Eosinophil Peroxidase-derived Reactive Brominating Species Target the Vinyl Ether Bond of Plasmalogens Generating a Novel Chemoattractant, α-Bromo Fatty Aldehyde*

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Plasmalogens are a subclass of glycerophospholipids that are enriched in the plasma membrane of many mammalian cells. The vinyl ether bond of plasmalogens renders them susceptible to oxidation. Accordingly, it was hypothesized that reactive brominating species, a unique oxidant formed at the sites of eosinophil activation, such as in asthma, might selectively target plasmalogens for oxidation. Here we show that reactive brominating species produced by the eosinophil peroxidase system of activated eosinophils attack the vinyl ether bond of plasmalogens. Reactive brominating species produced by eosinophil peroxidase target the vinyl ether bond of plasmalogens resulting in the production of a neutral lipid and lysophosphatidylcholine. Chromatographic and mass spectrometric analyses of this neutral lipid demonstrated that it was 2-bromohexadecanol (2-BrHDA). Reactive brominating species produced by eosinophil peroxidase attacked the plasmalogen vinyl ether bond at acidic pH. Bromide was the preferred substrate for eosinophil peroxidase, and chloride was not appreciably used even at a 1000-fold molar excess. Furthermore, 2-BrHDA production elicited by eosinophil peroxidase-derived reactive brominating species in the presence of 100 μM NaBr doubled with the addition of 100 mM NaCl. The potential physiological significance of this pathway was suggested by the demonstration that 2-BrHDA was produced by phorbol myristate acetate-stimulated eosinophils and by the demonstration that 2-BrHDA is a phagocyte chemoattractant. Taken together, the present studies demonstrate the targeting of the vinyl ether bond of plasmalogens by the reactive brominating species produced by eosinophil peroxidase and by activated eosinophils, resulting in the production of brominated fatty aldehydes.

Activated phagocytes generate a variety of reactive halogenating species, which attack adjacent cells as a part of the normal physiological defense function of phagocytes (1, 2). Eosinophil peroxidase amplifies the oxidizing potential of hydrogen peroxide by using it as a substrate in the presence of the micromolar concentrations of bromide to produce hypobromous acid and its conjugate base, which are the predominant oxygen-derived free radicals produced by activated eosinophils (3). Through the generation of reactive brominating species and their attack of viral and microbial proteins and membrane structures, eosinophil peroxidase plays a crucial role in the antiviral and antimicrobial defense mechanisms of eosinophils (3). Additionally, eosinophil peroxidase likely plays an important role through the production of reactive brominating species in asthma (4, 5). In fact the involvement of eosinophil peroxidase in asthma has been suggested by the presence of bromotyrosine in the airways and sputum of asthmatics (4–6).

Lipids are major targets of the reactive halogenating species produced by activated phagocytes. Reactive chlorinating and brominating species released by activated phagocytes attack unsaturated -C=C- bonds within the aliphatic chains of phospholipids and within the steroid nucleus of sterols forming chlorohydrins and bromohydrins, which may disrupt normal membrane fluid molecular dynamics (7–11). This mechanism may represent a major cytotoxic effect of activated phagocytes. Reactive brominating species have been shown to target unsaturated fatty acids resulting in the production of bromohydrins that may serve as markers not only of phagocyte-mediated inflammation but also specific inflammatory processes mediated by brominating oxidants (9).

Plasmalogens are a subclass of glycerophospholipids found both in the plasma membrane phospholipid pools of many mammalian tissues and in lung surfactant (12–18). Plasmalogens possess a masked aldehyde, vinyl ether linkage between the sn-1 aliphatic chain and the glycerol backbone and may have an important role in both the solvation of transmembrane ion channels and transport proteins as well as the storage of arachidonic acid (19–22). We have recently demonstrated that plasmalogens represent accessible molecular targets of the membrane-permeable, reactive chlorinating species generated by activated phagocytes resulting in the production of α-chloro fatty aldehydes (23, 24).

Because eosinophil peroxidase has a marked selectivity for the production of hypobromous acid at physiological halide concentrations (5, 25, 26), the present study was designed to
determine whether the vinyl ether bond of the sn-1 aliphatic chain of plasmalogens is susceptible to bromination by reactive brominating species produced by eosinophil peroxidase and to compare this putative halogenation with that mediated by reactive chlorinating species in eosinophils. The results herein demonstrate for the first time that eosinophil peroxidase utilizes micromolar concentrations of bromide at acidic pH resulting in the production of reactive brominating species that attack the vinyl ether bond of plasmalogens leading to the production of lysophospholipids and α-bromo fatty aldehydes. Additionally, these α-bromo fatty aldehydes are produced by PMA-stimulated eosinophils and thus represent a previously uncharacterized product of activated eosinophils. The biological role of α-bromo fatty aldehydes is also suggested by our results showing that they are a phagocyte chemotactrant. Taken together, the present study demonstrates that plasmalogens are attacked by eosinophil peroxidase-derived reactive brominating species and suggests that this may represent a novel mechanism for plasmalogen degradation during either antiviral/anti-microbial roles of eosinophils or, alternatively, during inflammatory tissue injury in eosinophil inflammatory states such as asthma.

EXPERIMENTAL PROCEDURES

Materials—Porcine eosinophil peroxidase (EPO) was isolated according to the method of Jorg (27) employing guanidine hydrochloride as the assay (28). The purity of EPO preparations was assayed before use by demonstrating an RZ of 0.9 (A415/A280, SDS-polyacrylamide gel electrophoresis analysis with Coomasie Blue staining, and in-gel tetramethylbenzidine peroxidase staining to confirm no contaminating myeloperoxidase activity (29). Pentafluorobenzyl hydroxylamine was purchased from Aldrich. Hexadecanoyl chloride was purchased from Nu-Chek Prep. All other reagents and chemicals were purchased from either Aldrich, Sigma, or Fisher.

Preparation of Lysoplasmenylcholine and Plasmenylcholine—The lysoplasmenylcholine molecular species, 1-O-hexadec-1-yl-GPC, was prepared from bovine heart lecithin and purified as described previously (31). Synthetically prepared plasmenylcholine and lysoplasmenylcholine were determined to be greater than 95% pure by thin-layer chromatography, straight-phase HPLC, reversed-phase HPLC, and capillary gas chromatography of the aliphatic constituents. Lysoplasmenylcholine and 16:0-16:0 plasmenylcholine were quantified by capillary gas chromatography.

Plasmalogen Treatment with Eosinophil Peroxidase-derived Reactive Halogenating Species: Analysis of Reaction Products by Thin-layer Chromatography and Capillary Gas Chromatography—In a typical assay, 50–200 nmol of either lysoplasmenylcholine or plasmenylcholine was incubated in 500 μl of phosphate buffer (20 mM NaPO₄) supplemented with the indicated concentrations of NaBr and/or selected concentrations NaCl, 0.1 mM diethylenetriaminepentaacetic acid (pH 4.0–7.0) in the presence or absence of indicated amounts of eosinophil peroxidase and H₂O₂ for indicated intervals at 37 °C. Reactions were terminated by the addition of methanol, and reaction products were extracted into chloroform by the method of Bligh and Dyer (32). Reaction products were separated by TLC utilizing silica gel 60-A plates (Whatman) and a mobile phase comprising petroleum ether/ethyl ether/acetate acid/acetic acid/water (6/8/2/1, v/v/v). Reaction products on TLC plates containing an aldehyde or a masked aldehyde were visualized by charring concentrated sulfuric acid-treated plates and phosphate-containing lipids were detected with mollybdate-containing spray. In some cases, reaction products or TLC-purified reaction products were extracted from silicas by a modified Bligh and Dyer technique (32). TLC-purified reaction products were then subjected to capillary gas chromatography following their derivatization with pentafluorobenzyl hydroxylamine (see below) or methanolic HCl (70 °C for 30 min). In some cases acid methanolation-derivatized products were subjected to capillary gas chromatography utilizing a Supelco Scientific DB-5 column and detected by FID under conditions previously described (15). Alternatively, acid methanolation-derivatized products were subjected to GC-MS with electron impact ionization.

Gas Chromatography-Mass Spectrometric Analyses of Pentafluorobenzylxime Derivative Products—GC-MS was performed on reaction products or TLC-purified reaction products either directly or following derivatization with acidic methanol or pentafluorobenzyl hydroxylamine. In brief, derivatization with pentafluorobenzyl hydroxylamine was performed by resuspending the reaction products in 300 μl of ethanol followed by the addition of 300 μl of 6 mg/ml pentafluorobenzyl hydroxylamine in water. The ethanol-water mixture was vortexed for 5 min at room temperature and allowed to further incubate at room temperature for an additional 25 min. Reaction products were diluted with 1.2 ml of water, extracted into cyclohexane/diethyl ether (4/1, v/v), and resuspended in 30~100 μl of petroleum ether prior to GC-MS analysis. GC-MS analysis of PFB oximes of α-halo fatty aldehydes was performed on a Hewlett Packard (Palo Alto, CA) 5973 mass spectrometer coupled to a Hewlett Packard 6890 gas chromatograph using the negative ion chemical ionization mode with methane as the reagent gas. The source temperature was set at 150 °C. The electron energy was 240 eV, and the emission current was 300 μA. The PFB derivatives were separated on a J&W Scientific (Folsom, CA) DB-1 column (12.5 m, 0.2 mm inner diameter, 0.33-μm film thickness). The injector and the transfer line temperatures were maintained at 250 °C. The GC oven was maintained at 150 °C for 5.5 min, increased at a rate of 25 °C/min to 310 °C, and held at 310 °C for an additional 5 min.

Eosinophil Activation—Whole blood (180 ml) was taken from hyper-sensitive volunteers, and eosinophils were isolated as described previously using a Percoll gradient were subsequently purified using a CD16 immunomagnetic bead sorting system on a Miltenyi MACS column as described previously (33). Purified eosinophils were resuspended in Hanks’ balanced salt solution (pH 7.3) supplemented with 100 μM NaBr and immediately subjected to experimental protocols. Eosinophils (1 x 10⁶ cells/ml) were treated with or without (control) 200 mM PMA for 1 h at 37 °C. Reactions were terminated by snap-freezing in liquid nitrogen, and subsequently lipids were extracted in the presence of internal standard (45 and 15 pmol of 2-Br-[d]-HDA and 2-Cl-[d]-HDA, respectively, for a total of 2.5 x 10⁶ cells extracted). Following Bligh and Dyer (32) extraction of eosinophil lipids, 2-BrHDA and 2-CHDA were quantified following derivatization to its PFB oxime by GC-MS utilizing selected ion monitoring GC-MS (SIM-GC-MS). Neutrophil Chemotaxis—Whole blood (50 ml) was taken from healthy volunteers and anticoagulated with EDTA (final concentration 5.4 mM) prior to the isolation of neutrophils using a Ficol-Hypaque gradient as described previously (23). Pelleted neutrophils were resuspended in chemotaxis buffer comprised of Hanks’ balanced salt solution (pH 7.3), 1% bovine serum albumin (w/v), and 10 mM HEPEs at a final concentration of 4 x 10⁶ neutrophils/ml. Neutrophil chemotaxis assay was assayed as described previously (34). 4MLP, 2-BrHDA in Me₂SO, or Me₂SO controls were diluted in modified Hanks’ balanced salt solution and loaded into the lower compartments of a Boyden chemotaxis chamber, which was separated from the top compartment containing 2 x 10⁶ neutrophils (50 μl) by cellulose nitrate filters (8 μm pore size). The chamber was incubated at 37 °C for 30 min and was then stained and dehydrated, and chemotaxis was assessed by the leading front method as described previously (34). Net migration through the filter was reported in μm.

RESULTS

The present studies were directed at determining the role of eosinophil peroxidase in the targeting of plasmalogens by reactive brominating species. As a first step, lysoplasmenylcholine (1-O-hexadec-1-yl-GPC) was incubated with eosinophil peroxidase in phosphate buffer containing hydrogen peroxide.
and 100 mM sodium bromide. TLC analysis of the reaction products revealed that a neutral lipid was produced, which migrated with an $R_f = 0.58$ in a solvent system that resolves neutral lipids (Fig. 1) when lysoplasmalogen is treated with eosinophil peroxidase, hydrogen peroxide, and sodium bromide at pH 4 (Fig. 1, EPO/HOBr system). This neutral lipid did not comigrate with either 2-chlorohexadecanal (2-ClHDA) ($R_f = 0.46$) or fatty acid ($R_f$ range for palmitic, palmitoleic, and arachidonic acid = 0.18–0.20) in this TLC system. The production of this neutral lipid was dependent on the presence of a complete reaction mixture comprising active eosinophil peroxidase (thermal treatment of eosinophil peroxidase ablated lysoplasmalogen loss), hydrogen peroxide, and sodium bromide (Fig. 1).

Similar studies were performed utilizing the plasmalogen molecular species, 1-O-hexadec-1-ethyl-2-hexadecanoyl-GPC, as the target phospholipid of the reactive brominating species generated by eosinophil peroxidase. Reaction products were subjected to TLC analysis with molybdate staining of inorganic phosphate, which demonstrated plasmalogen loss in the presence of the complete eosinophil peroxidase reactive brominating species system at pH 4 (Fig. 2A) concomitant with the production of a polar lipid that comigrated with authentic LPC (Fig. 2A). Additionally, under these conditions a neutral lipid that migrated with the solvent front was observed when TLC plates were charred with sulfuric acid (data not shown). Silica corresponding to regions 1 and 2 (indicated in Fig. 2) (taken from TLC plates that were developed concomitantly with those shown in Fig. 2A) was scraped from TLC plates, and purified lipids were extracted from the silica and subjected to GC following derivatization by acid methanolysis (Fig. 2B). This GC analysis confirmed the production of LPC in region 1 with plasmalogen as the target of the reactive brominating species produced by eosinophil peroxidase (+EPO in Fig. 2B) because only the fatty acid methyl ester of palmitic acid was present (peak b) and the vinyl ether derivative, the dimethylacetal of palmitaldehyde, was absent. Examination of region 2 (Fig. 2B), which stained positively in the +EPO sample using sulfuric acid charring, by GC analysis of acid methanolysis products revealed the presence of a peak that had an identical retention time to that of the dimethylacetal of 2-BrHDA (peak c). An additional analysis by GC of acid methanalysis derivatives of the neutral lipid produced by eosinophil peroxidase-derived reactive brominating species attack of lysoplasmenylcholine ablative lysoplasmalogen loss, hydrogen peroxide, and sodium bromide (Fig. 1).

FIG. 1. TLC analysis of eosinophil peroxidase-treated lysoplasmalogen. 200 nmol of lysoplasmalogen was incubated in the presence or absence of each of the EPO/HOBr-generating reagents including EPO (22.6 ng), H$_2$O$_2$ (1 mM), and NaBr (100 mM) in 500 µl of 20 mM phosphate buffer (pH 4.0 or 7.0) as indicated at 37 °C for 5 min. Incubations were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and subjected to TLC with a solvent system composed of petroleum ether/diethyl ether/acetic acid (90/10/1, v/v/v). Reaction products on developed TLC plates were visualized by sulfuric acid treatment and charring.

FIG. 2. TLC and GC analysis of eosinophil peroxidase-treated plasmalogen. A, 200 nmol of plasmalogen was incubated in the presence or absence of each of the EPO/HOBr-generating reagents including EPO (45 ng), H$_2$O$_2$ (1 mM), and NaBr (100 mM) in 500 µl of 20 mM phosphate buffer (pH 4) as indicated at 37 °C for 5 min. Incubations were terminated by the addition of methanol, and reaction products were sequentially extracted into chloroform and subjected to TLC with chloroform/acetone/methanol/acetic acid/water (6/8/2/2/1, v/v/v/v/v) as the mobile phase. Reaction products on developed TLC plates were visualized by molybdate treatment. B, parallel plates were developed identically, but silica was scraped from the regions denoted as 1 and 2, and purified lipids were extracted. These lipids were then subjected to acid methanalysis and analyzed by GC with FID detection. Peaks a, b, and c correspond to the solvent peak, the methyl ester of hexadecanoic acid, and the putative dimethylacetal of 2-bromohexadecanal, respectively.
plasmenylcholine (see Fig. 1) also demonstrated that the neutral lipid was most likely 2-BrHDA, as it yielded a peak with an identical retention time as that of peak \( c \) in Fig. 2B.

To confirm that the neutral lipid reaction product from the eosinophil peroxidase-mediated degradation of plasmalogens was 2-BrHDA, the TLC-purified neutral lipid product that was derivatized by acid methanolysis as well as the underivatized product were subjected to GC-MS utilizing electron impact ionization. The mass spectrum of the acid methanolysis product of the neutral lipid showed a base ion at \( m/z \) 75, which is a signature ion of dimethylacetals, acid methanolysis products of masked aldehydes (i.e. the vinyl ether bond of plasmalogens) and free aldehydes (Fig. 3A). The mass spectrum of the underivatized compound included the anticipated isotopomers of the brominated parent ions of 2-bromohexadecanal (2-BrHDA) (Fig. 3B). These ions at \( m/z \) 318 and 320 are present at a 1/1 ratio characteristic of monobrominated molecules because of the ratio of the natural isotopic abundance of \( ^{77}\text{Br} \) and \( ^{81}\text{Br} \) at 1/1. Two peaks consistent with the production of the syn- and anti-isomers of the PFB oxime derivative of 2-BrHDA were identified by GC-MS analyses using negative chemical ionization detection. The fragmentation pattern of the second peak is shown in Fig. 4. One of the major fragments at \( m/z \) 332 is monobrominated, having an ion of equal intensity observed at \( m/z \) 334. It should be recognized that the remaining fragmentation pattern is consistent with the structure of the PFB oxime of 2-BrHDA (Fig. 4, inset).

Further studies were designed to compare the production of 2-BrHDA in the presence and absence of physiological NaCl levels (100 mM). The data shown in Fig. 5 show that eosinophil peroxidase mediates the attack of plasmalogens in the presence of bromide more efficiently when also in the presence of physiological concentrations of NaCl in comparison with in the absence of NaCl. With only bromide present, 2-BrHDA production was maximal at 500 \( \mu \text{M} \) NaBr (Fig. 5). In contrast, the bromide requirement for maximal 2-BrHDA production was decreased to \( \sim \)100–250 \( \mu \text{M} \) in the presence of physiological concentrations of NaCl (100 mM) (Fig. 5). Furthermore, at
physiological concentrations of NaBr (50 μM), the presence of 100 mM NaCl increased 2-BrHDA production 2-fold (Fig. 5). The augmentation of 2-BrHDA production by the addition of sodium chloride is only minimally due to bromide contamination in the chloride, because 2-BrHDA production in the presence of 100 mM NaCl with no addition of bromide was only half of that found with only 25 mM sodium bromide added (Fig. 5). Because of the augmentation of 2-BrHDA production by NaCl supplementation, the pH dependence was characterized with 100 mM NaBr in the presence of 100 mM NaCl. The pH optima for 2-BrHDA production by reactive halogenating species produced by eosinophil peroxidase in the presence of physiological chloride and bromide concentrations were acidic, with maximal production observed at pH 4.5 (Fig. 6). Additionally, 2-CHDA production was not detected under these conditions (data not shown).

The specificity of the degradation of plasmalogens by eosinophil peroxidase-generated reactive brominating species was further assessed in reactions that included the peroxidase inhibitors sodium azide, catalase, and 3-aminotriazole using 500 μM NaBr. Sodium azide, catalase, and 3-aminotriazole inhibited eosinophil peroxidase-mediated degradation of the vinyl ether bond (Fig. 7). Additionally, the specificity of the reaction of the eosinophil peroxidase-generated reactive bromine species toward the vinyl ether bond of plasmalogens was demonstrated because destruction of the vinyl ether bond of plasmalogens by treatment with hydrochloric acid fumes for 10 min ablated the production of the eosinophil peroxidase-mediated neutral lipid product, 2-BrHDA (Fig. 7).

Because both eosinophil peroxidase and myeloperoxidase use both chloride and bromide, comparisons were made between these two peroxidases to determine their selectivity in using halides to produce α-halo fatty aldehydes. At pH 4, myeloperoxidase used either chloride or bromide to produce reactive halogenating species, resulting in the production of either 2-CHDA or 2-BrHDA, respectively (Fig. 8). Additionally, in the presence of both 100 μM bromide and 100 mM chloride, myeloperoxidase-derived reactive halogenating species attacked plasmalogens, resulting in the production of both 2-CHDA and 2-BrHDA (Fig. 8). In contrast, eosinophil peroxidase was very specific for bromide as substrate, with only small amounts of 2-CHDA produced in the presence of 100 mM chloride in the absence of bromide (Fig. 8). Furthermore, 2-BrHDA was selectively produced by eosinophil peroxidase in the presence of 100 μM bromide and 100 mM chloride (Fig. 8).

The physiological significance of eosinophil peroxidase-mediated production of 2-BrHDA from plasmalogens was assessed by determining that this biochemical mechanism is present in intact activated eosinophils as well as by determining that 2-BrHDA is a phagocyte chemoattractant. To determine that halogenated aldehydes are produced in activated eosinophils, GC-MS analyses of their PFB derivatives were measured using a deuterated internal standard as described previously (23). For these experiments, unstimulated (control) and PMA-stimulated human eosinophils were incubated in Hanks’ balanced salt solution supplemented with 100 μM NaBr, and the production of 2-BrHDA and 2-CHDA was determined by GC-MS of their respective PFB oximes. PMA activation of the eosinophils led to the production of 2-BrHDA and a small increase in 2-CHDA compared with nonactivated eosinophils (Fig. 9). PMA-stimulated 2-BrHDA and 2-CHDA production were blocked by the peroxidase inhibitor, sodium azide (Fig. 9). The potential physiological role of 2-BrHDA as a phagocyte chemoattractant was tested. Both 90 nM and 90 μM 2-BrHDA induced neutrophil chemotaxis to a significantly greater extent than their Me₂SO controls (Fig. 10). For comparison, the known potent neutrophil chemoattractant, fMLP, is shown as a positive control for chemoattraction (Fig. 10).

**DISCUSSION**

Plasmalogens are a predominant phospholipid molecular subclass found in many mammalian tissues including endothelial cells and vascular smooth muscle (16, 35). Vascular tissue is one of the major pathophysiological host targets of activated leukocytes leading to inflammation. The present study now demonstrates that eosinophils also produce reactive halogenating species that attack plasmalogens. For these studies several lines of evidence have demonstrated that 2-BrHDA is produced from the attack on the vinyl ether bond of the plasmalogen 1-O-hexadec-1'-eny-GPC by reactive brominating species produced by eosinophil peroxidase. First, utilizing GC-MS analyses of the acid methanolysis product of the reaction of reactive

![Graph](http://example.com/five.png)
brominating species with plasmalogen indicated that a dimethylacetal derivative of an aldehyde was present. Second, the characteristic isotopic cluster of a monobrominated molecule was observed at the predicted mass of 2-BrHDA utilizing GC-MS analyses of the underivatized compound. These ions at m/z 318 and m/z 320 were present at a 1:1 ratio, which is a signature for a monobrominated molecule because of the 1:1 natural abundance of 79Br and 81Br, respectively. Finally, derivatization of the neutral lipid with pentafluorobenzyl hydroxylamine followed by GC-MS analysis with negative chemical ionization was consistent with the derivatization of 2-BrHDA to its pentafluorobenzyl oxime. Collectively these analyses have demonstrated that eosinophil peroxidase-derived reactive brominating species attack the vinyl ether bond of plasmalogens.

**FIG. 6.** pH dependence of plasmalogen degradation by the eosinophil peroxidase/reactive brominating species-generating system in the presence of physiological concentrations of NaCl. 50 nmol of lysoplasmenylcholine was incubated in the presence of the eosinophil peroxidase/reactive brominating species-generating system, which includes eosinophil peroxidase (22.6 ng), H2O2 (1 mM), NaCl (100 mM), and NaBr (0.1 mM) in 2 ml of phosphate buffer at the indicated pH at 37 °C for 5 min. Lipid reaction products were extracted and subjected to acid methanolysis in the presence of 1-hexadecanoyl-GPC (internal standard), and derivatives were analyzed by capillary gas chromatography with FID detection as described under “Experimental Procedures.” Values are the means ± S.E. of at least three independent experiments.

**FIG. 7.** Inhibition of eosinophil peroxidase-mediated plasmalogen degradation by eosinophil peroxidase pathway inhibitors and pretreatment of plasmalogens with acid vapors. 200 nmol of lysoplasmenylcholine was incubated with 22.6 ng of eosinophil peroxidase (or thermal denatured eosinophil peroxidase (Denat. EPO), 15 min at 90 °C) in the HOBr-generating buffer system (100 mM NaCl and 0.5 mM NaBr) at pH 4.0 at 37 °C for 5 min in the presence or absence of 3-aminotriazole (ATZ, 50 mM; preincubated with eosinophil peroxidase at 37 °C for 5 min prior to initiation of reaction), 10 µg/ml catalase (preincubated with reaction mixture at 37 °C for 5 min prior to the addition of eosinophil peroxidase), or 100 µM sodium azide (preincubated with eosinophil peroxidase at 37 °C for 5 min prior to initiation of reaction) as indicated. Alternatively, 200 nmol of lysoplasmenylcholine was pretreated with HCl vapors (as indicated) and then incubated with 22.6 ng of eosinophil peroxidase in the HOBr-generating buffer system at pH 4.0 at 37 °C for 5 min. Lipid reaction products were extracted and subjected to acid methanolysis in the presence of 1-hexadecanoyl-GPC (internal standard), and derivatives were analyzed by capillary gas chromatography with FID detection as described under “Experimental Procedures.” The mass of product formed was calculated by comparing the integrated areas of the peak derived from plasmalogen degradation to that of the internal standard. Values are the means ± S.E. of at least three independent experiments.
gens resulting in 2-BrHDA production. Furthermore, this is the first demonstration that lipid halogenation occurs through the activation of eosinophils.

The present studies also suggest that /H9251-bromo fatty aldehydes are produced by intact activated eosinophils through a mechanism that is mediated by eosinophil peroxidase-derived reactive brominating species that target the plasmalogen vinyl ether bond. Eosinophil peroxidase-derived reactive brominating species are produced during eosinophil activation (4, 5), and the present results show that inhibition of their production by the inclusion of the peroxidase inhibitor azide drastically attenuates the production of 2-BrHDA by PMA-stimulated eosinophils. It should be appreciated that in both intact eosinophil

Fig. 8. Comparisons of α-halo fatty aldehyde production by eosinophil peroxidase- and myeloperoxidase-derived reactive halogenating species. 100 nmol of lysosphasmenylcholine was incubated in the presence of either the myeloperoxidase- or eosinophil peroxidase-derived reactive halogenating species-generating systems, which includes either myeloperoxidase (0.6 units) or eosinophil peroxidase (22.6 ng), H2O2 (1 mM), and the indicated concentrations of NaBr and/or NaCl in 2 ml of phosphate buffer at pH 4 and at 37 °C for 5 min. Lipid reaction products were extracted and subjected to acid methanolysis in the presence of 1-hexadecanoyl-GPC (internal standard), and derivatives were analyzed by capillary gas chromatography with FID detection as described under “Experimental Procedures.” Values are the means ± S.E. of at least three independent experiments.

Fig. 9. 2-BrHDA and 2-CIHDA accumulation in PMA-stimulated eosinophils. Eosinophils isolated from allergen-hypersensitive individuals were suspended in Hanks' balanced salt solution at a concentration of 1 × 10⁶ cells/ml for 1 h under the indicated conditions. The complete system contained 100 μM NaBr and 200 nM PMA. Following incubation, cell suspensions were snap frozen, and subsequently lipids were extracted in the presence of deuterated 2-BrHDA and 2-CIHDA. Halogenated aldehydes were then converted to their PFH oximes and quantitated by selected ion monitoring using GC-MS with chemical ionization. Experiments were performed in duplicate as shown.

Fig. 10. 2-Bromohexadecanal induces neutrophil chemotaxis. 2 × 10⁵ neutrophils were loaded into the upper compartments of a Boyden chamber, and neutrophil chemotaxis was elicited by 100 nM fMLP, 90 nM and 90 μM 2-BrHDA, or chemotaxis buffer (as indicated), measured as described under “Experimental Procedures.” * and ** indicate treatments with p < 0.05 and p < 0.005, respectively, compared with the appropriate controls. Each value indicates the distance of neutrophil migration for at least four independent measurements.

The present studies also suggest that α-bromo fatty aldehydes are produced by intact activated eosinophils through a mechanism that is mediated by eosinophil peroxidase-derived reactive brominating species that target the plasmalogen vinyl ether bond. Eosinophil peroxidase-derived reactive brominating species are produced during eosinophil activation (4, 5), and the present results show that inhibition of their production by the inclusion of the peroxidase inhibitor azide drastically attenuates the production of 2-BrHDA by PMA-stimulated eosinophils. It should be appreciated that in both intact eosinophil
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that plasmalogens can terminate the cytotoxicity of reactive mechanisms initiated by the attack of the vinyl ether-masked 2-BrHDA. Some 2-ClHDA production may be attributed to the production of reactive brominating species and the production of oxidase demonstrated that it is relatively selective for the production of reactive halogenating species. On the other hand, it is possible that plasmalogens may serve as protective agents to the host cell if the products of reactive brominating species attack of plasmalogens are relatively less cytotoxic compared with the attack of other targets of reactive brominating species, such as proteins and nucleic acids, which could potentially lead to greater long-term damage to the host cell. Taken together, the present results demonstrate the targeting of vinyl ether bonds of plasmalogens by reactive brominating species produced by eosinophils.

This may represent a potentially important and as yet unrecognized mechanism mediating the generation of α-bromo fatty aldehydes and lysophospholipids, which may both play important roles in the antimicrobial role of eosinophils as well as in inflammatory host cell injury in diseases such as asthma.

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