Class A Scavenger Receptor-mediated Macrophage Adhesion Requires Coupling of Calcium-independent Phospholipase A$_2$ and 12/15-Lipoxygenase to Rac and Cdc42 Activation*

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Class A scavenger receptors (SR-A) participate in multiple macrophage functions including adhesion to modified extracellular matrix proteins present in various inflammatory disorders such as atherosclerosis and diabetes. By mediating macrophage adhesion to modified proteins and increasing macrophage retention, SR-A may contribute to the inflammatory process. Eicosanoids produced after phospholipase A$_2$ (PLA$_2$)-catalyzed release of arachidonic acid (AA) are important regulators of macrophage function and inflammatory responses. The potential roles of AA release and metabolism in SR-A-mediated macrophage adhesion were determined using macrophages adherent to modified protein. SR-A-dependent macrophage adhesion was abolished by selectively inhibiting calcium-independent PLA$_2$ (iPLA$_2$) activity and absent in macrophages isolated from iPLA$_2$$^{β^-/-}$ mice. Our results further demonstrate that 12/15-lipoxygenase (12/15-LOX)-derived, but not cyclooxygenase- or cytochrome P450-dependent epoxygenase-derived AA metabolites, are specifically required for SR-A-dependent adhesion. Because of their role in regulating actin polymerization and cell adhesion, Rac and Cdc42 activation were also examined and shown to be increased via an iPLA$_2$ and LOX-dependent pathway. Together, our results identify a novel role for iPLA$_2$-catalyzed AA release and its metabolism by 12/15-LOX in coupling SR-A-mediated macrophage adhesion to Rac and Cdc42 activation.

Cell adhesion to the substratum involves cell surface receptors that bind components of the extracellular matrix and initiate intracellular signaling cascades that regulate actin polymerization and focal adhesion formation (for review, see Refs. 1–4). Class A macrophage scavenger receptors (SR-A)$^4$ are homotrimeric membrane glycoproteins that mediate multiple functions including calcium-independent macrophage adhesion to modified extracellular matrix components. Adhesion substrates for SR-A include glycated and cigarette smoke-modified collagen type IV, denatured collagen type I, and β-amyloid fibrils (5–9). In addition, proteoglycans that are up-regulated during inflammation, such as biglycan and decorin, are ligands for SR-A (10). Macrophages isolated from transgenic mice overexpressing SR-A display increased spreading in culture and enhanced macrophage accumulation in carrageenan-induced granulomas in vivo (11). Furthermore, macrophage activation increases SR-A expression and SR-A-dependent macrophage adhesion (12, 13). In contrast, SR-A-deficient macrophages fail to acquire spread morphology when plated on modified protein (14). Taken together these observations suggest an important role for SR-A-mediated macrophage adhesion in various inflammatory processes characterized by macrophage activation and modification of the extracellular matrix.

Macrophages respond to various physiological and pathological stimuli via the activation of intracellular signaling cascades including phospholipase A$_2$ (PLA$_2$)-catalyzed hydrolysis of arachidonic acid (AA) from membrane phospholipids (15). Based on their location and Ca$^{2+}$ requirements for enzymatic activity, PLA$_2$s can be classified into three groups: secretory PLA$_2$s that are secreted from cells and require millimolar Ca$^{2+}$, cytosolic (cPLA$_2$s) that require micromolar Ca$^{2+}$, and calcium-independent (iPLA$_2$s) that reside in the cytosol of resting cells but do not require Ca$^{2+}$ for enzymatic activity (16). Several members of the iPLA$_2$ family are now recognized, and they are designated group VI PLA$_2$ enzymes (17). The first recognized and best characterized is the group VIA PLA$_2$ (18–20), which is also designated iPLA$_2$$^β$ (21, 22).

Most cellular AA is esterified to the glycerol backbone of phospholipids. The free AA that is accessible to AA-metabolizing oxygenases is generally thought to be released by the action of either cPLA$_2$ or iPLA$_2$. Whether AA release is involved in regulating SR-A function is not yet known, but this possibility is suggested by the finding that acetylated low density lipoprotein promotes tumor necrosis factor α production in macrophages via a pathway that depends on Ca$^{2+}$ and PLA$_2$ activation, arachidonic acid; BEL, bromoelone lactone (6E-[bromoethylene]-tetrahydro-3-(1-naphthyl)-2H-pyran-2-one); COX, cyclooxygenase; LOX, lipoxygenase; CytoP450, cytochrome P450-dependent epoxygenase; NDGA, nordihydroguaiaretic acid; MDA-BSA, malondialdehyde-modified bovine serum albumin; MPM, mouse peritoneal macrophage.
although the specific receptor mediating this response has not been identified (23). In addition, a role for specific PLA₂ isoforms in regulating the calcium-independent SR-A adhesion has not been investigated.

PLA₂-derived AA is subsequently metabolized to produce a variety of biologically active eicosanoids. Enzymes that metabolize AA include cyclooxygenases (COX), which catalyze the production of prostaglandins and thromboxanes, lipoxigenases (LOX), which catalyze the production of hydroxyeicosatetraenoic acid, lipoxins, and leukotrienes, and cytochrome P450-dependent epoxigenases (CypP450), which synthesize epoxyeicosatrienoic acids (24–26). Although AA-derived metabolites are involved in intercellular signaling, recent evidence indicates that AA metabolites also participate in regulating intracellular signaling processes including those involved in actin polymerization (15, 27–29).

Activation of Rho-GTPases, including Rac and Cdc42, plays a key role in coordinating cell adhesion (30–32). Binding of cell surface receptors to the extracellular matrix promotes Rac and Cdc42 activation, which in turn activates additional signaling molecules that ultimately result in the projection of actin-containing fibers and changes in the cytoskeleton that are required for acquiring a spread morphology and firm cell adhesion. In contrast, Rho activation promotes fiber retraction and detachment that are characteristic of migrating cells (33, 34). We have previously shown that SR-A mediates macrophage spreading on modified protein (14), but the potential involvement of Rac and Cdc42 in coupling surface SR-A binding to modified protein with increased macrophage adhesion has not been investigated.

The aim of the current study was to determine the potential role for specific PLA₂ isoforms and AA metabolites in regulating Rac and Cdc42 activation and SR-A-mediated macrophage adhesion to modified protein. For this we used pharmacologic inhibitors of specific AA signaling pathways and macrophages isolated from mice lacking specific AA metabolic enzymes. Our results indicate that iPLA₂-catalyzed release of AA and its subsequent metabolism by 12/15-LOX couples SR-A to Rac and Cdc42 activation and macrophage adhesion.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Dulbecco’s modified Eagle’s medium (DMEM) with 1-glutamine, DMEM with 25 mM HEPES without phenol red, and penicillin, streptomycin, heat-inactivated fetal bovine serum, and human plasma fibronectin were purchased from Invitrogen. Serum-free DMEM was purchased from BioWhittaker. DMEM with 25 mM HEPES was purchased from Biological Industries, Inc. (Kibbutz Beit Haemek, Israel). DMEM with phenol red and 1-glutamine was purchased from Biofluids. DMEM with 1-glutamine was purchased from Invitrogen. DMEM with 1-glutamine and phenol red was purchased from Invitrogen. Ultra low attachment polystyrene 6-well culture plates were purchased from Costar (Corning, NY). SKF525A was purchased from Biomol Research Laboratories, Inc. (Plymouth, PA). AACOCF₃ (arachidonoyltrifluoromethyl ketone), bromoelaidoyl lactone (BEL, 6F-bromoethylene-tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one), cPLA₂ inhibitor, MK-886 (3-[p-chlorobenzy]-5-[(isopropyl)-3-t-butylthioindol-2-yl]-2,2-dimethylpropanoic acid, Na), and indomethacin (1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid) were purchased from Calbiochem. Nordihydroguaiaretic acid (NDGA) and prostaglandin E₂ enzyme-linked immunoassay kits were purchased from Cayman Chemical (Ann Arbor, MI). Malondialdehyde bis(dimethyl acetal) was purchased from Sigma Aldrich.

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**Malondialdehyde Modification of Bovine Serum Albumin**—Malondialdehyde-modified BSA (MDA-BSA) was prepared using malondialdehyde bis(dimethyl acetal) as previously described (35). Protein modification was confirmed by immunoblotting with anti-MDA-specific antibody (Academy Bio-Medical Co., Inc; Houston, TX).

**Cell Isolation and Culture**—Mouse peritoneal macrophages (MPM) were harvested from wild-type mice (The Jackson Laboratory, Bar Harbor, ME), SR-A−/− (University of Kentucky), iPLA₂β−/− mice (prepared and characterized elsewhere (36–39)), 5-LOX−/− mice, or 12/15-LOX−/− mice (The Jackson Laboratory) via peritoneal lavage with ice-cold sterile saline and cultured in Dulbecco’s modified Eagle’s medium containing fetal bovine serum (10% v/v), penicillin, and streptomycin as previously described (40). Animal care and use for all procedures was done according to protocols reviewed and approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

**Cell Adhesion Assays**—To assess cell spreading, MPM were plated (30,000 cells/well) into four-chambered LAB-TEK slides (Nalge Nunc International; Naperville, IL) precoated with either MDA-BSA or fibronectin and treated with inhibitors as described in the figure legends. Trypan blue exclusion was used to confirm that the treatments did not affect cell viability. After treatments, macrophages were gently washed with warm phosphate-buffered saline, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100. The cells were then blocked with 1% BSA for 30 min before staining with Alexa Fluor 568-conjugated phallolidin and 4′,6-diamidino-2-phenylindole (Molecular Probes; Eugene, OR). Cells were mounted in Prolong Antifade reagent (Molecular Probes), and random images of at least 25 cells from at least three independent experiments were digitally captured using a Leica TCS SP confocal microscope. Individual cells were outlined, and total cell area was quantified using Metamorph® software.

**Rac/Cdc42 Activation Assay**—To examine Rac and Cdc42 activation during SR-A-dependent macrophage adhesion, cells were treated as described in figure legend 5 and then lysed in radioimmune precipitation assay buffer containing phosphatase and protease inhibitors. Protein concentration was determined using the Bio-Rad DC assay. Equal amounts of cell lysate protein were incubated with the p21 binding domain of PAK1 fused to glutathione S-transferase beads for 1 h at 4°C. The p21 binding domain of PAK binds the GTP-bound (active) form of Rac and Cdc42 but not the GDP-bound state (41). Glutathione S-transferase-PAK beads were isolated, and the amount of GTP-bound (active) Rac and Cdc42 was quantified by immunoblotting isolated proteins with Rac1 and Cdc42-specific antibodies (BD Transduction Laboratories). Proteins were visualized by enhanced chemiluminescence using anti-mouse horseradish peroxidase-coupled secondary antibody. Images were digitally captured and quantified using an Eastman Kodak Co. Image Station 4000MM. The amount of GTP-bound Rac was normalized to the total amount of Rac or Cdc42 detected in each cell lysate.
RESULTS

SR-A-mediated Macrophage Adhesion Requires PLA2-mediated AA Release—Macrophages respond to various stimuli via activation of intracellular signaling cascades including the PLA2-catalyzed hydrolysis of AA from membrane phospholipids. Therefore, we examined the potential role for AA in regulating SR-A-dependent macrophage adhesion to MDA-BSA. Malondialdehyde-modified proteins are SR-A ligands and have been previously used to study SR-A function (35, 42).

To determine whether PLA2-mediated AA release plays a role in SR-A-dependent adhesion, peritoneal macrophages isolated from SR-A<sup>+/+</sup> or SR-A<sup>−/−</sup> mice were pretreated with AACOCF3 (30 µM), an inhibitor of cPLA2 and iPLA2, before plating on MDA-BSA-coated slides. After 2 h, macrophage morphology was assessed by staining polymerized actin (F actin) with fluorescently conjugated phalloidin. Consistent with our previous report (14), control (SR-A<sup>+/+</sup>) macrophages that were allowed to adhere to MDA-BSA for 2 h exhibited enhanced spreading compared with SR-A<sup>−/−</sup> macrophages (Fig. 1). Importantly, SR-A<sup>+/+</sup> and SR-A<sup>−/−</sup> macrophages spread to the same extent when allowed to adhere to an integrin ligand (14). Like SR-A<sup>−/−</sup> macrophages, macrophages treated with AACOCF3 remained rounded when allowed to adhere to MDA-BSA, which indicates that PLA2 activation is required for SR-A-mediated macrophage adhesion. The inhibitory effect of AACOCF3 on macrophage spreading was significantly reversed adding exogenous AA (10 µM), which confirms that AA is required for SR-A-dependent macrophage adhesion. Maximal reversal of the effects of PLA2 inhibition was achieved by adding exogenous AA at a concentration of 10 µM, and no further reversal occurred at higher AA concentrations (data not shown).

SR-A-mediated Macrophage Adhesion Requires Calcium-independent PLA2-mediated Arachidonic Acid Release—To examine the potential involvement of different PLA2 enzymes in SR-A-mediated adhesion, macrophages were pretreated with BEL, a suicide substrate inhibitor of iPLA2 (43), or with a specific pyrrolidine inhibitor of cPLA2<sub>α</sub> (44) and then plated on MDA-BSA (Fig. 2). Like macrophages treated with AACOCF3, macrophages treated

Statistical Analysis—Experiments were repeated at least three times, and significance among treatment groups was determined by one-way analysis of variance with appropriate post hoc tests using GraphPad Prism. Values with p < 0.05 were considered to be statistically significant.

FIGURE 1. SR-A-mediated macrophage adhesion requires PLA2-mediated AA release. MPM isolated from SR-A<sup>+/+</sup> (control) or SR-A<sup>−/−</sup> mice were pretreated as indicated with AACOCF3 (30 µM) for 25 min in suspension. AA (10 µM) was added to pretreated cells immediately before plating into MDA-BSA-coated slides. Cells were allowed to adhere for 2 h at 37 °C, fixed, and stained with Alexa-Fluor568-conjugated phalloidin. Nuclei were stained with 4',6-diamidino-2-phenylindole. Confocal images were digitally captured, and cell surface area was quantified. The scale bar represents 50 µm. The graph depicts the mean ± S.E. of at least three separate experiments. Asterisk, denotes significantly different (p < 0.05) from SR-A<sup>+/+</sup> control; #, denotes significantly different (p < 0.05) from SR-A<sup>−/−</sup> cells.

FIGURE 2. Calcium-independent PLA2β-catalyzed AA release is required for SR-A-mediated macrophage adhesion. MPM isolated from wild-type or iPLA2β<sup>−/−</sup> mice were pretreated as indicated with the specific iPLA2 suicide substrate inhibitor (inh) BEL (3 µM) or the cPLA2<sub>α</sub> inhibitor (5 µM) for 25 min in suspension. AA (10 µM) was added to pretreated cells immediately before plating into MDA-BSA-coated slides. Cells were allowed to adhere for 2 h at 37 °C, fixed, and stained with Alexa-Fluor568-conjugated phalloidin. Nuclei were stained with 4',6-diamidino-2-phenylindole. Confocal images were digitally captured, and cell surface area was quantified. The scale bar represents 50 µm. The graph depicts the mean ± S.E. of at least three separate experiments. Asterisk, denotes significantly different (p < 0.05) from control; #, denotes significantly different (p < 0.05) from iPLA2β<sup>−/−</sup> macrophages.
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with BEL (3.0 μM; 30 min) before plating on MDA-BSA remained rounded compared with untreated control macrophages. Furthermore, adding exogenous AA (10 μM) restored SR-A-mediated macrophage spreading after iPLA₂ inhibition with BEL. The specific requirement for iPLA₂β activity was confirmed using macrophages isolated from iPLA₂−/− mice, which exhibited significantly less spreading on MDA-BSA than did untreated iPLA₂β+/+ control macrophages. The spreading of iPLA₂−/− macrophages plated on MDA-BSA was increased by adding exogenous AA (data not shown). In contrast to the effect of inhibiting iPLA₂, treating macrophages with the pyrrolidine cPLA₂ inhibitor at a concentration (5 μM) that abolishes calcium-mediated prostaglandin formation (data not shown and Ref. 44) had no effect on SR-A-mediated cell spreading. This result indicates that cPLA₂ activity is not required for SR-A-mediated cell adhesion. Taken together, these results demonstrate that iPLA₂β-catalyzed AA release is specifically required for SR-A-dependent macrophage adhesion to modified protein.

SR-A-mediated Macrophage Adhesion Requires 12/15-LOX-derived AA Metabolites—To examine the potential role of AA metabolism in SR-A-mediated adhesion, SR-A+/+ macrophages were pretreated with NDGA, an inhibitor of both 12/15-LOX and 5-LOX enzymes, and the ability of macrophages to spread on MDA-BSA was assessed. Pretreatment with NDGA abolished macrophage spreading on MDA-BSA (Fig. 3). The addition of exogenous AA (10 μM) did not restore the spreading of NDGA-treated macrophages plated on MDA-BSA, which indicates that LOX-derived AA metabolites rather than AA itself are required for SR-A-dependent cell adhesion/spreading.

The inhibition of SR-A-mediated macrophage spreading by NDGA suggests a role for LOX-derived products in that process. To determine the specific LOX enzyme(s) required for SR-A-mediated adhesion, we used macrophages isolated from 12/15-LOX−/− mice or from 5-LOX−/− mice or macrophages pretreated with the specific 5-LOX inhibitor MK-866 (Fig. 3). Macrophages isolated from 12/15-LOX−/− mice showed diminished ability to spread on MDA-BSA, which indicates that 12/15-LOX activity is required. In contrast, macrophages that were isolated from 5-LOX−/− mice or treated with the selective 5-LOX inhibitor (MK-886) at a concentration (5 μM) previously shown to inhibit macrophage production of leukotriene B₄ and 5-hydroxyeicosatetraenoic acid (45) spread normally on MDA-BSA. This finding indicates that SR-A-dependent macrophage adhesion does not require 5-LOX-derived AA metabolites.

In addition to the LOX pathway, CytP450 and COX isoenzymes catalyze the metabolism of AA to biologically active compounds (46). To examine the potential role for a CytP450 pathway, cells were treated with an inhibitor of CytP450 (SKF525A) at a concentration (10 μM) that inhibits epoxygenase-saturated fatty acid production (47), and the ability of cells to spread was assessed. Treating macrophages with SKF525A (Fig. 3) did not affect SR-A-dependent macrophage spreading. To examine the potential involvement of the COX pathway, macrophages were treated with a nonselective inhibitor of COX-1 and COX-2 (indomethacin) at a concentration (10 μM) that inhibits prostaglandin production in macrophages (48, 49). Treating macrophages with indomethacin also failed to affect SR-A-dependent spreading (Fig. 3). Taken together, the data in Fig. 3 indicate that 12/15-LOX-derived products of AA are specifically required for SR-A-dependent macrophage adhesion but that AA metabolites produced by CytP450 or COX are not.

LOX Metabolism of iPLA₂-derived AA Coupled SR-A to Rac and Cdc42 Activation—The Rho family GTPases Rac and Cdc42 are important regulators of actin polymerization and formation of the cortical cytoskeleton structures (e.g. lamellipodia and filopodia) involved in cell adhesion. To investigate whether Rac and Cdc42 are activated by SR-A-dependent macrophage adhesion to modified protein, macrophages were plated for various intervals on MDA-BSA, and cell lysates were then prepared. The active forms of Rac and Cdc42 were isolated from cell lysates using a fusion protein that binds only the active, GTP-bound form of these GTPases. As illustrated in Fig. 4A, Rac and Cdc42 were both rapidly activated in macrophages that adhere to MDA-BSA. Activation increased significantly after 10 min of adhesion to MDA-BSA and tended to remain above basal after 120 min. Importantly, neither Rac nor Cdc42 activation was detected in SR-A−/− macrophages plated on MDA-
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**FIGURE 4.** Rac and Cdc42 are rapidly activated in a SR-A-dependent manner during macrophage adhesion/spreading. A representative blot and the mean ± S.E. of at least three separate experiments are shown. Asterisk denotes significantly different (p < 0.05) from non-adhered (suspension) SR-A−/− cells.

**FIGURE 5.** SR-A-dependent Rac and Cdc42 activation requires LOX-mediated metabolism of iPLA2-derived AA. A representative blot and the mean ± S.E. of at least three separate experiments are shown. Asterisk denotes significantly different (p < 0.05) from cells in suspension.

BSA (Fig. 4B), which confirms that Rac and Cdc42 activation is SR-A-dependent.

To determine whether iPLA2-catalyzed AA release and subsequent metabolism by LOX are required for SR-A-dependent Rac activation, cells were pretreated with inhibitors of iPLA2 or of LOX before assessing Rac activation. Pretreating macrophages with inhibitors of iPLA2 (AACOCF3 or BEL) or LOX (NDGA) prevented activation of both Rac and Cdc42 during SR-A-dependent macrophage adhesion/spreading (Fig. 5). Taken together, these results indicate that the Rac and Cdc42 activation during SR-A-dependent macrophage adhesion requires metabolism of iPLA2-derived AA by LOX.

**DISCUSSION**

Specific roles for intracellular signaling cascades in regulating SR-A function continue to be elucidated (14, 23, 40, 50–54). In the current study we examined the potential role for PL2-catalyzed AA release and subsequent AA metabolism in SR-A-dependent macrophage adhesion using pharmacological inhibitors and macrophages isolated from genetically modified mice lacking iPLA2β or specific AA oxygenases.

Both calcium-dependent and calcium-independent PLA2 enzymes are involved in intracellular signaling in macrophages (55–57). We found that AACOCF3, an inhibitor of both cPLA2 and iPLA2, abolished SR-A-mediated macrophage spreading on modified protein. Using a specific pyrrolidine cPLA2α inhibitor and the specific iPLA2 inhibitor (BEL) and macrophages isolated from iPLA2β−/− mice, we further show that iPLA2β activity is specifically required for SR-A-mediated macrophage adhesion/spreading. PLA2-catalyzed hydrolysis of membrane phospholipids produces a free fatty acid (e.g. AA) and a 2-lysophospholipid, each of which can have biologic activity. The ability of exogenous AA to restore SR-A-mediated spreading to iPLA2β−/− macrophages or to macrophages treated with BEL confirms that AA is an important mediator of SR-A-mediated macrophage adhesion.

Intracellular AA can be rapidly metabolized to bioactive eicosanoids by various oxygenases, including those of the LOX, COX, and CyP450 families. Inhibition of LOX with NDGA abolished macrophage spreading on MDA-BSA, but neither the COX inhibitor indomethacin nor the CyP450 inhibitor SKF525A affected macrophage spreading. These findings suggest that AA metabolites produced by LOX are specifically required for SR-A-mediated adhesion/spreading. A specific requirement for 12/15-LOX activity was demonstrated using macrophages isolated from 12/15-LOX−/− mice, in which SR-A-mediated adhesion/spreading is abolished.

**TABLE 1.**

| Condition | Active Rac1 | Total Rac1 |
|-----------|------------|-----------|
| Susp      | 3          | 5         |
| Adh       | 5          | 6         |

**TABLE 2.**

| Condition | Active cdc42 | Total cdc42 |
|-----------|--------------|-------------|
| Susp      | 2            | 5           |
| Adh       | 4            | 6           |
spreading was abolished. In contrast, macrophages isolated from 5-LOX−/− mice spread normally as did macrophages isolated from wild-type mice and treated with the 5-LOX inhibitor MK-866. These data indicate that 5-LOX is not involved in SR-A-mediated macrophage spreading. Exogenous AA failed to restore spreading to wild-type macrophages in which 12/15-LOX was inhibited by NDGA or to macrophages from 12/15-LOX−/− mice, which confirms that a metabolite of AA produced by 12/15-LOX rather than AA itself is required for the spreading response.

Activation of Rac and Cdc42, members of the Rho-like GTPase family, coordinates changes in cell morphology during cell adhesion by promoting assembly and organization of the actin cytoskeleton (30–32). Like other G proteins, activation of Rac and Cdc42 requires GDP/GTP exchange, which is mediated by guanine nucleotide exchange factors. Several nucleotide exchange factors (GEFs) have been identified, and mechanisms that regulate these GEFs continue to be elucidated (for review, see Refs. 58 and 59). In addition to nucleotide exchange factors, RhoGTPases are regulated by interactions with guanine-nucleotide dissociation inhibitors (for review, see Refs. 60 and 61). The ability of AA metabolites to regulate Rac and Cdc42 has been suggested previously (62–64). For example, it has been reported that AA promotes Rac and Cdc42 activation in a COX-2-dependent manner (63). It has also been suggested that various biologically active lipids, including AA, can disrupt the interaction of Rac with Rac-guanine-nucleotide dissociation inhibitors (62). In addition to regulating Rac/Cdc42 activation, other studies have demonstrated that Rac activation promotes cPLA2-catalyzed AA release (65, 66). The findings that Rac activation may either precede or follow AA release suggests a complex relationship between RhoGTPase activation and AA release. To our knowledge ours is the first study to demonstrate that 12/15-LOX metabolism of AA released by iPLA2 mediates Rac or Cdc42 activation. Thus, our findings identify a novel pathway for regulating Rac and Cdc42 activation.

Diverse chronic inflammatory diseases including diabetes, atherosclerosis, and Alzheimer disease are characterized by modifications of extracellular matrix components. Such modifications result in formation of SR-A ligands, and SR-A may contribute to macrophage accumulation at specific inflammatory sites. For example, SR-A recognizes glycated proteins formed as a result of the hyperglycemia accompanying diabetes (7). The finding that SR-A contributes to enhanced glomerular macrophage accumulation in diabetic animals suggests a role for SR-A in diabetes-induced complications, such as nephropathy (67). Similarly, extracellular matrix proteoglycans that are up-regulated in atherosclerotic plaques are adhesion substrates for SR-A, which suggests that SR-A may contribute to macrophage adhesion and retention in atherosclerotic lesions (10). An additional role for SR-A in Alzheimer disease is suggested by the finding that SR-A mediates adhesion of microglia to β-amyloid fibril-coated surfaces (6). Accumulation of SR-A ligands at sites where macrophage accumulate suggests that SR-A-mediated adhesion may participate in chronic inflammatory diseases.

Macrophage expression of both SR-A and 12/15-LOX can be altered during chronic inflammation, such as that associated with atherosclerosis and diabetes (68–72). Changes in SR-A and 12/15-LOX expression can have important effects on the inflammatory process. For example, the involvement of 12/15-LOX in lipid peroxidation, cytokine expression, and monocyte recruitment indicates that 12/15-LOX-derived metabolites are proinflammatory (73–76). This notion is supported by the observation that 12/15-LOX−/− mice have reduced atherosclerosis and brain oxidative stress (73, 77, 78) and reduced sensitivity to diabetes in models associated with islet inflammation (79). Similarly, SR-A−/− mice are protected from atherosclerosis and diabetic nephropathy (80, 81). Thus, inhibiting SR-A or components of its downstream adhesion signaling pathway, including iPLA2β and 12/15-LOX, might be therapeutically beneficial in chronic inflammatory diseases.

Overall, our results identify a novel role for iPLA2β and 12/15-LOX in coupling SR-A to Rac and Cdc42 activation and macrophage adhesion. An implication of our results is that SR-A-mediated adhesion might promote inflammation by increasing macrophage retention at sites of extracellular matrix modification and by activating signaling pathways in which AA and its metabolites participate.

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REFERENCES
1. Schwartz, M. A., and Ginsberg, M. H. (2002) Nat. Cell Biol. 4, 65–68
2. Berton, G., and Lowell, C. A. (1999) Cell. Signal. 11, 621–635
3. DeMali, K. A., and Burridge, K. (2003) J. Cell. Sci. 116, 2389–2397
4. Zamir, E., and Geiger, B. (2001) J. Cell Sci. 114, 3583–3590
5. Gowen, B. B., Borg, T. K., Ghaffar, A., and Mayer, E. P. (2000) Matrix Biol. 19, 61–71
6. El Khoury, J., Hickman, S. E., Thomas, C. A., Cao, L., Silverstein, S. C., and Loike, J. D. (1996) Nature 382, 716–719
7. El Khoury, J., Thomas, C. A., Loike, J. D., Hickman, S. E., Cao, L., and Silverstein, S. C. (1994) J. Biol. Chem. 269, 10197–10200
8. Kirkham, P. A., Spooner, G., Fouilhoux-Jones, C., and Calvez, R. (2003) Free Radic. Biol. Med. 35, 697–710
9. Santiago-Garcia, J., Mas-Oliva, J., Innerarity, T. L., and Pitas, R. E. (2001) J. Biol. Chem. 276, 30655–30661
10. Santiago-Garcia, J., Kodama, T., and Pitas, R. E. (2003) J. Biol. Chem. 278, 6942–6946
11. Daugherty, A., Kosswig, N., Cornicelli, J. A., Whitman, S. C., Wolfe, S., and Rateri, D. L. (2001) J. Lipid Res. 42, 1049–1055
12. Haworth, R., Platt, N., Keshav, S., Hughes, D., Darley, E., Suzuki, H., Kurishara, Y., Kodama, T., and Gordon, S. (1997) J. Exp. Med. 186, 1431–1439
13. van Velzen, A. G., Suzuki, H., Kodama, T., and van Berkel, T. J. (1999) Exp. Cell Res. 250, 264–271
14. Nikolic, D. M., Cholewa, J., Gass, C., Gong, M. C., and Post, S. R. (2007) Am. J. Physiol. Cell Physiol. 292, 1450–1458
15. Akiba, S., Ohno, S., Chiba, M., Kume, K., Hayama, M., and Sato, T. (2002) Biochem. Pharmacol. 63, 1969–1977
16. Scholoske, R. H., and Dennis, E. A. (2006) Biochim. Biophys. Acta 1761, 1246–1259
17. Six, D. A., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1448, 1–19
18. Tang, I., Kriz, R. W., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) J. Biol. Chem. 272, 8567–8575
19. Balboa, M. A., Balsinde, J., Jones, S. S., and Dennis, E. A. (1997) J. Biol. Chem. 272, 8576–8580
20. Ma, Z., Ramanadham, S., Kempe, K., Chi, X. S., Ladenson, J., and Turk, J.
