Myeloperoxidase-derived Reactive Chlorinating Species from Human Monocytes Target Plasmalogens in Low Density Lipoprotein

A role for myeloperoxidase (MPO) in atherosclerosis has received considerable attention recently. To identify potential chlorinated lipid products in human low density lipoprotein (LDL), studies were designed to demonstrate that MPO-derived reactive chlorinating species (RCS) target the plasmalogen pool of LDL isolated from peripheral human blood in vitro. The vinyl ether bond of LDL plasmalogens was targeted by MPO-derived RCS, resulting in the release of the 16- and 18-carbon-containing α-chloro fatty aldehydes, 2-chlorohexadecanal and 2-chlorooctadecanal, respectively, from the plasmalogen glycerol backbone. Targeting of the LDL plasmalogen vinyl ether bond was dependent on the presence of MPO-derived RCS. Electrospray ionization mass spectrometric analysis of MPO-treated LDL demonstrated that a novel population of unsaturated lysophosphatidylcholine molecular species was produced by a phospholipase A2-independent mechanism. Unsaturated lysophosphatidylcholine molecular species elicited cyclic AMP response element binding protein phosphorylation in RAW 264.7 cells. Additionally, MPO-mediated targeting of both monococyte and LDL plasmalogen pools was demonstrated in phorbol myristate acetate-stimulated human monocytes, resulting in the production of both 2-chlorohexadecanal and 2-chlorooctadecanal. In contrast, α-chloro fatty aldehydes were not produced in phorbol myristate acetate-stimulated mouse monocytes. Collectively, the present studies demonstrate a novel MPO-specific mechanism that mediates the production of a novel group of unsaturated lysophosphatidylcholine molecular species and chlorinated aldehydes from both LDL and monococyte plasmalogen pools that may have important effects during inflammatory reactions mediated by monocytes, most notably atherosclerosis.

Myeloperoxidase (MPO), a bactericidal enzyme secreted by activated phagocytes, specifically catalyzes the production of hypochlorous acid (HOCl) from chloride and hydrogen peroxide (1, 2). HOCl, in equilibrium with both its conjugate anion ·OCl and molecular Cl2, collectively compose the major reactive chlorinating species (RCS) produced by phagocytes. These RCS mediate a number of potentially deleterious reactions, which include the chlorination of unsaturated aliphatic groups (3) and tyrosine (4), oxidative bleaching of heme iron and sulfur protein centers (1, 5–7), as well as the generation of equally reactive and potentially damaging chloramines with primary amine groups (8).

Atherosclerosis is an inflammatory arterial pathology occurring most significantly in coronary and carotid arteries. Atherosclerosis is characterized by the development of immature fatty streaks that progress into mature plaques, attributable in part to the accumulation of necrosing oxidized low density lipoprotein (LDL)-laden macrophages termed foam cells in the arterial tunica intima. Although atherosclerotic lesions in wild-type mice do not contain MPO-derived products and MPO-knock-out mice have larger atherosclerotic lesions in comparison with wild-type mice (9), multiple studies have implicated MPO with human atherosclerotic pathophysiology. Active MPO has been isolated from human atherosclerotic tissue (10), and the MPO-specific product 3-chlorotyrosine (4) as well as HOCl-modified proteins (11) has been detected at elevated levels in atherosclerotic lesions as compared with normal vascular tissue. Additionally, elevated blood and leukocyte MPO levels have been shown to significantly correlate with the presence of coronary artery atherosclerosis in patients (12). Lastly, HOCl has been shown to activate matrixin (matrix metalloproteinase-7) in vitro, and MPO colocalizes with this matrix metalloproteinase in tissue sections from vulnerable plaques, suggesting a role for MPO in the process of plaque rupture as well (13).

Plasmalogens are a glycerophospholipid species enriched in the plasma membranes of many cells present in the mammalian cardiovascular system and are characterized by the presence of a vinyl ether bond-linked aliphatic chain at the sn-1 position (14–17). The physiological importance of plasmalogens likely includes roles as storage depots of esterified arachidonic acid; RCS, reactive chlorinating species; LDL, low density lipoprotein; LPC, lysophosphatidylcholine; 2-Cl-[d1]-HDA, 2-chloro-[7,8,9-d4]-hexadecanal; FFB, pentafluorobenzyl; CREB, cyclic AMP response element binding protein; pCREB, phospho-CREB; ATZ, 3-aminotriazole; GC-MS, gas chromatography-mass spectrometry; ESI-MS, electrospray ionization mass spectrometry; NICI, negative ion chemical ionization; 2-CH2DCA, 2-chlorohexadecanal; SIM, selected ion monitoring; DDE, Dulbecco’s Modified Eagle medium; HBSS, Hank’s balanced salt solution; PMA, phorbol myristate acetate.

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acid (15–17) as well as the solvation of transmembrane proteins such as the sodium-calcium exchanger (18–20).

Because plasmalogens have been identified in human lipoproteins (21) and because LDL attack by MPO-derived RCS produced from macrophages has been considered to be at least one mechanism leading to LDL modification and subsequent foam cell development (22), the present studies were performed to determine the susceptibility of human LDL plasmalogens pools to RCS attack. The present findings now demonstrate that human LDL plasmalogens are rapidly modified by MPO-derived RCS, resulting in the production of α-chloro fatty aldehydes as well as unsaturated lysophosphatidylcholine (LPC) molecular species. Furthermore, activated human monocytes produce RCS that attack both monocyte and LDL plasmalogens pools to produce α-chloro fatty aldehydes as well. Taken together, these data demonstrate that RCS-derived from activated monocytes target human lipoprotein plasmalogens pools, resulting in the production of novel lipid species that may participate in monocyte signaling pathways as well as have a role in atherosclerosis.

EXPERIMENTAL PROCEDURES

Materials—2-Cl-[7,8,9,10]d4-hexadecanal (2-Cl-[d4]HDA) was synthesized and purified as described previously (23). Chloride-free sodium hyochlorite was prepared in chlorine demand-free conditions and chloride-free glassware by a modification of a method described previously (24, 25). The concentration of sodium hyochlorite was determined spectrophotometrically (ε250 = 350 x cm−1) (26), and sodium hyochlorite was used immediately for experiments. MPO was purchased from Calbiochem. 1-Tetradecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (14:0 LPC), 1,2-di-octadec-9′-enoyl-sn-glycero-3-phosphocholine (di-18:2 PC), and 1,2-dieicosatetra-5′/H11032/5′/H11032/5′/H11032/5′/H11032/5′-enoyl-sn-glycero-3-phosphocholine (di-20:4 PC) were purchased from Avanti. Pentafluorobenzyl (PFB) hydroxylamine was purchased from Aldrich. DNase I was purchased from Roche Diagnostics. PVDF-plus membranes were purchased from X-OMAT AR and reaction products were sequentially extracted into chloroform by the method of Bligh and Dyer (29). Reactions were then terminated by the addition of methanol containing 14.0 LPC, and reaction products were sequentially extracted into chloroform. These extracts were then analyzed by electrospray ionization mass spectrometry (ESI-MS) for the production of LPC molecular species as described below.

Preparation of PFB Oximes of α-Chloro Fatty Aldehydes and Quantitative GC-MS Analysis—Lipid extracts isolated from the various LDL treatments were evaporated to dryness under N2, and α-chloro fatty aldehydes were converted to their respective PFB oxime derivatives by using a modification of a method described previously (23, 31). Specifically, the total integrated peak area arising from m/z 288, the structurally informative fragment ion produced from the PFB oxime of 2-CH2H2ODA, was compared with the total integrated peak area produced by m/z 292, the structurally informative fragment ion of the PFB oxime of 2-Cl-[d4]HDA. Also, 2-chloro-5-oxodecan-2-CIOA, the 18-carbon-containing α-chloro fatty aldehyde, was monitored by detection of m/z 316, the structurally informative fragment ion produced from the PFB oxime of 2-CIOA. For monocyte experiments, 2-CIOA was quantified by comparison of the integrated peak area produced by m/z 316 to that produced by m/z 292 from the PFB oxime of 2-Cl-[d4]HDA.

ESI-MS Analysis of LPC Species—Liquid chromatography-electrospray mass spectrometric analysis was conducted on a Finnigan (San Jose, CA) TSQ 7000 mass spectrometer equipped with the ICIS data system. The phospholipid extracts were dissolved in chloroform:methanol:1% v/v aqueous methanolic LiCl or sodium hypochlorite (1:1:1). The solution was then filtered through 0.45-μm filter, and the filtrate was analyzed by ESI-MS. Quantification was performed by comparison of the integrated peak area produced by m/z 316 to that produced by m/z 292 from the PFB oxime of 2-Cl-[d4]HDA.

For the quantification of LPC molecular species produced by MPO treatment of LDL, LDL (500 μg) placed in sodium phosphate buffer pH 4 supplemented with 1 mM H2O2 and 100 mM NaCl was incubated for 1 h at 37 °C in the presence or absence of MPO (10 units). Reactions were then terminated by the addition of methanol containing 14.0 LPC, and reaction products were sequentially extracted into chloroform. These extracts were then analyzed by electrospray ionization mass spectrometry (ESI-MS) for the production of LPC molecular species as described below.

Unsaturated LPC Synthesis and HPLC Purification—Unsaturated LPC molecular species were synthesized from 1,2-diacyl-sn-glycero-3-phosphocholine species as described previously (32). In brief, di-18:2 PC and di-20:4 PC were evaporated to dryness under N2, and resuspended in 100 mM borate buffer pH 6.5 and diethyl ether (1:1, v/v) respectively. After the addition of Rhizopus lipase (9 × 104 units/mg diacyl PC), the mixture was shaken vigorously for 1 h at 24 °C. Reactions were then terminated by the addition of ice-cold methanol, and lipids were extracted into chloroform by the method of Bligh and Dyer (29). Reactions were confirmed by thin layer chromatography (silica gel G plates) with chloroform:methanol:ammonium hydroxide (65:25:5, v/v/v) as the mobile phase. The unsaturated LPC molecular species were resolved using a silica stationary phase (Dynamax 80A column) and a nonlinear gradient. The mobile phase consisted of solvent A (hexane:isopropanol:water:96:5:6.5, v/v/v) as the mobile phase. For the purification of the HPLC-identified lipid species, quantification was performed by GC analysis using flame ionization detection of their respective fatty acid methyl ester derivatives using arachidic acid (20:0 fatty acid) as an internal standard (33).
Plasmenogen Targeting by Reactive Chlorinating Species

Monocyte Activation—Human monocytes were isolated from peripheral blood as described previously (34). In brief, anticoagulated blood was diluted with Ca²⁺/Mg²⁺-free HBSS and subjected to Ficoll density gradient centrifugation (density, 1.077 g/ml; 400 × g for 45 min). The middle layer of cells containing monocytes were collected and diluted 1:1 with Ca²⁺/Mg²⁺-free HBSS, which was then subjected to further Ficoll density gradient centrifugation (density, 1.070 g/ml; 400 × g for 15 min). The mid-floating layer was plated on 60-mm culture dishes (5.7 × 10⁵) in Medium 199 and cultured at 37 °C for 1 h in a 5% CO₂ atmosphere for 70 h in Dulbecco’s Modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, and 6 mM l-glutamine. The cells were incubated with 2 units of MPO in the presence of 1 mM H₂O₂ and 100 μM Na⁺VO₄, and 50 mM NaF (phosphatase inhibitors). The samples were boiled for 3 min and sequentially subjected to SDS-PAGE using 15% polyacrylamide gels followed by the transfer of proteins to PVDF plus membranes for Western blot analysis. Anti-CREB and anti-pCREB antibodies were used as primary antibodies (1:1333 dilution, plus membranes for Western blot analysis. Anti-CREB and anti-pCREB antibodies were used as primary antibodies (1:1333 dilution, respectively) along with the horseradish peroxidase-conjugated secondary antibody (1:7000 dilution). Immunoreactive bands were then visualized by chemiluminescence detected on x-ray film using the ECL chemiluminescence system after 1-h exposure. Multiple exposures of film to blot were developed. Exposures that had linear levels of grain development were used for quantitation of band intensity using NIH Image software after scanning and conversion of autoradiographic data to TIFF file formats using a Macintosh 5500/225 computer and a Lincolor-Hell-Jade scanner. Quantitative analysis of autoradiographic data was performed using the public domain NIH Image program.²

Experimental Procedures.

Fig. 1. NICI mass spectrum of 2-ClHDA derived from LDL treated with the MPO/RCS generating system. 100 μg of LDL was incubated with 2 units of MPO in the presence of 1 mM H₂O₂ and 100 mM NaCl in phosphate buffer (pH 4) for 30 min at 37 °C. Reactions were terminated by the addition of methanol, and reaction products were extracted into chloroform. After conversion of reaction products to their respective PFB oximes, NICI GC-MS analysis was performed as described under “Experimental Procedures.” Shown is the mass spectrum from a peak derivatized lipid extracts from MPO/RCS treatment that had an identical retention time to that of the authentic PFB oxime of 2-ClHDA (∼8.4 min).

Western Blot Analysis of CREB/pCREB Expression in RAW 264.7 Cells—RAW 264.7 cells (4 × 10⁶) were plated on 35-mm culture dishes and cultured at 37 °C in a 5% CO₂ atmosphere for 70 h in Dulbecco’s Modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, and 6 mM l-glutamine. One h before treatment, cells were incubated with DMEM supplemented with 2% fetal calf serum. The cells were incubated with the indicated concentrations of sonicated unsaturated LPC molecular species added in 25 μl of DMEM. After the cells were incubated for 30 min at 37 °C, the medium was removed from the dishes, and the cells were scraped in SDS sample buffer containing dithiothreitol, 400 units/ml DNase I, 200 μM Na₃VO₄, and 50 mM NaF (phosphatase inhibitors). The samples were boiled for 3 min and sequentially subjected to SDS-PAGE using 15% polyacrylamide gels followed by the transfer of proteins to PVDF membranes for Western blot analysis. Anti-CREB and anti-pCREB antibodies were used as primary antibodies (1:1333 dilution, respectively) along with the horseradish peroxidase-conjugated secondary antibody (1:7000 dilution). Immunoreactive bands were then visualized by chemiluminescence detected on x-ray film using the ECL chemiluminescence system after 1-h exposure. Multiple exposures of film to blot were developed. Exposures that had linear levels of grain development were used for quantitation of band intensity using NIH Image software after scanning and conversion of autoradiographic data to TIFF file formats using a Macintosh 5500/225 computer and a Lincolor-Hell-Jade scanner. Quantitative analysis of autoradiographic data was performed using the public domain NIH Image program.²

Fig. 2. NICI SIM GC-MS analysis of PFB oximes of 2-ClHDA produced from LDL treated with the MPO/RCS generating system. LDL suspensions (100 μg) were treated as indicated as described under “Experimental Procedures.” Reactions were terminated by the addition of methanol containing 2-Cl-[d₆]-HDA, and reaction products were extracted into chloroform. After conversion of reaction products to their respective PFB oximes, SIM GC-MS analysis using NICI for m/z 288 and m/z 292 was performed on derivatized reaction products from treatments without MPO (A), with MPO (B), with MPO in the presence of 10 mM 3-amino triazole (C), and with MPO but in the absence of LDL (D).

vage of C57BL mice and plated on 60-mm culture dishes as described previously (35) at the same plating density as that used for human monocytes. Mouse monocyte experimental conditions were identical to those used for human monocytes. Experimental intervals were terminated by decanting media and rapidly subjecting media and cells to Bligh-Dyer extraction of lipids in the presence of 3 pmol of 2-Cl-[d₆]-HDA and 50 μg of diolein (added as carrier lipid). 2-ClHDA and

² Internet address: rsb.info.nih.gov/nih-image/
2-ClODA were then quantitated in each fraction by GC-MS of their PFB oxime derivatives (see above). The relative amount of 16- and 18-carbon vinyl ether aliphatic chains in LDL and monocyte plasmalogens pools was also determined by subjecting dried lipid extracts from these respective lipid sources to HCl fumes for 10 min before conversion of the resultant fatty aldehyde product (from the plasmalogen vinyl ether aliphatic group) to their PFB aldehyde derivatives that were analyzed by GC-MS under identical conditions as those for 2-ClHDA and 2-ClODA.

RESULTS

To determine that MPO-derived RCS target LDL plasmalogens, human LDL isolated from peripheral blood was incubated with RCS generated from MPO, and lipidic products were converted to their PFB oximes and subjected to NICI GC-MS analysis. Fig. 1 shows the mass spectrum of a derivative that has an identical retention time (~8.4 min) with that of the authentic PFB oxime of 2-ClHDA and has a fragmentation pattern very similar to that of the authentic PFB oxime of 2-ClODA (~9.1 min) [A]. Lipid extracts from untreated (control) [B] and MPO-treated [C] LDL samples were also converted to their PFB oximes and analyzed by SIM GC-MS for m/z 316 and m/z 35.

2-ClODA were then quantitated in each fraction by GC-MS of their PFB oxime derivatives (see above). The relative amount of 16- and 18-carbon vinyl ether aliphatic chains in LDL and monocyte plasmalogens pools was also determined by subjecting dried lipid extracts from these respective lipid sources to HCl fumes for 10 min before conversion of the resultant fatty aldehyde product (from the plasmalogen vinyl ether aliphatic group) to their PFB aldehyde derivatives that were analyzed by GC-MS under identical conditions as those for 2-ClHDA and 2-ClODA.

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Next, using synthetic 2-Cl-[d$_4$]-HDA as an internal standard with selective ion monitoring (SIM GC-MS) for m/z 288 and m/z 292, 2-ClHDA production in LDL was quantified under selected experimental conditions. In the absence of MPO, 2-ClHDA was not detected in LDL, as shown by the absence of
Fig. 6. MPO/RCS targeting of LDL plasmalogens generates unsaturated LPC molecular species. LDL (500 µg) was incubated with the MPO/RCS generating system as described under “Experimental Procedures.” After incubation, reactions were terminated by the addition of methanol containing 14:0 LPC, and lipids were extracted into chloroform and analyzed by ESI-MS as described under “Experimental Procedures.” Values represent the mean ± S.E.

m/z 288 (Fig. 2A). However, treating LDL with RCS generated from MPO resulted in the detection of 2-CHDA, as demonstrated by the appearance of two peaks for m/z 288, corresponding to the syn- and anti-isomers (36) of the PFB oxime derivative of 2-CHDA, which had identical retention times (−8.3 and −8.4 min) with the authentic PFB oxime of 2-CHDA (Fig. 2B). Additionally, control experiments demonstrated that the addition of the MPO inhibitor ATZ to conditions used in Fig. 2B eliminated 2-CHDA production (Fig. 2C). Furthermore, the MPO/RCS generating system alone did not produce 2-CHDA in the absence of LDL plasmalogen substrate (Fig. 2D). −1.3 pmol of 2-CHDA/nmol of inorganic phosphate was produced in LDL treated with MPO-generated RCS (Fig. 3). 2-CHDA production was attenuated by the presence of the MPO inhibitors sodium cyanide, ATZ, or sodium azide, respectively, which further supports the idea that MPO-generated RCS target LDL plasmalogens (Fig. 3). Furthermore, the requirements for a source of RCS as well as a plasmalogen substrate are supported by LDL treated with the RCS HOCI, producing −2.8 pmol of 2-CHDA/nmol of inorganic phosphate. The enhanced production of 2-CHDA in HOCI-treated LDL in comparison to that in MPO-treated LDL is expected because LDL plasmalogen vinyl ether substrates are not limiting in this system, whereas RCS production by MPO is rate-limiting. Specifically, ESI-MS analysis of LDL plasmalogens did not significantly decrease during treatments with RCS derived from MPO (data not shown). 2-CHDA production in LDL incubated with MPO was pH-dependent (Fig. 4) with optimal attack of plasmalogens observed at pH 6 and below whereas targeting of plasmalogens at neutral pH was minimal.

Because plasmalogen molecular species also include those with 18-carbon aliphatic groups at their sn-1 positions (33), the possibility that these LDL plasmalogens also are targeted was investigated. Accordingly, reaction products from MPO-treated LDL were converted to their PFB oxime derivatives and subjected to NICI GC-MS analysis. For this analysis, the mass spectrum of the PFB oxime of 2-CIODA should yield structurally informative fragment ions (m + 28)/z in comparison to the analogous ions produced by the fragmentation of the PFB oxime of 2-CHDA (as shown in Fig. 1). Analysis of PFB-derivatized lipids from LDL treated with the MPO/RCS generating system as samples is shown in Fig. 5A, which shows the mass spectrum for a peak that had an identical retention time as that of the PFB oxime of authentic 2-CIODA eluting at −9.05 min. As predicted, this mass spectrum shows the ion pair m/z 316/318 present at a 3:1 ratio, respectively, which is specific to monochlorinated compounds, and the mass of these ions is increased by 28 mass units compared with that corresponding to the PFB oxime of 2-CHDA. Also, the mass spectrum shows m/z 442 (M-HF-Cl), the ion pair m/z 35/37 at 3:1 ratio, m/z 178, and m/z 196. Taken together, these data demonstrate that 2-CIODA is also produced by MPO-derived RCS targeting of LDL plasmalogens.

Saturated LPC (1-hexadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine; 16:0 LPC) produced by phospholipase A2 has been considered previously as a mediator of atherosclerosis (33, 37–42). Because LDL lipid pools possess plasmalogens with unsaturated aliphatic groups at the sn-2 position, it would be predicted that the targeting of the sn-1 vinyl ether bond of LDL plasmalylcholine by RCS would yield unsaturated LPC molec-
ular species. Accordingly, lipid extracts prepared from LDL treatments with and without MPO-generated RCS were analyzed by ESI-MS in the positive ion mode (Fig. 6). Incubation of LDL with MPO resulted in the generation of unsaturated LPC molecular species including 18:2 LPC, 20:4 LPC, and 1-hydroxy-2-docosahexaenoyl-sn-glycero-3-phosphocholine (22:6 LPC), which are present at significantly increased levels compared with control treatments (i.e., in the absence of MPO-generated RCS). Only unsaturated LPC molecular species were generated by RCS attack of LDL plasmalogens, thus confirming the targeting of the sn-1 aliphatic chain of the putative LPC precursors (plasmalogens). Collectively, this demonstrates for the first time a novel mechanism based on MPO-derived RCS targeting of plasmalogens that produces a population of novel unsaturated LPC species through a phospholipase A₂-independent mechanism.

Additional studies were performed to determine whether activated monocytes produce RCS that target LDL plasmalogens. Initial experiments showed that the ratio of 16-carbon to 18-carbon vinyl ether aliphatic chains in LDL plasmalogen pools was nearly twice that in the human monocyte plasmalogen pool (data not shown). Data in Fig. 8A show that PMA stimulation of human monocytes elicits the production of both 2-CIHDA and 2-CIODA that is associated with cells. Additionally, cell-associated α-chloro fatty aldehydes levels further increased in PMA-stimulated human monocytes in the presence of LDL. In both the presence and absence of LDL, human monocyte-associated α-chloro fatty aldehydes produced in response to PMA were comprised of about twice the amount of both 50 μM 18:2 and 50 μM 20:4 LPC elicited increases in CREB phosphorylation in relation to control. Moreover, CREB phosphorylation increased relative to control as LPC concentrations of both molecular species were increased to 100 μM, respectively. It should be appreciated that these concentrations of unsaturated LPC molecular species are below the critical micellar concentration in this medium containing serum. In contrast, 2-CIHDA does not elicit CREB phosphorylation (data not shown).

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2-ClODA compared with 2-CHDA (Fig. 8A). The presence of sodium azide blocked the production of PMA-stimulated α-chloro fatty aldehyde production (Fig. 8A). Furthermore, analysis of the media revealed that α-chloro fatty aldehydes were present predominantly when PMA-stimulated human monocytes were incubated in the presence of LDL (Fig. 8B). However, the relative increase in the amount of 2-CHDA versus 2-ClODA in the media in comparison to the relative amounts associated with the human monocytes suggests that LDL plasmalogen pools were the targets of released RCS from monocytes. Additional studies were performed using mouse monocytes, which contain ~40% of the plasmalogen content that we found in human monocytes. In sharp contrast to the results with human monocytes, activation of mouse monocytes with PMA did not result in α-chloro fatty aldehyde production that was cell-associated (Fig. 8A). Furthermore, in these studies with mouse monocytes, α-chloro fatty aldehydes in the media (in both the presence and absence of human LDL in the media) was not increased during mouse monocyte stimulation with PMA (Fig. 8D).

DISCUSSION

Although MPO has been implicated as a mediator of atherosclerosis (4, 10, 12, 13), the putative mechanism by which MPO initiates or promotes atherogenesis has not been delineated. Furthermore, chlorinated lipid species produced from monocyte-derived RCS attack of cellular and lipoprotein lipids had not been identified. In the studies herein, MPO-derived RCS have been shown to target LDL plasmalogen pools in both in vitro and human monocyte cell culture systems. Specifically, the masked aldehyde vinyl ether bond of LDL plasmalogens are cleaved, resulting in the release of the α-chloro fatty aldehydes, 2-CHDA and 2-ClODA, from the glycerol backbone of plasmalogens. Moreover, RCS attack of LDL plasmalogen pools produced novel unsaturated LPC molecular species that may contribute to atherosclerotic lesion development through activation of gene expression. It should also be noted that although multiple studies have suggested a role for MPO in human atherosclerosis (4, 10–13), catalytically active MPO is not present in murine atherosclerotic lesions (9). The present findings further support this important difference between humans and mice; although activation of human monocytes leads to the production of the plasmalogen catabolites, α-chloro fatty aldehydes, these products are not observed in activated mouse monocytes. This difference likely is reflected by the nearly 10-fold excess of MPO in human monocytes compared with mouse monocytes (9).

The overall effects elicited by 2-CHDA (and 2-ClODA) from RCS attack of lipoprotein plasmalogens remain to be determined. However, other aldehyde products of lipid (per)oxidation reactions have been implicated with atherosclerosis. For example, hydroxynonenal has been shown to mediate atherogenic effects, such as down-regulating IκB phosphorylation, leading to a suboptimal immune response and the low-level, chronic inflammatory response thought to characterize atherosclerotic progression (43). Additionally, hydroxynonenal-modified lysine residues have been detected in atherosclerotic tissue as well (44). It is speculated that 2-CHDA produced by RCS attack of LDL and monocyte plasmalogens may form Schiff base adducts with both proteins and primary amine-containing phospholipids such as phosphatidylserine and phosphatidylethanolamine. Formation of these Schiff base adducts would likely disrupt normal protein function and membrane dynamics.

Phospholipase A₂-mediated production of LPC has been suggested to be a proatherogenic mechanism because saturated LPC mediates numerous atherogenic effects, including the up-regulation of endothelial cell surface P-selectin expression (45) and induction of monocyte chemotaxis (46). The production of unsaturated LPC molecular species by RCS targeting of plasmalogens represents a unique phospholipase A₂-independent mechanism for LPC production. It should be appreciated that in comparison to saturated LPC generated by phospholipase A₂, the production of unsaturated LPC by RCS targeting of plasmalogens would not be subject to the highly regulated activity characteristic of such enzymes. Unsaturated LPC molecular species and lysoplasmenylcholine have been shown previously to activate cyclic AMP-dependent protein kinase, leading to CREB phosphorylation (47, 48). Furthermore, evidence has emerged that genes thought to play roles in atherosclerosis contain cyclic AMP response elements in their promoter regions, most notably cyclooxygenase-2 (49).

Plasmalogens have traditionally been regarded as storage depots for esterified arachidonic acid (15–17) and as structural elements stabilizing transmembrane proteins (18, 19). Previous studies have suggested that plasmalogens may be protective by serving as antioxidants during lipoprotein oxidation because the vinyl ether bond acts as a non-propagating sink for reactions mediated by reactive oxidizing species (21). In contrast, the attack of the vinyl ether bond by RCS produces two potentially important bioactive lipidic compounds, α-chloro fatty aldehydes and unsaturated LPC molecular species, that may serve as important signaling molecules in monocyte-mediated inflammatory processes.
Plasmalogen Targeting by Reactive Chlorinating Species

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