

5-Lipoxygenase products regulate basophil functions: 5-Oxo-ETE elicits migration, and leukotriene B₄ induces degranulation

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Background: 5-Lipoxygenase (5-LO) products have been strongly implicated in the pathogenesis of allergic diseases. In addition to their physiologic effects on residential cells, 5-LO products are capable of stimulating various eosinophil functions. However, little is known regarding the effects of 5-LO products on basophil functions.

Objective: This study was designed to elucidate the effects of the main 5-LO products (ie, leukotriene [LT] B₄, LTD₄, and 5-oxo-6,8,11,14-eicosatetraenoic acid [5-oxo-ETE]), as well as their receptor expression on human basophils.

Methods: We studied the effects of 5-LO products on Ca²⁺ mobilization, migration, CD11b expression, and degranulation of human basophils. Expression of the receptors for LTC₄/D₄/E₄ (cysteinyl leukotriene 1 [CysLT₁] and CysLT₂), LTB₄ (BLT₁ and BLT₂), and 5-oxo-ETE (oxoeicosanoid [OXE]) was assessed by means of real-time PCR and flow cytometry.

Results: At the mRNA level, basophils strongly expressed OXE and predominantly expressed CysLT₁ and BLT₂. The expression level of OXE mRNA in basophils was approximately 20-fold higher than in neutrophils and similar to that in eosinophils. At the protein level, basophils expressed CysLT₁, CysLT₂, BLT₁, and OXE, but not BLT₂. All products elicited a transient increase of cytosolic calcium, with the order of magnitude being LTB₄ > 5-oxo-ETE > LTD₄. 5-Oxo-ETE induced a strong basophil migratory response that was almost equivalent to that of prostaglandin D₂. LTB₄ elicited significant degranulation of IL-3–primed basophils. In contrast, no functional significance was observed for LTD₄.

Conclusion: Among 5-LO products, 5-oxo-ETE induces a potent basophil migratory response, and LTB₄ elicits degranulation under certain conditions. Our results strongly suggest that 5-oxo-ETE might afford opportunities for

therapeutic targeting in allergic inflammation. (*J Allergy Clin Immunol* 2005;116:578-85.)

Key words: Basophils, leukotriene D₄, leukotriene B₄, 5-oxo-6,8,11,14-eicosatetraenoic acid, receptor, CD11b, chemotaxis, degranulation, Ca²⁺ influx, human

Basophils comprise only 0.5% to 1% of peripheral leukocytes. However, they possess FcεRI on their surface, and cross-linking of FcεRI by specific allergens leads to the release of various types of proinflammatory mediators, including histamine. Earlier studies demonstrated increased numbers of basophilic cells in exudates from the upper¹ and lower² airways and in skin³ after antigen challenge of allergic patients. Furthermore, by using basophil-specific mAbs, more recent studies clearly confirmed basophilic infiltration in allergic inflammation of various organs.^{4,5} It is now recognized that basophils belong to a class of allergic inflammatory cells that migrate from the blood compartment to inflammatory sites, where they act as effector cells for late-phase reactions in concert with eosinophils.⁶

Metabolism of arachidonic acid through the 5-lipoxygenase (5-LO) pathway leads to the formation of 5-hydroperoxyeicosatetraenoic acids, which are further transformed to leukotriene (LT) A₄ and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE). Although 5-oxo-ETE is a stable metabolite, LTA₄ is unstable and is converted by secondary enzymes into LTB₄ and cysteinyl LTC₄. The latter is further converted into the stable metabolites LTD₄ and LTE₄.⁷ The biologic functions of these eicosanoids are mediated by G protein–coupled 7-transmembrane domain receptors. To date, 4 types of receptors for 5-LO products have been cloned, which include cysteinyl leukotriene 1 (CysLT₁) and CysLT₂ for LTC₄/D₄/E₄ and BLT₁ and BLT₂ for LTB₄.⁷ Furthermore, a putative receptor for 5-oxo-ETE has recently been cloned^{8,9} and designated oxoeicosanoid (OXE) receptor.¹⁰

5-LO products have been strongly implicated in the pathogenesis of allergic diseases: their pharmacologic actions include constricting smooth muscle, enhancing mucus secretion, and increasing vascular permeability, and they are strongly involved in the clinical symptoms of allergic disorders.⁷ In addition to these potent physiologic

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Supported by a grant from the Ministry of Education, Science, Sports and Culture of Japan, and grants-in-aid from the Ministry of Health, Labor and Welfare of Japan.

Received for publication August 16, 2004; revised April 19, 2005; accepted for publication April 21, 2005.

Available online June 30, 2005.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2005.04.029

Abbreviations used

CysLT:	Cysteinyl leukotriene
ED ₅₀ :	Median effective dose
5-LO:	5-Lipoxygenase
LT:	Leukotriene
MACS:	Magnetic cell sorting
OXE:	Oxoeicosanoid
5-Oxo-ETE:	5-Oxo-6,8,11,14-eicosatetraenoic acid
PAF:	Platelet-activating factor
PG:	Prostaglandin

effects on residential cells, recent studies have demonstrated that 5-LO products are capable of directly stimulating allergic inflammatory leukocytes (ie, eosinophils). LTB₄, known as a potent activator of neutrophils, has also been shown to stimulate chemotaxis^{11,12} and CD11b expression¹² in eosinophils. In addition, we and others have demonstrated that eosinophils migrate,^{13,14} adhere,¹⁵ and degranulate¹⁴ in response to LTD₄. Furthermore, several groups have demonstrated that 5-oxo-ETE strongly stimulates eosinophil chemotaxis¹⁶⁻¹⁸ and modulates adhesion molecule expression¹² and degranulation.¹⁸ The effects of 5-LO products on eosinophils have thus become increasingly understood, but their effects on basophils have not been studied to date.

In the present study we have explored the effects of 5-LO products on basophil functions for the first time. We studied the expression of receptors, as well as the effects of the principal 5-LO products (ie, LTB₄, LTD₄, and 5-oxo-ETE) on Ca²⁺ mobilization, migration, degranulation, and CD11b expression in human basophils. The effects of 5-LO products were compared with those of 2 established basophil-activating lipid mediators, prostaglandin (PG) D₂ and platelet-activating factor (PAF).

METHODS

Reagents

LTB₄, 5-oxo-ETE, PGD₂, and PAF C-16 were purchased from Cayman Chemical (Ann Arbor, Mich). LTD₄ was purchased from Biomol Research Laboratory (Plymouth Meeting, Pa). These lipid mediators were dissolved in dimethyl sulfoxide at a concentration of 1.5 mmol/L as stock solutions. Other batches of PAF, C-18 (Cayman Chemical) and C-16 (Biomol), were also used in some experiments. Human recombinant eotaxin/CC chemokine ligand 11 was purchased from PeproTech (London, United Kingdom). Rabbit anti-CysLT₂ antibody and anti-BLT₂ antibody were purchased from Cayman Chemical. Goat anti-OXE antibody was purchased from Abcam (Cambridge, Mass). The other reagents used in the experiments were exactly the same as described previously.^{14,19,20}

Cell preparation

Leukocytes were isolated from venous blood obtained from consenting volunteers with no history of atopic disease. In most experiments basophils were semipurified by means of Percoll density centrifugation.²⁰ The purity of Percoll-separated basophil preparations was 11.5% ± 1.0% (n = 50). A vast majority of the contaminating cells were lymphocytes, and the preparations usually contained

no eosinophils or neutrophils. For some experiments, Percoll-separated basophils were further purified by means of negative selection with magnetic cell sorting (MACS) beads (Basophil Isolation Kit; Miltenyi BioTech, Belgisch-Gladbach, Germany), as previously described.²⁰ The recovery rates of basophils after Percoll and after Percoll plus MACS procedures were approximately 60% and 30%, respectively. Eosinophils were purified by means of density gradient centrifugation, followed by negative selection with anti-CD16-bound micromagnetic beads (Miltenyi BioTech), as previously described.²¹ Neutrophils were isolated by means of density gradient centrifugation, as previously described.²¹

Real-time quantitative PCR analysis for receptors of 5-LO products

Real-time quantitative PCR analysis was performed as previously described.²¹ In brief, total RNA was extracted from MACS-separated basophils (purity, 98.5% ± 0.5%), eosinophils (purity, 99.2% ± 0.6%), or neutrophils (purity, 98.7% ± 0.7%) by using a QIAGEN RNeasy Mini Kit (Qiagen, Hilden, Germany). Real-time PCR was performed with the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, Calif). Gene-specific primers and probes were purchased from PE Applied Biosystems. The primers and probes for CysLT₁ (5'-TGGATGAAACAGGAAATCTGACAGT, 5'-TGGATGAAACAGGAAATCTGACAGT, and 5'-FAM-CTGCCACATGCCATGACACTATTGTATGA-3'-TAMRA), CysLT₂ (5'-TATTTCTCTGACCGTGCTGAGTGT, 5'-TGACATGCAGAAGCCGAAAG, and 5'-FAM-TGCGTTTCTGGCAATGTGTTACAC-3'-TAMRA), BLT₁ (5'-CCTCGCGTACCGCACAGTA, 5'-GGGTACCGCGGGAAGC, and 5'-FAM-TGCCCTGGAAAACGAACATGAGCC-3'-TAMRA), and BLT₂ (5'-GCCTTCTTCAGTTCTAGCGTCAA, 5'-AAGAGCCGCGTGAGGAAAC, and 5'-FAM-CCGGTGCTCTACGTCTTACCGCT-3'-TAMRA) were designed according to the published sequences. The primers and probes for OXE were designed by PE Applied Biosystems. A standard curve was constructed with serial dilutions of specific PCR products, which were obtained by amplifying peripheral leukocyte cDNA, as previously described.²¹

Flow cytometric analysis

Purified basophils were washed in PBS supplemented with 3% FCS and 0.1% NaN₃, preincubated with 5 mg/mL human IgG (Yoshitomi Pharmaceuticals, Osaka, Japan), and then incubated with anti-5-LO receptor antibody (CysLT₂, 20 µg/mL; OXE, 20 µg/mL; BLT₂, 20 µg/mL) for 60 minutes at 4°C. Rabbit IgG (DAKO, Glostrup, Denmark) or goat IgG (Sigma Chemical Co, St Louis, Mo) was used as a negative control. After washing, the cells were stained with FITC-conjugated F(ab')₂ fragment anti-rabbit or anti-goat IgG (Jackson ImmunoResearch, West Grove, Pa) at 10 µg/mL for 30 minutes. For staining of BLT₁, cells were stained with FITC-conjugated mouse anti-BLT₁ mAb (R&D, Minneapolis, Minn) or control IgG1 for 30 minutes. Because anti-CysLT₁ antibody recognizes the intracellular domain of this receptor, cells were fixed with 4% paraformaldehyde in PBS for 30 minutes and permeabilized with 0.1% Tween 20 in PBS for 30 minutes at 4°C. The cells were then incubated with anti-CysLT₁ antibody (20 µg/mL) or control rabbit IgG for 60 minutes and stained with FITC-conjugated F(ab')₂ fragment anti-rabbit IgG at 10 µg/mL for 30 minutes. Stained cells were analyzed by means of flow cytometry with an EPICS XL System II (Coulter, Miami, Fla).

CD11b expression experiments were performed with Percoll-separated basophils, as previously described.¹⁹ Treated cells were incubated with phycoerythrin-conjugated anti-CD11b mAb (10 µg/mL) and biotinylated anti-FcεRIα mAb (5 µg/mL). The cells were

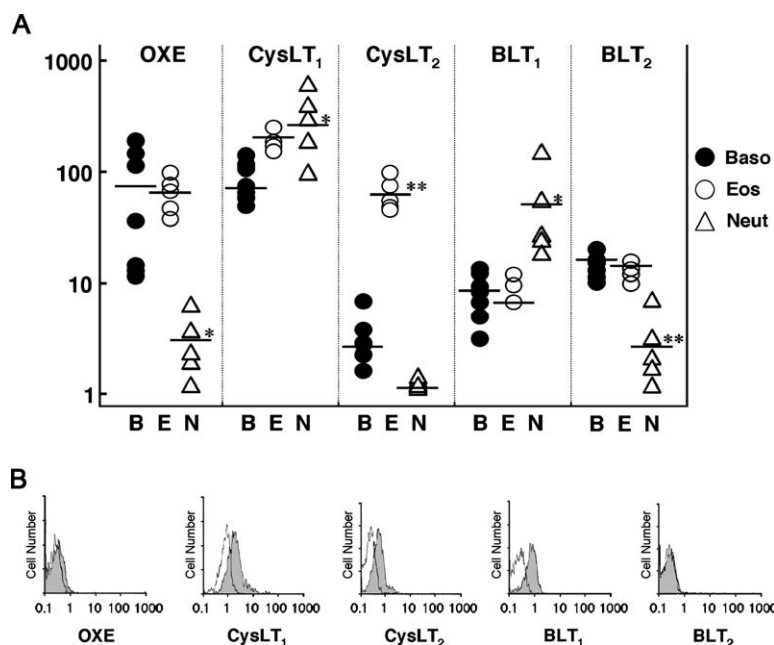


FIG 1. A, Quantitative analysis of transcripts of receptors for 5-LO products in basophils (B, filled circles, n = 7), eosinophils (E, open circles, n = 5), and neutrophils (N, open triangles, n = 5). Transcripts were quantified by means of real-time PCR. The data are expressed as a ratio: copy number of receptor gene/copy number of β -actin gene $\times 100,000$. Horizontal bars represent mean values. **P < .01 and *P < .05 versus basophils. **B**, 5-LO receptor protein expression in basophils. Purified basophils were stained with anti-5-LO receptor antibody and analyzed by means of flow cytometry. The nonshaded area shows the fluorescence of cells stained with isotype-matched antibody. The data are representative of 3 independent experiments, and the others showed similar results.

further stained with FITC-conjugated streptavidin (10 μ g/mL) and analyzed by means of flow cytometry. The median values of fluorescence intensity were converted to the numbers of molecules of equivalent soluble fluorochrome units, as previously described.¹⁹ Surface receptor levels were semiquantified by using the following formula:

$$\Delta \text{MESF} = (\text{MESF of cells stained with anti-CD11b mAb}) - (\text{MESF of cells stained with control IgG}).$$

Ca²⁺ influx

Ca²⁺ influx was determined as previously described.²⁰ In brief, MACS-separated basophils (purity, 98.5% $\pm$ 0.7%) were labeled with Fura-2 AM, and Ca²⁺ influx was measured on the basis of the ratio of the fluorescence at 340 nm to that at 380 nm on a Hitachi F-2500 fluorescence spectrometer (Hitachi Ltd, Tokyo, Japan). The calibration of the signal was performed for each sample by adding 0.1% Triton X-100 to obtain the maximal fluorescence, followed by addition of 10 mM ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid to obtain the minimal fluorescence.

Chemotaxis

Basophil chemotaxis was performed with Percoll-separated basophils and chemotactic cell (Kurabo, Osaka, Japan), as previously described.²⁰ After incubation for 2 hours at 37°C, the cells, which had migrated into the lower chamber, were collected, stained with FITC-conjugated goat anti-human IgE (Biosource, Camarillo, Calif), and counted by means of flow cytometry. Migration was expressed as a percentage of the inoculated cells after subtracting the spontaneous migration.

Degranulation

Basophil degranulation experiments were performed with Percoll-separated basophils, as previously described.²⁰ The released histamine was measured by using an automated fluorometric technique. Histamine release was expressed as a percentage of the total cellular histamine after subtracting the spontaneous release (consistently <2%). Enhancement of histamine release was calculated by using the following formula:

$$\begin{aligned} \% \text{ Enhancement of histamine release} \\ = 100 \times (\text{Histamine released by the stimulus plus reagent} \\ - \text{Histamine released by the stimulus}) / \text{Total histamine content}.^{20} \end{aligned}$$

Statistics

All data are expressed as the mean $\pm$ SEM. Unless otherwise indicated, differences between values were analyzed with the 1-way ANOVA test. When this test indicated a significant difference, the Fisher protected least significant difference test was used to compare individual groups.

RESULTS

Expression of mRNA for receptors of 5-LO products in granulocytes

In our first series of experiments, we studied the expression of mRNA for receptors of 5-LO products in basophils in comparison with that in eosinophils and neutrophils. Cells were purified to apparent homogeneity (>98.5%), and the expression levels of transcripts of

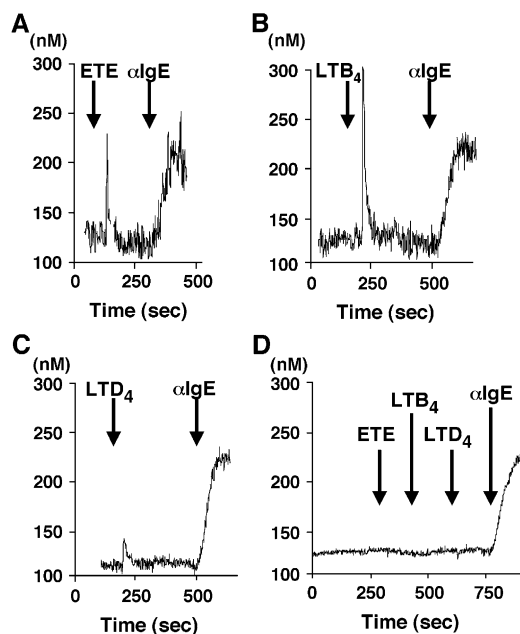


FIG 2. A transient increase of $[Ca^{2+}]^i$ by 5-oxo-EETE, LTB_4 , and LTD_4 . Highly purified basophils were stimulated with an agonist ($1 \mu\text{mol/L}$) or anti-IgE ($\times 1500$) at the time points indicated by arrows (A, B, and C). D, Basophils were pretreated with pertussis toxin (100 ng/mL) at 37°C for 2 hours and stimulated. The data shown are representative of 2 independent analyses from separate donors, with another showing similar results.

CysLT₁, CysLT₂, BLT₁, BLT₂, and OXE were quantified by means of real-time PCR (Fig 1, A). Basophils expressed CysLT₁ mRNA at a level almost equivalent to that of eosinophils. On the other hand, although eosinophils expressed CysLT₂ transcripts, CysLT₂ transcripts were hardly detected in basophils or neutrophils. BLT₁ was strongly expressed in neutrophils but at significantly lower levels in basophils and eosinophils. In contrast, BLT₂ was predominantly expressed in basophils and eosinophils. Importantly, a considerable level of OXE transcripts was found in basophils: judging from the copy number ratio against β -actin, the expression level of OXE mRNA in basophils was approximately 20-fold higher than in neutrophils and similar to that seen in eosinophils.

Protein expression of receptors for 5-LO products in basophils

In agreement with their mRNA transcripts, basophils expressed CysLT₁, BLT₁, and OXE at the protein level (Fig 1, B). Despite the low mRNA expression, apparent surface expression of CysLT₂ was detected on basophils. However, we did not detect surface BLT₂ expression on basophils, which was in clear contrast to the apparent mRNA expression, although surface BLT₂ expression was detected on eosinophils (data not shown).

Effects of 5-LO products on cytosolic Ca^{2+} mobilization of basophils

Basophils were purified to apparent homogeneity ($>98\%$), and cytosolic Ca^{2+} mobilization was examined

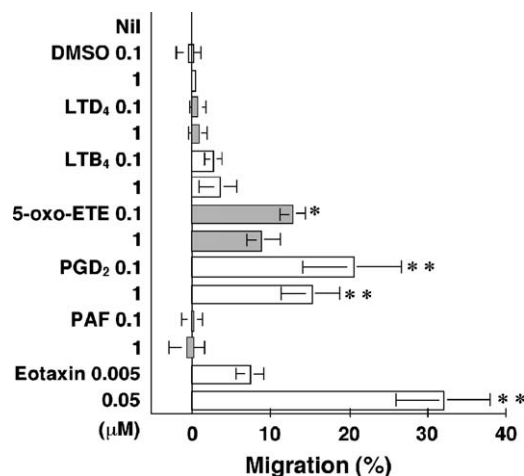


FIG 3. Effects of 5-LO products on basophil migration. Percoll-separated basophils were stimulated with 5-oxo-EETE, LTB_4 , LTD_4 , PGD_2 , PAF, or eotaxin. Control migration in the absence of any agonist (Nil) was $6.0\% \pm 0.8\%$. Bars represent the SEM (n = approximately 5-8). ** $P < .01$ and * $P < .05$ versus Nil.

by means of fluorescent spectrophotometry of Fura-2 fluorescence. As shown in Fig 2, A through C, stimulation of basophils with LTB_4 , LTD_4 , and 5-oxo-EETE resulted in a transient increase of $[Ca^{2+}]^i$, with the order of magnitude being $LTB_4 > 5\text{-oxo-EETE} > LTD_4$, and both LTB_4 - and 5-oxo-EETE-induced Ca^{2+} mobilization was more potent than that induced by PAF (data not shown). In cells stimulated with these 5-LO products, free cytosolic calcium increased rapidly within 1 second after stimulation and then decreased rapidly to the baseline level without any apparent sustained phase. The pattern of calcium mobilization by these 5-LO products was identical with that reported in cells stimulated with PGD_2 .²² Furthermore, Gi-class G proteins were involved in the 5-LO product-induced increase of $[Ca^{2+}]^i$. The 5-LO product-induced increase in the free cytosolic calcium level was completely attenuated by pretreatment of cells with pertussis toxin (100 ng/mL), although these cells responded to the positive control anti-IgE antibody (Fig 2, D).

Effects of 5-LO products on migration of basophils

Using Percoll-separated basophils, we studied the effects of 5-LO products on basophil migration, and the results are depicted in Fig 3. Consistent with previous reports by us²² and others,²³ a cyclooxygenase product, PGD_2 , induced potent migratory responses in basophils. Among 5-LO products, 5-oxo-EETE induced a strong basophil migratory response. LTB_4 failed to elicit statistically significant migration of basophils, although weak migration was observed in some subjects (2 of the total 8 subjects). On the other hand, LTD_4 showed no effects on basophil migration. Treatment of basophils with a priming cytokine, IL-3, greatly enhanced spontaneous migration to the lower wells, but IL-3 did not enhance the migratory response to these 5-LO products (data not shown).

TABLE I. Effects of 5-LO products on CD11b expression of basophils

Stimulus		Stimulus	
DMSO	100.0 ± 0.0	Nil	100.0 ± 0.0
5-oxo-EETE	107.9 ± 2.6	IL-3	248.7 ± 16.4*
LTD ₄	104.4 ± 1.8		
LTB ₄	111.4 ± 3.2		
PGD ₂	120.3 ± 5.8†		
PAF	111.6 ± 2.2		

Percoll-separated basophils were incubated with several 5-LO products (1 μmol/L) or PAF (1 μmol/L), IL-3 (300 pmol/L), or vehicle (dimethyl sulfoxide or Nil) at 37°C for 30 minutes. The CD11b levels shown were calculated as percentages of molecules of equivalent soluble fluorochrome units of control cells. The baseline molecules of equivalent soluble fluorochrome units of control preparations were 183,920 ± 20,490 and 197,209 ± 18,378 for DMSO-treated cells and nontreated cells (Nil), respectively (n = 4).

DMSO, Dimethyl sulfoxide.

**P* < .01 and †*P* < .05 versus control.

Concentration-response experiments revealed that 5-oxo-EETE showed a typical bell-shaped curve, with the median effective dose (ED₅₀) being between 10⁻⁸ and 10⁻⁷ mol/L. When compared with PGD₂, the effects of 5-oxo-EETE were almost equiefficient but slightly less potent (n = 4, data not shown). 5-Oxo-EETE-mediated basophil chemotaxis was attributed to a direct effect on basophils and not to an indirect one by contaminating cells. When the migratory responses were simultaneously studied in MACS-separated basophils (purity, 99.0% ± 0.6%) and Percoll-separated basophils (purity, 19.5% ± 5.1%), 5-oxo-EETE (3 × 10⁻⁷ mol/L) induced significant migration, even of the highly-purified basophils (9.2% ± 3.4% and 9.7% ± 0.7% for Percoll- and MACS-separated cells, respectively; n = 4). Furthermore, checkerboard analyses revealed that 5-oxo-EETE-induced migration was chemotactic, as well as chemokinetic. When the gradient was eliminated by adding the same concentration of 5-oxo-EETE (10⁻⁷ mol/L) to both the upper and lower wells, the basophil migration was significantly but not completely inhibited (42.0% ± 8.8% of the control; *P* < .01, paired *t* test; n = 4).

Effects of 5-LO products on CD11b expression of basophils

Percoll-separated basophils were used to study the effects of 5-LO products on basophil CD11b expression (Table I). Consistent with previous reports,^{22,24} CD11b expression was upregulated markedly by IL-3 (148.7% ± 16.4% above control value, *P* < .01) and slightly by PGD₂ (20.3% ± 5.8% above control value, *P* < .05). In contrast, none of the 5-LO products or PAF stimulated CD11b expression at all.

Effects of 5-LO products on degranulation of basophils

Using Percoll-separated basophils, we studied the effects of 5-LO products on basophil degranulation. As shown in Table II, freshly isolated basophils did not

TABLE II. Induction of histamine release by 5-LO products

Stimulus	Nonprimed	IL-3 primed
DMSO	0.0 ± 0.0	0.6 ± 0.2
5-Oxo-EETE	0.2 ± 0.2	1.7 ± 0.7
LTD ₄	0.3 ± 0.1	0.8 ± 0.4
LTB ₄	0.7 ± 0.6	6.8 ± 1.4*
PGD ₂	0.3 ± 0.1	2.2 ± 1.0
PAF	0.1 ± 0.2	4.7 ± 1.6†
Anti-IgE	18.5 ± 2.3*	35.5 ± 4.1*

Basophils were treated with or without IL-3 (300 pmol/L) for 30 minutes and then stimulated for an additional 45 minutes with 5-LO products or PAF (1 μmol/L), anti-IgE (× 10,000), or vehicle (dimethyl sulfoxide). Data are presented as percentage release, as described in the Methods section. The effects of other batches of PAF were essentially similar to those described in this table.

DMSO, Dimethyl sulfoxide.

**P* < .01 and †*P* < .05 versus vehicle.

significantly degranulate in response to 5-LO products. Because IL-3 is known to modulate basophil histamine release in a quantitative fashion, a qualitative fashion, or both,²⁵ we also tested the effects of 5-LO products on IL-3-treated cells. In line with previous reports by others,²⁶ PAF induced significant degranulation of IL-3-primed basophils. Significant histamine release was also elicited in IL-3-primed basophils by LTB₄ to a slightly greater extent compared with the release induced by various batches of PAF, whereas 5-oxo-EETE and LTD₄ failed to cause significant histamine release, even in these cells (Table II). Concentration-response experiments revealed that LTB₄ showed a sigmoid response curve, with the ED₅₀ being between 10⁻⁸ and 10⁻⁷ mol/L (n = 4, data not shown). Furthermore, LTB₄-induced basophil degranulation was not the result of an indirect effect by contaminating cells. When degranulation was simultaneously studied in MACS-separated basophils (purity, 99.3% ± 0.4%) and Percoll-separated basophils (purity, 14.8% ± 2.8%), LTB₄ (10⁻⁶ mol/L) elicited significant histamine release, even in the highly purified basophils (6.7% ± 1.8% and 7.4% ± 1.2% for Percoll- and MACS-separated cells, respectively; n = 4). Furthermore, we checked the priming ability of 5-LO products on basophil degranulation. Percoll-separated cells were treated with 5-LO products for 30 minutes, and histamine release was initiated by addition of phorbol 12-myristate 13-acetate, ionophore, or anti-IgE antibody. As reported by us²² and others,²⁷ PGD₂ primed basophils for increased releasability in response to phorbol 12-myristate 13-acetate or ionophore. However, none of the 5-LO products showed any priming effects at all (data not shown).

DISCUSSION

To date, numerous reports have demonstrated that 5-LO products are capable of stimulating various eosinophil functions,¹¹⁻¹⁸ but little is known regarding the effects of

5-LO products or other lipid mediators on human basophil functions. Some earlier studies demonstrated that lipid mediators modulate basophil degranulation.^{28,29} Among them, a phospholipid mediator, PAF, is known to induce basophil degranulation,²⁶ migration,³⁰ and adhesion to endothelium.³¹ More recent studies by us and others showed that PGD₂, a cyclooxygenase product of arachidonic acid metabolism, induces potent migratory responses,²³ upregulates CD11b expression,²² and enhances degranulation²² of human basophils through chemoattractant receptor-homologous molecules expressed on T_H2 cells. On the other hand, to our knowledge, the effects of 5-LO products on human basophils have been studied in only one article²⁹ to date. In the present study we have explored the receptor expression profiles and functions of 5-LO products in human basophils for the first time. Real-time PCR experiments indicated that basophils strongly expressed OXE and predominantly expressed CysLT₁ and BLT₂ for CysLT and LTB₄ receptors, respectively. The profile of receptor mRNA expression in basophils was rather similar to that found in eosinophils compared with that found in neutrophils. Furthermore, apparent protein expression of OXE, CysLT₁, CysLT₂, and BLT₁ was confirmed in basophils by means of flow cytometric analysis. Our results clearly showed that OXE expressed in basophils has functional relevance. A high-affinity OXE ligand, 5-oxo-EETE, induced a strong basophil migratory response, with efficacy almost equivalent to that of PGD₂ and higher than that of PAF. The ED₅₀ value of 5-oxo-EETE for basophil migration (10^{-8} to 10^{-7} mol/L) determined by using the Boyden chamber technique was similar to that reported for eosinophil migration assayed with the Boyden chamber technique (2×10^{-8} mol/L).¹⁷ Although 5-oxo-EETE was also shown to be chemoattractive for neutrophils, a comparative study demonstrated that on a molar basis, 5-oxo-EETE elicits a more potent response in eosinophils.¹⁸ The potency of 5-oxo-EETE might be closely related to its expression level in each cell type. In fact, our present results showed that the level of OXE mRNA in eosinophils was approximately 20-fold higher than that in neutrophils and that basophils expressed OXE transcripts at a level similar to that seen in eosinophils. The relatively higher mRNA level of OXE expression strongly suggests that basophils, as well as eosinophils, represent a major target for the action of 5-oxo-EETE.

In contrast to eosinophils, we failed to observe any significant effects of 5-oxo-EETE on CD11b expression or degranulation in basophils. These findings might indicate that the role of OXE in basophils is limited mainly to the migratory response. Nevertheless, because influx of basophils to inflamed tissues represents a characteristic aspect of allergic inflammation,⁶ our findings that 5-oxo-EETE induces basophil migratory responses with high potency and efficacy strongly extend the repertoire of proinflammatory effects of 5-oxo-EETE in allergic inflammation. Recently, Stamatiou et al³¹ reported that 5-oxo-EETE directly stimulated monocytes to release GM-CSF. As a result, 5-oxo-EETE indirectly prolonged the eosinophil lifespan by stimulating monocytes to

liberate an antiapoptogenic cytokine for eosinophils.³² This novel property of 5-oxo-EETE might also be involved in basophil infiltration of allergic inflammatory sites because GM-CSF is also capable of enhancing survival of basophils by blocking their apoptosis.¹⁹

Treatment with a leukotriene receptor antagonist resulted in a significant decrease in the eosinophil count in the induced sputum of asthmatic subjects³³ and in the bronchoalveolar lavage fluid of asthmatic model mice.³⁴ Blockade of chemotactic signals through CysLT on eosinophils^{13,14} or mast cells^{35,36} might be at least partly involved in the reduction of eosinophilic infiltration by CysLT antagonists. Apparent expression of receptors for CysLTs was also identified in basophils. That is, basophils expressed both CysLT₁ and CysLT₂, and an apparent Ca²⁺ influx was induced by the ligands, with the order of potency being LTD₄ > LTC₄ > LTE₄ (data not shown), which suggests the functional dominance of CysLT₁ over CysLT₂. However, our present results failed to show any functional significance for LTD₄ other than calcium mobilization. This lack of LTD₄-induced basophil function suggests that basophils are less sensitive to LTD₄ than eosinophils, which might be due to the relatively low CysLT₂ expression at both the mRNA and protein levels (data not shown) in basophils. On the other hand, basophils expressed functionally relevant receptors for LTB₄, especially BLT₁. Although the expression level of BLT₁ mRNA in basophils was significantly lower than in neutrophils, basophils expressed similar levels of BLT₁ mRNA and protein with those in eosinophils. The neutrophil-chemotactic properties of LTB₄ have been well established, but some reports have demonstrated that LTB₄ also induces migratory responses in eosinophils.^{11,18} In the present study LTB₄ failed to induce statistically significant basophil migration, although it elicited a weak migratory response in a small number of subjects. LTB₄ consistently induced significant degranulation under certain conditions, suggesting that the LTB₄-induced functional outcome might be regulated by cell type-specific mechanisms. LTB₄ failed to induce significant histamine release in freshly isolated basophils, but it liberated significant amounts of histamine from basophils pretreated with IL-3. Although we observed apparent surface BLT₁ expression on basophils by means of flow cytometry, there was no difference in surface BLT₁ levels between nonprimed and IL-3-primed cells (data not shown). Thus stimulation with LTB₄ alone is unable to cause transduction of signals sufficient to evoke degranulation, but IL-3 renders basophils susceptible to stimulation with LTB₄. Inasmuch as IL-3 induces a wide array of changes in basophil functions, a similar situation has been observed for many other types of secretagogues, including PAF,²⁶ C3a,³⁷ and secretory IgA.³⁸ Because basophils at inflammatory sites are highly likely to be primed by IL-3 during ongoing allergic reactions, LTB₄ might contribute at least partly to the pathogenesis of allergic inflammation by promoting degranulation of tissue basophils.

Our results revealed that each of the lipid mediators tested in this study demonstrates a unique and partially

overlapping action on basophils. Although the receptors for lipid mediators belong to the G protein-coupled class, the functional diversity of the lipid mediators strongly suggest that the intracellular signal pathways of the receptors might be complex. Thus detailed analysis of the intracellular events in basophils, as well as in other cell types, such as eosinophils, will be of special interest in future studies.

In summary, we have explored the receptor expression profile and functions of 5-LO products in human basophils for the first time. 5-Oxo-EETE induced a potent migratory response, and LTB₄ elicited degranulation of primed basophils. In combination with previous reports by others,^{11,12,16-18,34,39,40} our results strongly suggest that 5-oxo-EETE might afford opportunities for therapeutic targeting in allergic inflammation.

We thank M. Imanishi and C. Tamura for their skilled technical assistance. Thanks are also extended to S. Takeyama for her excellent secretarial help.

REFERENCES

- Guo CB, Liu MC, Galli SJ, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Identification of IgE-bearing cells in the late-phase response to antigen in the lung as basophils. *Am J Respir Cell Mol Biol* 1994;10:384-90.
- Iliopoulos O, Baroody FM, Naclerio RM, Bochner BS, Kagey SA, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992;148:2223-8.
- Charlesworth EN, Hood AF, Soter NA, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. *J Clin Invest* 1989;83:1519-26.
- Irani AM, Huang C, Xia HZ, Kepley C, Nafie A, Fouda ED, et al. Immunohistochemical detection of human basophils in late-phase skin reactions. *J Allergy Clin Immunol* 1998;101:354-62.
- Ying S, Robinson DS, Meng Q, Barata LT, McEuen AR, Buckley MG, et al. C-C chemokines in allergen-induced late-phase cutaneous responses in atopic subjects: association of eotaxin with early 6-hour eosinophils, and of eotaxin-2 and monocyte chemoattractant protein-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (monocyte chemoattractant protein-3 and RANTES). *J Immunol* 1999;163:3976-84.
- Schroeder JT, Kagey-Sobotka A, Lichtenstein LM. The role of the basophil in allergic inflammation. *Allergy* 1995;50:463-72.
- Brink C, Dahlen SE, Drazen J, Evans JF, Hay DW, Nicosia S, et al. International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. *Pharmacol Rev* 2003;55:195-227.
- Hosoi T, Koguchi Y, Sugikawa E, Chikada A, Ogawa K, Tsuda N, et al. Identification of a novel human eicosanoid receptor coupled to G(i/o). *J Biol Chem* 2002;277:31459-65.
- Jones CE, Holden S, Tenaillon L, Bhatia U, Seuwen K, Tranter P, et al. Expression and characterization of a 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid receptor highly expressed on human eosinophils and neutrophils. *Mol Pharmacol* 2003;63:471-7.
- Brink C, Dahlen SE, Drazen J, Evans JF, Hay DW, Rovati GE, et al. International Union of Pharmacology XLIV. Nomenclature for the oxoeicosanoid receptor. *Pharmacol Rev* 2004;56:149-57.
- Lindsay MA, Haddad EB, Rousell J, Teixeira MM, Hellewell PG, Barnes PJ, et al. Role of the mitogen-activated protein kinases and tyrosine kinases during leukotriene B₄-induced eosinophil activation. *J Leukoc Biol* 1998;64:555-62.
- Powell WS, Gravel S, Halwani F. 5-oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of L-selectin shedding, surface expression of CD11b, actin polymerization, and calcium mobilization in human eosinophils. *Am J Respir Cell Mol Biol* 1999;20:163-70.
- Fregonese L, Silvestri M, Sabatini F, Rossi GA. Cysteinyl leukotrienes induce human eosinophil locomotion and adhesion molecule expression via a CysLT1 receptor-mediated mechanism. *Clin Exp Allergy* 2002;32:745-50.
- Ohshima N, Nagase H, Koshino T, Miyamasu M, Yamaguchi M, Hirai K, et al. A functional study on CysLT1 receptors in human eosinophils. *Int Arch Allergy Immunol* 2002;129:67-75.
- Nagata M, Saito K, Tsuchiya K, Sakamoto Y. Leukotriene D₄ upregulates eosinophil adhesion via the cysteinyl leukotriene 1 receptor. *J Allergy Clin Immunol* 2002;109:676-80.
- Schwenk U, Schroder JM. 5-Oxo-eicosanoids are potent eosinophil chemotactic factors. Functional characterization and structural requirements. *J Biol Chem* 1995;270:15029-36.
- Powell WS, Chung D, Gravel S. 5-Oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of human eosinophil migration. *J Immunol* 1995;154:4123-32.
- O'Flaherty JT, Kuroki M, Nixon AB, Wijkander J, Yee E, Lee SL, et al. 5-Oxo-eicosatetraenoate is a broadly active, eosinophil-selective stimulus for human granulocytes. *J Immunol* 1996;157:336-42.
- Yoshimura-Uchiyama C, Yamaguchi M, Nagase H, Fujisawa T, Ra C, Matsushima K, et al. Comparative effects of basophil-directed growth factors. *Biochem Biophys Res Commun* 2003;302:201-6.
- Iikura M, Miyamasu M, Yamaguchi M, Kawasaki H, Matsushima K, Kitaura M, et al. Chemokine receptors in human basophils: inducible expression of functional CXCR4. *J Leukoc Biol* 2001;70:113-20.
- Nagase H, Okugawa S, Ota Y, Yamaguchi M, Tomizawa H, Matsushima K, et al. Expression and function of Toll-like receptors in eosinophils—activation by Toll-like receptor 7 ligand. *J Immunol* 2003;171:3977-82.
- Yoshimura-Uchiyama C, Iikura M, Yamaguchi M, Nagase H, Ishii A, Matsushima K, et al. Differential modulation of human basophil functions through PGD₂ receptors CRTH2/DP2 and DP1. *Clin Exp Allergy* 2004;34:1283-90.
- Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D₂ selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 2001;193:255-61.
- Bochner BS, McKelvey AA, Sterbinsky SA, Hildreth JE, Derse CP, Klunk DA, et al. IL-3 augments adhesiveness for endothelium and CD11b expression in human basophils but not neutrophils. *J Immunol* 1990;145:1832-7.
- Hirai K, Miyamasu M, Takaishi T, Morita Y. Regulation of the function of eosinophils and basophils. *Crit Rev Immunol* 1997;17:325-52.
- Brunner T, de Weck AL, Dahinden CA. Platelet-activating factor induces mediator release by human basophils primed with IL-3, granulocyte-macrophage colony-stimulating factor, or IL-5. *J Immunol* 1991;147:237-42.
- Peters SP, Kagey-Sobotka A, MacGlashan DW Jr, Lichtenstein LM. Effect of prostaglandin D₂ in modulating histamine release from human basophils. *J Pharmacol Exp Ther* 1984;228:400-6.
- Marone G, Kagey-Sobotka A, Lichtenstein LM. Effects of arachidonic acid and its metabolites on antigen-induced histamine release from human basophils in vitro. *J Immunol* 1979;123:1669-77.
- Peters SP, Siegel MI, Kagey-Sobotka A, Lichtenstein LM. Lipoxygenase products modulate histamine release in human basophils. *Nature* 1981;292:455-7.
- Tanimoto Y, Takahashi K, Kimura I. Effects of cytokines on human basophil chemotaxis. *Clin Exp Allergy* 1992;22:1020-5.
- Bochner BS, Peachell PT, Brown KE, Schleimer RP. Adherence of human basophils to cultured umbilical vein endothelial cells. *J Clin Invest* 1988;81:1355-64.
- Stamatiou PB, Chan CC, Monneret G, Ethier D, Rokach J, Powell WS. 5-Oxo-6,8,11,14-eicosatetraenoic acid stimulates the release of the eosinophil survival factor granulocyte-macrophage colony stimulating factor from monocytes. *J Biol Chem* 2004;279:28159-64.
- Minoguchi K, Kohno Y, Minoguchi H, Kihara N, Sano Y, Yasuhara H, et al. Reduction of eosinophilic inflammation in the airways of patients with asthma using montelukast. *Chest* 2002;121:732-8.
- Eum SY, Maghni K, Hamid Q, Campbell H, Eidelman DH, Martin JG. Involvement of the cysteinyl-leukotrienes in allergen-induced airway eosinophilia and hyperresponsiveness in the mouse. *Am J Respir Cell Mol Biol* 2003;28:25-32.

35. Mellor EA, Maekawa A, Austen KF, Boyce JA. Cysteinyl leukotriene receptor 1 is also a pyrimidinergic receptor and is expressed by human mast cells. *Proc Natl Acad Sci U S A* 2001;98:7964-9.
36. Mellor EA, Frank N, Soler D, Hodge MR, Lora JM, Austen KF, et al. Expression of the type 2 receptor for cysteinyl leukotrienes (CysLT2R) by human mast cells: Functional distinction from CysLT1R. *Proc Natl Acad Sci U S A* 2003;100:11589-93.
37. Bischoff SC, de Weck AL, Dahinden CA. Interleukin 3 and granulocyte/macrophage-colony-stimulating factor render human basophils responsive to low concentrations of complement component C3a. *Proc Natl Acad Sci U S A* 1990;87:6813-7.
38. Iikura M, Yamaguchi M, Fujisawa T, Miyamasu M, Takaishi T, Morita Y, et al. Secretory IgA induces degranulation of IL-3-primed basophils. *J Immunol* 1998;161:1510-5.
39. Muro S, Hamid Q, Olivenstein R, Taha R, Rokach J, Powell WS. 5-oxo-6,8,11,14-eicosatetraenoic acid induces the infiltration of granulocytes into human skin. *J Allergy Clin Immunol* 2003;112:768-74.
40. Stamatiou P, Hamid Q, Taha R, Yu W, Issekutz TB, Rokach J, et al. 5-oxo-EETE induces pulmonary eosinophilia in an integrin-dependent manner in Brown Norway rats. *J Clin Invest* 1998;102:2165-72.

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