Recently α-chloro fatty aldehydes have been shown to be products of reactive chlorinating species targeting the vinyl ether bond of plasmalogens utilizing a cell-free system. Accordingly, the present experiments were designed to show that α-chloro fatty aldehydes are produced by activated neutrophils and to determine their physiologic effects. A sensitive gas chromatography-mass spectrometry technique was developed to detect pentafluorobenzyl oximes of α-chloro fatty aldehydes utilizing negative ion chemical ionization. Phorbol 12-myristate 13-acetate activation of neutrophils resulted in the production of both 2-chlorohexadecanal and 2-chlorooctadecanal through a myeloperoxidase-dependent mechanism that likely involved the targeting of both 16 and 18 carbon vinyl ether-linked aliphatic groups present in the sn-1 position of neutrophil plasmalogens. 2-Chlorohexadecanal was also produced by FMLP-treated neutrophils. Additionally, reactive chlorinating species released from activated neutrophils targeted endothelial cell plasmalogens resulting in 2-chlorohexadecanal production. Physiologically relevant concentrations of 2-chlorohexadecanal induced neutrophil chemotaxis in vitro suggesting that α-chloro fatty aldehydes may have a role in neutrophil recruitment. Taken together, these studies demonstrate for the first time a novel biochemical mechanism that targets the vinyl ether bond of plasmalogens during neutrophil activation resulting in the production of α-chloro fatty aldehydes that may enhance the recruitment of neutrophils to areas of active inflammation.

Activated neutrophils consume oxygen to produce an array of damaging reactive oxidizing species including superoxide anions and hydrogen peroxide. Although hydrogen peroxide is substantially less reactive and harmful than its precursor, superoxide anion, the oxidizing potential of hydrogen peroxide is amplified by its conversion, mediated by myeloperoxidase at plasma chloride concentrations, to the reactive chlorinating species hypochlorous acid (HClO) (6, 7). In activated neutrophils, hydrogen peroxide is produced and released concomitant with the release of the heme protein myeloperoxidase resulting in the generation of hypochlorous acid.

Hypochlorous acid, in equilibrium with its conjugate anion hypochlorite (−OCl) and molecular chlorine (Cl2), mediates several critical reactions that are responsible for some of the cytotoxic properties of activated neutrophils (6). Specifically, these mechanisms include the oxidative bleaching of protein heme and iron sulfur centers (7–11) and, in conjunction with available primary amine groups, the generation of the equally reactive and damaging oxidizing chloramines (12). Alkene groups (−C=C−) of cholesterol or unsaturated aliphatic groups of fatty acids are particularly susceptible targets for modification collectively by reactive chlorinating species resulting in the production of chlorohydrins (9). Moreover, the generation of chlorohydrins and other chlorinated lipid-derived products (13) is well documented and may indeed mediate, in part, the cytotoxicity attributed to reactive chlorinating species through alterations in membrane dynamics (14, 15).

Plasmalogens are a glycerophospholipid molecular subclass possessing a vinyl ether bond between their glycerol backbone and the sn-1 aliphatic group. Plasmalogens are enriched in the plasma membranes of mammalian tissues (16–20), and they are likely important components of the plasma membrane by both solvating critical transmembrane proteins (21–23) and storing esterified arachidonic acid (18–20). Although plasmalogenase activity has been described that targets the vinyl ether bond (24, 25), this activity is not substantial in intact cells, and thus the vinyl ether bond was considered until recently to be metabolically stable. Recently, we demonstrated in a cell-free system that myeloperoxidase-derived reactive chlorinating species target the sn-1 vinyl ether bond of plasmalogens (26). In this cell-free system, a neutral lipid was released from plasmalogens treated with a reactive chlorinating species that was identified as an α-chloro fatty aldehyde. The present results now demonstrate for the first time that the α-chloro fatty aldehydes, 2-chlorohexadecanal (2-CHDA) and 2-chloroocta-
decanal (2-CIODA) are produced when neutrophils are activated through a mechanism that includes the production of myeloperoxidase-derived reactive chlorinating species that attack both neutrophil and endothelial cell plasmalogens. Additionally, 2-CIHDA induces neutrophil chemotaxis in vitro, and this may play an important role in the ongoing recruitment and perpetuation of neutrophil-mediated inflammation in vivo.

**Experimental Procedures**

**Materials**—Pentafluorobenzyl (PFB) hydroxylamine, Hank’s Balanced Salt Solution (HBSS), 3-amino-triazole, sodium cyanide, and sodium azide were purchased from Sigma. Cellulose nitrate chemotaxis filters were purchased from NeuroProbe. [7,7,8,8-d4]-hexadecane acid was purchased from Medical Isotopes, Inc. 2-Chloroacetodecanole was prepared from 1-Octadeoxy-1-iodo-PG (lyso phosphomonoylethyl) utilizing sodium hypochlorite as previously described (26–29). All other reagents and chemicals were purchased from Aldrich, Sigma, Fisher, or Nu-Check Prep.

**Synthesis and Purification of 2-CIHDA and 2-Cl-[d4]-HDA—2-Chloro-[7,7,8,8-d4]-Hexadecanal (2-Cl-[d4]-HDA) was synthesized by the following scheme: 1) synthesis of [7,7,8,8-d4]-hexadecanone from [7,7,8,8-d4]-hexadecanoyl acid (Medical Isotopes, Inc.) using sodium bis(2-methoxyethoxy)aluminum hydride; 2) synthesis of [7,7,8,8-d4]-hexadecanone by partial oxidation at ~70 °C utilizing oxayl chloride-activated dimethyl sulfoxide (Me2SO) as a catalyst (30); 3) synthesis of the di-methyl acetal of [7,7,8,8-d4]-hexadecanoyl acid methanolysis; and 4) synthesis of the dimethyl acetal of 2-CL-[d4]-HDA utilizing an acetal chlorination system employing MnO2-trimethylchlorosil dane (31); and 5) synthesis of 2-Cl-[d4]-HDA by refluxing in 1:1 trifluoroacetic acid/di-chloromethane. The synthetic 2-Cl-[d4]-HDA was purified by HPLC utilizing a Dynaxan Si column (21.4 × 250 mm; 8 µm) and gradient elution with hexane as the initial mobile phase and hexane/chloroform 90:10, v/v, as the final mobile phase at a flow rate of 6 ml/min. Utilizing this system, 2-Cl-[d4]-HDA eluted in 3% chloroform and the purity was confirmed by TLC, as well as by GC-MS of its PFB oxime derivative (see below). Synthetic 2-CIHDA was prepared and purified by a similar scheme as that for the deuterated compound except that hexadecanol (Nu-Chek Prep) was the starting reagent.

**Neutrophil Activation and 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 Ficoll-Hypaque gradient as previously described (32). Pelleted neutrophils were resuspended in HBSS (pH 7.3) and immediately subjected to experimental protocols. In indicated experiments neutrophils were re-suspended in HBSS supplemented with 5 × 107 neutrophils, 1 mM sodium cyanide, and 1 mM sodium azide. Neutrophils (0.5 × 106 cells/ml) were stimulated with selected concentrations of PMA or FMLP for indicated times with reactions terminated by the addition of methanol containing the internal standard (20 pmol of 2-Cl-[d4]-HDA). Following Bligh and Dyer extraction of neutrophil lipids (33), 2-CIHDA was quantitated following derivatization to its PFB oxime by GC-MS. In selected studies 2-CIHDA and its deuterated internal standard were purified from lipid extracts of experimentally treated neutrophils (2.5 × 106 cells) by TLC utilizing silica gel G plates as the stationary phase and petroleum ether/ethyl ether/acetic acid (90:10:1, v/v/v) as the mobile phase. The Boyden chamber was separated from the top compartment containing 2 × 104 neutrophils (50 µl) by 5 µm pore-size cellulose nitrate filters. The Boyden chamber was incubated for 35 min at 37 °C and the filters were then stained and dehydrated, and chemotaxis was assessed by the leading front method as previously described (34). Net migration through the filter was reported in micrometers.

**Preparation of PFB Oximes of α-Chloro Fatty Aldehydes and Quantitative Gas Chromatography-Mass Spectrometric Analysis**—Neutrophil lipid extracts were dried under nitrogen and resuspended in 300 µl of ethanol followed by the addition of 300 µl of PFB hydroxylamine (6 mg/ml). The mixtures were vortexed for 5 min and incubated at 24 °C for 25 min. Following the addition of 1.2 ml of water, the reaction products were sequentially extracted into 1 ml of cyclohexane/diethyl ether (4:1, v/v), dried under a stream of nitrogen and resuspended in 50 µl of petroleum ether for GC-MS analysis. GC-MS analysis of PFB oximes of α-chloro fatty aldehydes was performed on a Hewlett-Packard 6890 gas chromatograph using the negative ion chemical ionization (NICI) mode with methane as the reagent gas. 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 (125 m, 0.2 mm inner diameter, 0.33-µm film thickness). The injection 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 30 °C/min to 270 °C, and held at 270 °C for an additional 2 min. Quantitation of 2-CIHDA was performed utilizing SIM by comparing the integrated peak area corresponding to m/z = 288 to that produced from the deuterated internal standard (i.e., m/z = 292).

**Neutrophil Vinyl Ether Studies—Vinyl ether (plasmalogen) content was measured as described previously (35). In brief, crude lipid extracts from control and PMA-activated neutrophils were subjected to 1 ml of 0.3 mM iodine and 90.4 mM KI in methanol/water (1:1, v/v) for 30 min prior to the addition of 4 ml of 95% ethanol. Vinyl ether content was determined by spectrophotometry at 355 nm utilizing the iodine extinction coefficient ε254 = 27,500 M⁻¹ cm⁻¹.

**Neutrophil and Endothelial Cell Studies in Transwell Culture Chambers**—Bovine pulmonary artery endothelial cells were cultured to confluence in minimum Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum and washed in warm phosphate-buffered saline immediately prior to experiments utilizing 35-µm collagen-coated culture dishes. Neutrophils (2 × 10⁶ cells) in the presence and absence of PMA were added in transwell chambers (Falcon) containing a polyethylene terephthalate membrane above endothelial cells (2 × 10⁵ cells). Control experiments were performed with heat-inactivated neutrophils. Following experimental intervals, lipids from wells containing neutrophils and endothelial cells were extracted separately for 2-CIHDA measurements by GC-MS following derivatization of the lipid extracts to prepare the PFB oxime of 2-CIHDA.

**Results**

We have previously demonstrated that reactive chlorinating species produced from the heme-containing enzyme myeloperoxidase attack the vinyl ether bond of plasmalogens resulting in the production of an α-chloro fatty aldehyde (26). Accordingly, techniques were devised to quantitate the α-chloro fatty aldehyde, 2-CIHDA, in biological samples. For these studies, a deuterated internal standard, 2-Cl-[d4]-HDA, was synthesized and purified by HPLC. Both 2-Cl-[d4]-HDA and synthetic 2-CIHDA were converted to their PFB oxime derivatives, and then subjected to NICI GC-MS analysis. Fig. 1, A and B shows the mass spectrum of the PFB oximes derived from synthetically prepared 2-CIHDA and the deuterated compound, 2-Cl-[d4]-HDA, respectively. In the former spectrum, the molecular anion (M⁻) containing a 35Cl, and 37Cl atom is observed at m/z 469 and 471, respectively. The ratio of the abundance of these two ions is close to 3:1, a unique isotope pattern specific for molecules containing a chlorine atom. This is further evidenced by the observation of the fragment ions m/z 35 (35Cl) and 37 (37Cl) with an abundance ratio of 3:1 in the same spectrum. The mass spectrum also contains structurally informative ions at m/z 196 (CH₃ - HF - Cl), 206 (CH₃ - HF - Cl), 216 (HF - Cl₂), and 226 (HFCl₂). For comparison, these ions are characteristic to PFB oxime derivatives (36). The analogous (m/z + 4) ions at m/z 473/471 (M⁺), 483 (M⁺ - HFCl₂), 493 (M⁺ - Cl₂), were also observed for the 2-Cl-[d4]-HDA, as well as the common fragment ions at m/z 196, 178, and 35/37 (Fig. 1, B and D). Importantly, NICI GC-MS analysis of the PFB oxime of 2-CIHDA and of 2-Cl-[d4]-HDA produced the structurally informative fragments at m/z = 288 and 292, respectively (Fig. 1, A and B) suggesting that SIM of m/z = 288 and 292 from the
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PFB oxime derivatives of 2-ClHDA and 2-Cl-[d₄]-HDA, respectively, could be used to quantitate 2-ClHDA (Fig. 2, A and B). Furthermore, the utility of the synthetic deuterated internal standard and SIM-GC-MS of m/z = 288 and 292 for the quantitation of 2-ClHDA was demonstrated by the linear relationship between the ratio of integrated peak area of m/z = 288 to m/z = 292 and the ratio of 2-ClHDA mass to 2-Cl-[d₄]-HDA mass (slope = 0.99, R² = 0.999) (Fig. 2C).

Selected ion monitoring of m/z = 288 (from 2-ClHDA) and 292 (from the deuterated internal standard) of samples prepared by treating crude chloroform extracts of control and PMA-stimulated neutrophils with PFB hydroxylamine revealed that m/z = 288 (i.e. the fragment derived from 2-ClHDA) was only found in samples prepared from PMA-stimulated neutrophils in comparison to control neutrophils (Fig. 3, A and B). There are two peaks (8.35 and 8.45 min) in the chromatogram, corresponding to the anti- and syn- isomer of the PFB oxime derivatives, respectively (36). Furthermore, these two peaks for m/z = 288 derived from PMA-activated neutrophils had an identical retention time to that of the authentic PFB oxime of 2-ClHDA (compare with Fig. 2A). Fig. 3, C and D, are the chromatograms for SIM of m/z = 288 to m/z = 292 in samples prepared from control and PMA-treated neutrophils, respectively, that were first subjected to TLC purification of 2-ClHDA prior to conversion to its PFB oxime and SIM-GC-MS. Thus, nearly identical ratios of m/z = 288 to m/z = 292 are observed in neutrophil samples prepared directly from crude lipid extracts as well as those subjected to TLC purification of 2-ClHDA (e.g. compare Fig. 3, A and B to C and D). In separate analyses, the acquired mass spectrum for the peak corresponding to m/z = 288 from PMA-activated neutrophil treatments (Fig. 3E) was similar to that produced by synthetic 2-ClHDA (Fig. 1A). Collectively, these data demonstrate for the first time that PMA-activated neutrophils produce 2-ClHDA.

Next, SIM of m/z = 288 to m/z = 292 was used to quantitate 2-ClHDA in neutrophils stimulated with selected concentrations of PMA as well as fMLP, a potent neutrophil chemottractant (37). PMA stimulation of neutrophils resulted in at least twice the amount of 2-ClHDA compared with fMLP stimulation even at PMA concentrations that were 20-fold less than fMLP (Fig. 4A). The disparity between PMA- and fMLP-elicited 2-ClHDA production results from the degradation of neutrophil plasmalogens by myeloperoxidase-derived reactive chlorinating species because the myeloperoxidase inhibitors such as sodium azide, sodium cyanide, and 3-amino triazole severely attenuated 2-ClHDA production in neutrophils stimulated with selected concentrations of PMA and fMLP (38). It is likely that neutrophil-mediated 2-ClHDA production results from the degradation of neutrophil plasmalogens by myeloperoxidase-derived reactive chlorinating species, and the myeloperoxidase inhibitors such as sodium azide, sodium cyanide, and 3-amino triazole severely attenuated 2-ClHDA production in neutrophils stimulated with selected concentrations of PMA and fMLP (38).

Because neutrophil plasmalogens contain vinyl ether-linked aliphatic groups of predominantly 16 and 18 carbons in length (39), the production of 2-ClODA was also examined. As a first step in these studies, synthetic 2-ClODA was derivatized to its PFB oxime and subjected to GC-MS analysis utilizing NICI as described under “Experimental Procedures.” The mass spectrum of the PFB oxime of 2-ClHDA and 2-Cl-[d₄]-HDA are shown. The structures and putative fragmentation patterns of the PFB oxime of 2-ClHDA and 2-Cl-[d₄]-HDA are shown (C and D, respectively).

Fig. 1. The NICI mass spectrum of the PFB oximes of 2-chlorohexadecanal and 2-chloro-[7,7,8,8-d₄]-hexadecanal. 75 nmol of
In contrast, no detectable peaks were observed utilizing SIM of m/z 288 with samples prepared from either control neutrophils or PMA-stimulated neutrophils in the presence of the myeloperoxidase inhibitor 3-amino-
notriazole (Figs. 5, C and D, respectively). Additionally, in separate analyses of the PFB oxime of synthetic 2-ClHDA, the mass spectrum was acquired for the peak corresponding to m/z 316 at 9.2 min (Fig. 5E). This mass spectrum was similar to that derived from the corresponding PFB oxime of 2-ClHDA (Fig. 1A). The fragment ions m/z 316, 318, 442, 444, 497, and 499, reflect a mass increase of 28, corresponding to the two carbon increase in chain length. Furthermore, the mass spectrum of the peak corresponding to m/z 316 at 9.2 min from samples prepared from PMA-stimulated neutrophils (Fig. 5F) was similar to that derived from the PFB oxime of synthetically prepared 2-ClODA (Fig. 5E).

Because it is likely that the sn-1 vinyl ether bond of the neutrophil plasmalogens is the only precursor of α-chloro fatty aldehydes produced by the action of reactive chlorinating species released during neutrophil activation, the vinyl ether content in control and PMA-stimulated neutrophils was quantitated utilizing the method of Gottfried and Rapport (35). The vinyl ether (plasmalogen) content was not substantially different in control and PMA-stimulated neutrophils, respectively (19 nmol of vinyl ether groups/10^6 cells in control versus 19.5 nmol of vinyl ether groups/10^6 cells in the PMA group). It should be appreciated that the changes in 2-ClHDA measured in PMA-stimulated neutrophils ranged between 34 and 37 fmoI/10^6 neutrophils although the mass of plasmalogen pool was three orders of magnitude greater. Thus, it is unlikely that significant changes in plasmalogen molecular species content following PMA activation would be observed under the conditions employed. However, these findings do underscore both the large abundance of plasmalogens that can be attacked by

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**Fig. 2.** Selected ion monitoring GC-MS analysis of the PFB oxime derivative of 2-chlorohexadecanal and 2-chloro-[7,7,8,8-d_4]-hexadecanal. 20 pmol of 2-ClHDA and 2-Cl-[d_4]-HDA were converted to their PFB oximes and subjected to GC-MS analysis as described under “Experimental Procedures.” Selected ion monitoring for m/z = 288 from the PFB oxime of 2-ClHDA (A) and m/z = 292 for the PFB oxime of 2-Cl-[d_4]-HDA (B) was performed. The PFB oximes of mixtures containing 20 pmol of 2-Cl-[d_4]-HDA and increasing amounts of 2-ClHDA (5–60 pmol) were prepared and subjected to GC-MS analysis. The ratio of the integrated peak area for m/z = 288 to the integrated peak area for m/z = 292 was calculated and plotted versus the ratio of the mass of 2-ClHDA to 2-Cl-[d_4]-HDA that was derivatized (C). The equation describing the line produced by linear regression of the data points was y = 0.99x + 8.5 × 10^{-3} with R^2 = 0.999.
released reactive chlorinating species as well as the likely maintenance of neutrophil membrane integrity during PMA stimulation accompanied by the production of reactive chlorinating species and chlorinated fatty aldehydes.

Although PMA-stimulated neutrophils produce \( \alpha \)-chloro fatty aldehydes (see above), further studies were designed to determine whether reactive chlorinating species produced by neutrophils target endothelial cell plasmalogen pools to produce 2-ClHDA. For these studies, neutrophils were separated from endothelial cells by a polyethylene terephthalate mem-

FIG. 3. NICI GC-MS analysis of PFB oximes of 2-ClHDA derived from 2-ClHDA produced by PMA-stimulated neutrophils. \( 1 \times 10^6 \) neutrophils were treated with either 200 nM PMA or vehicle only for 30 min at 37 °C. Reactions were terminated by the addition of methanol containing 20 pmol of 2-Cl-[d\(_4\)]-HDA, and the reaction products were extracted into chloroform. Following conversion to their PFB oximes, SIM-GC-MS analysis using NICI for \( m/z = 288 \) and \( m/z = 292 \) was performed for the control (A) and PMA-treated (B) neutrophil samples, respectively. In additional experiments, lipid extracts from control samples and PMA-treated samples were subjected to TLC purification of 2-ClHDA utilizing petroleum ether/diethyl ether/acetic acid (90:10:1, v/v/v) as the mobile phase and TLC purified 2-ClHDA, and comigrating 2-Cl-[d\(_4\)]-HDA were then converted to their PFB oximes followed by SIM-GC-MS analysis using NICI for \( m/z = 288 \) and \( m/z = 292 \) in both control (C) and PMA-treated (D) samples, respectively. The mass spectrum of the PFB oxime of 2-ClHDA from PMA-stimulated neutrophils is shown in E.
brane, and the lower compartment containing endothelial cells was examined for 2-ClHDA. Importantly, 2-ClHDA was not observed in the lower compartment under conditions with activated neutrophils (+PMN, +PMA) in the upper compartment but no endothelial cells in the lower compartment (Fig. 6). This indicates that 2-ClHDA produced by neutrophils in the upper well does not transverse the membrane. Additionally, endothelial cells did not produce 2-ClHDA in the absence of neutrophils despite the presence of PMA. 2-ClHDA in the lower chamber containing endothelial cells was only observed when PMA-stimulated neutrophils were in the upper chamber (Fig. 6). Taken together, these results suggest that plasmalogen of neighboring cells of activated neutrophils can be attacked to produce 2-ClHDA.

The potential physiological role of 2-ClHDA as a neutrophil chemoattractant was tested. Both 90 μM and 90 nm 2-ClHDA induced neutrophil chemotaxis significantly greater than their Me2SO controls (Fig. 7). 2-ClHDA-mediated neutrophil chemotactic activity was slightly less than migration induced by the known neutrophil chemoattractant fMLP (Fig. 7). In contrast, PMA-elicited chemotaxis was 2-fold less than that elicited by fMLP and 2-ClHDA. It should be noted that this disparity between fMLP and PMA as chemoattractants is similar to that previously reported (37). Additional experiments that included 2-ClHDA in the upper chamber of the Boyden chamber demonstrated that 2-ClHDA did not increase neutrophil chemokinesis (data not shown).

**DISCUSSION**

Recently, we reported that in a cell-free system the sn-1 vinyl ether bond of plasmalogen phospholipids is specifically attacked by myeloperoxidase-derived reactive chlorinating species to generate the novel α-chloro fatty aldehyde, 2-ClHDA (26). Because activated neutrophils release myeloperoxidase and hydrogen peroxide (40), the present study was designed to show the production of α-chloro fatty aldehydes by activated neutrophils. Utilizing NICI GC-MS analysis, 2-ClHDA in crude lipid extracts from the activated neutrophils was detected and quantitated as its PFB oxime derivative. The identification of the PFB oxime of 2-ClHDA from activated neutrophils was achieved by comparison of the mass spectrum and the mass chromatogram retention times with that arising from the synthetic 2-ClHDA standard. The formation of the monochlorinated compounds is highlighted by the observation of the predicted molecular anion pairs at m/z 469/471, the m/z 288/290 (M–PFB), and the Cl– ion at m/z 35/37 with abundance ratios of 3:1. Additionally, the 18 carbon α-chloro fatty aldehyde, 2-ClODA, was also observed. The detection of 2-ClODA was expected because the sn-1 aliphatic composition of plasmalogens of neutrophils is enriched with both 16:0 and 18:0 aliphatic groups (39). Taken together, these results demonstrate for the first time that the sn-1 vinyl ether bond of plasmalogens can be targeted during neutrophil activation with subsequent production of the α-chloro fatty aldehydes, 2-ClHDA and 2-ClODA.

It is likely that α-chloro fatty aldehydes are produced by activated neutrophils through a mechanism that is mediated by myeloperoxidase-derived reactive chlorinating species that target the plasmalogen vinyl ether bond. Myeloperoxidase-derived reactive chlorinating species are produced during neutrophil activation, and the present results show that inhibition of their production by the inclusion of the myeloperoxidase inhibitor azide, cyanide, or 3-aminotriazole dramatically attenuates the production of 2-ClHDA in PMA-stimulated neutrophils. It should be noted that our previous studies have demonstrated that the production of α-chloro fatty aldehydes is dependent on the presence of the masked aldehyde at the sn-1 position (vinyl ether bond) of plasmalogens only (26). Specifically, glycerophospholipid species containing ester linkages at the sn-1 and sn-2 positions or free fatty aldehydes themselves do not produce α-chloro fatty aldehydes when treated with myeloperoxidase-derived reactive chlorinating species (26). Given that 2-ClHDA and 2-ClODA are the predominant α-chloro fatty aldehydes produced by PMA-treated neutrophils and neutrophil plasmalogens are highly enriched with 16:0 and 18:0 aliphatic groups at their sn-1 positions respectively (39), it is highly likely that that neutrophil plasmalogens are targeted by myeloperoxidase-derived reactive chlorinating species released by activated neutrophils. Although significant changes in plasmalogen content were not detected in crude lipid extracts from control and PMA-treated neutrophil samples, it is unlikely that significant alterations in neutrophil vinyl ether content would
be detected given that the mass of α-chloro fatty aldehydes produced from PMA-treated neutrophils is three orders of magnitude less than the total mass of the neutrophil plasmalogen pool. It should be appreciated that, despite the targeting of plasmalogens and production of α-chloro fatty aldehydes, the relative maintenance of neutrophil membrane plasmalogen composition most likely precludes deleterious changes in membrane dynamics and function in the activated neutrophil stemming from altered membrane plasmalogen content.

Although fMLP induces significant neutrophil chemotaxis, the possibility that 2-ClHDA itself acts as a neutrophil chemoattractant was investigated. Many different aldehydes, such as 4-hydroxynonenal and 4-hydroxytetradecenal (41), thought to be produced by lipid peroxidation in the course of inflammatory reactions have been shown to induce significant neutrophil chemotaxis in vitro. 2-ClHDA induced neutrophil chemotactic activity less than that of fMLP, which corroborates with previously reported chemotactic activity induced by other aldehydes in relation to fMLP (41). Importantly, the generation of 2-ClHDA by neutrophils might represent a previously unrecognized factor driving ongoing neutrophil recruitment to sites of active inflammation while also lending support to the hypothesis that aldehydic lipid oxidation or peroxidation products play important roles in such mechanisms. The present studies suggest that neutrophils recruited to sites of active inflamma-

FIG. 5. Myeloperoxidase-dependent production of 2-chlorooctadecanal in PMA-treated human neutrophils. 20 pmol of synthetic 2-CIODA was converted to its respective PFB oxime and subjected to SIM-GC-MS analysis for \( m/z = 316 \) (A). 1 \( \times 10^6 \) neutrophils were treated with either 200 nm PMA (B), vehicle (C), or 200 nm PMA in the presence of 10 mM 3-aminotriazole (ATZ) (D) for 30 min at 37 °C. Reactions were terminated by the addition of methanol, and products were extracted into chloroform, converted to their PFB oximes, and analyzed by SIM-GC-MS for \( m/z = 316 \) (B–D). The mass spectrum of the PFB oximes of synthetic 2-CIODA (E) and 2-CIODA from PMA-stimulated neutrophils (F) eluting at 9.1 min are shown.
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2-Chlorohexadecanal induces neutrophil chemotaxis. 2 × 10⁵ neutrophils were loaded into the upper compartments of a Boyden chamber, and neutrophil chemotaxis was elicited by either 100 nM MLP, 90 μM 2-CHDA, 200 nM PMA, or chemotaxis buffer (as indicated) was measured as described under “Experimental Procedures.” Asterisks indicate treatments with p < 0.01 compared appropriate controls.

FIG. 7. 2-Chlorohexadecanal induces neutrophil chemotaxis.

Taken together, the present results demonstrate the targeting of vinyl ether bonds of plasmalogens in a biological system. This may represent a potentially important and as of yet unrecognized mechanism mediating the generation of α-chloro fatty aldehydes and lysophospholipids which may both play important roles in the pathophysiological sequelae of phagocyte-mediated diseases.

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