in rats,³¹ whereas intradermal injection causes the infiltration of eosinophils and, at higher doses, also neutrophils into human skin.³² Our current observations that human basophils express OXE mRNA and are equally or even more sensitive to 5-oxo-EETE in chemotaxis assays than eosinophils suggest that the eicosanoid might induce basophil recruitment *in vivo*. Moreover, 5-oxo-EETE causes the contraction of guinea pig airway smooth muscle, pointing at a potential bronchoconstrictor effect *in vivo*.³³ Inflammatory cells, including neutrophils, monocytes, lymphocytes, macrophages, and dendritic cells, are able to produce substantial amounts of 5-oxo-EETE,^{1,2,34} and oxidative stress is a major stimulus for its release.¹ Therefore, 5-oxo-EETE-induced bronchoconstriction and recruitment of eosinophils and basophils to the tissue might be important pathogenic mechanisms in asthma and allergic inflammation.

Memorial: The authors wish to say farewell to Dr Rainer Amann, a wonderful friend and brilliant scientist, who unexpectedly passed away on May 17, 2005. We will miss Rainer ever so much.

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