Apolipoprotein CIII in Apolipoprotein B Lipoproteins Enhances the Adhesion of Human Monocytic Cells to Endothelial Cells

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Background—Lipoproteins containing apolipoprotein (apo) CIII predict coronary heart disease and associate with components of the metabolic syndrome. ApoCIII inhibits lipoprotein catabolism in plasma. However, it is unknown whether apoCIII itself, or in association with VLDL, LDL, or HDL, directly affects atherogenic mechanisms in vascular cells. Thus, we investigated the direct effect of lipoproteins that do or do not have apoCIII, and apoCIII itself, on adhesion of THP-1 cells, a human monocytic cell line, to vascular endothelial cells (ECs).

Methods and Results—VLDL CIII/H11001 and LDL CIII/H11001 (100 µg apoB/mL) from fasting plasma of 18 normolipidemic volunteers increased THP-1 cell adhesion to ECs under static conditions by 2.40.3-fold and 1.80.7-fold, respectively (P<0.01), whereas VLDL or LDL without apoCIII did not affect THP-1 cell adhesion. ApoCIII (100 µg/mL), but not apoCI, apoCII or apoE, also increased THP-1 cell adhesion by 2.1±0.6-fold. Studies with human peripheral blood monocytes yielded similar results. ApoCIII also had strong proadhesive effects under shear flow conditions. VLDL CIII+, LDL CIII+, or apoCIII itself activated PKC and RhoA in THP-1 cells, which resulted in β1-integrin activation and enhancement of THP-1 cell adhesion. Interestingly, HDL CIII+ did not affect THP-1 cell adhesion, whereas HDL without apoCIII decreased their adhesion.

Conclusions—ApoB lipoproteins that contain apoCIII increase THP-1 cell adhesion to ECs via PKC and RhoA-mediated β1-integrin activation. These results indicate that apoCIII not only modulates lipoprotein metabolism but also may directly contribute to the development of atherosclerosis. (Circulation. 2006;113:691-700.)

Key Words: apolipoproteins ■ atherosclerosis ■ cell adhesion molecules ■ leukocytes

Apolipoprotein (apo)CIII, a 8.8-kDa protein, resides on the surface of some VLDL and LDL (apoB lipoproteins) and strongly affects their metabolism.1 ApoCIII inhibits lipoprotein lipase activity,2 an enzyme that metabolizes triglyceride in apoB lipoproteins and facilitates their clearance from the circulation. ApoCIII also impairs clearance of apoB lipoproteins from the circulation by interfering with their binding to hepatic lipoprotein receptors.3 A high apoCIII level correlates with insulin resistance, obesity, and hypertriglyceridemia.4

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Alaupovic and colleagues5,6 proposed that apoB lipoproteins that have apoCIII would have distinct metabolism and relationships to atherosclerosis compared with those that do not have apoCIII and found that the concentration of apoCIII containing lipoproteins correlates with the progression of coronary atherosclerosis. We reported that the plasma concentration of apoCIII in apoB lipoproteins and the apoB concentration of LDL particles that have apoCIII independently predict risk of coronary heart disease (CHD).7,8 ApoB lipoproteins with apoCIII appear to augment risk out of proportion to their low concentration in plasma, eg, 50 to 100 µg apoB/mL. Thus, we hypothesized that apoCIII-containing lipoproteins have enhanced atherogenicity relative to their counterparts that do not contain apoCIII.

The adhesion of circulating monocytes to endothelial cells (ECs) contributes importantly to the inflammatory aspects of atherosclerosis. Monocytes from hypercholesterolemic patients have increased expression of integrins and other adhesion molecules9,10 and show increased adhesion to ECs in vitro.9 In this regard, we previously reported that remnant lipoproteins (RLPs) induced U937 monocytic cells to adhere to ECs.11 Because RLPs have a high content of apoCIII,12 we hypothesized that apoCIII may be involved in this process.

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The present study tested the hypothesis that apoB lipoproteins with apoCII induce monocyte activation and subsequent adhesion to ECs. It also examined the direct effects of apoCIII on signal transduction involved in these processes.

**Methods**

**Cell Culture and Reagents**

THP-1 cells, a human monocytic cell line, were obtained from American Type Culture Collection. Human saphenous vein ECs (HSVECs) (passage 3) and human peripheral monocytes were collected under a protocol approved by the Human Research Committee of the Brigham and Women’s Hospital and cultured as described previously.11 Human apoCI, apoCII, apoCIII, and apoE were purchased from Academy Biomedical. Antibodies used in the present study include mouse anti-β1-integrin antibody (JB1A) (Chemicon International); mouse anti-activated β1-integrin antibody (B44) (Chemicon International); mouse anti–VCAM-1 (C3P4) (Chemicon International); mouse anti–ICAM-1 (P2A4) (Chemicon International); mouse anti–PKCβ antibody (BD Biosciences); mouse anti–PKCδ antibody (BD Biosciences); goat anti–PKCε antibody (Chemicon International); rabbit anti–PKCζ antibody (Upstate); mouse anti-RhoA monoclonal antibody (Santa Cruz Biotechnology); goat anti-apoCI, anti-apoCII, anti-apoCIII, and anti-apoE antibody (Academy Biomedical); and mouse anti-HLA class I monoclonal antibody (W6/32) (American Type Culture Collection).

**Lipoprotein and Lipid Preparation**

Blood was drawn in tubes containing EDTA from 18 healthy volunteers after a 12-hour fast. The study was approved by the Institutional Review Board of Harvard School of Public Health. The subjects were not taking cardiovascular medications, antioxidants, or estrogen. Immunoaffinity chromatography was conducted with affinity-purified anti-human apoCIII on Sephrose4B resin (apoCIII resin) (Academy Biomedical) to separate the plasmas into a lipoprotein fraction with (CIII+) and one without (CIII−) apoCIII. VLDL (d<1.006), LDL (1.006<d<1.050), and HDL (1.063<d<1.210) were isolated from 2 lipoprotein fractions (CIII+, CIII−) by very-fast ultracentrifugation with an Optima TLX ultracentrifuge (Beckman Coulter) as described previously.8 The lipid fraction of a lipoprotein preparation was extracted with chloroform and methanol, dried under N₂ gas, and stored in dimethyl sulfoxide before use. To evaluate the effect of apoCIII, we incubated VLDL CIII− or LDL CIII− with apoCIII (100 µg/mL) for 2 hours at 37°C and reisolated them by ultracentrifugation.13,14 Endotoxin levels in the lipoprotein fractions measured with a Limulus amebocyte lysate chromogenic test (Associates of Cape Cod) were <0.03 EU/mL.

**Lipid and Apolipoprotein Measurements**

Cholesterol was determined in plasma and lipoprotein preparations enzymatically, and apoB and apoCIII were determined by ELISA as described previously.9 The molecular ratio of apoCIII to apoB reflects apoCIII enrichment on the particles was calculated by using their respective molecular mass (apoB, 550 kDa; apoCIII, 8.8 kDa).

**Adhesion Assay**

**Static Conditions**

The HSVEC monolayer in a 96-well plate was stimulated with IL-1β (10 ng/mL) (Genzyme) for 4 hours before the adhesion assay was begun. THP-1 cells (1×10⁶/mL) were labeled with BCECF-AM (Calbiochem), placed on an HSVEC monolayer (6 wells per condition) at 1×10⁵ THP-1 cells per well, and allowed to adhere for 10 minutes. After nonadherent cells were removed, the fluorescent intensity of adhered cells and that of total cells applied to the well was measured by CytoFlour II (Perceptive Biosystems). The ratio of adherent to total cells was expressed as adhesion (percent).

**Flow Conditions**

Adhesion experiments used a parallel-plate flow chamber, as previously described.13 Briefly, confluent HSVEC monolayers, grown on 25-mm glass coverslips (Carolina Biological Supply) and coated with 5 µg/mL fibronectin (Sigma), were stimulated with IL-1β (10 ng/mL) for 4 hours and inserted into the flow chamber. THP-1 cells (0.5×10⁶/mL) suspended in flow buffer (PBS/0.1% human serum albumin) were drawn through the chamber at decreasing flow rates corresponding to an estimated shear stress of 1.0, 0.76, and 0.5 dynes/cm². THP-1 cell interaction was determined after the initial minute of each flow rate by counting the number of adherent cells in
4 different fields recorded with a video microscope. Arrested (firmly adhered) and rolling THP-1 cells were counted.15

Flow Cytometry
THP-1 cells (1×10^6/mL) were treated with mouse anti–β1-integrin antibody (JB1A) or mouse anti–activated β1-integrin antibody (B44) for 10 minutes, followed by incubation with FITC-conjugated goat anti-mouse antibody. Cell surface (activated) β1-integrin expression was analyzed with a FACS Caliber (BD Biosciences).

Immunoblotting
Total cell lysates and the membrane fraction of THP-1 cells (1×10^6/mL) were prepared as described previously.16 An equal amount of protein (10 μg) from each fraction was subjected to 12% SDS-PAGE. The activation of RhoA and PKC was examined by detecting the membrane-bound protein that translocated from cytosol fraction with an ECL Plus (Amersham Biosciences, Piscataway, NJ). Blots were quantified by densitometry, and the membrane-associated fraction was expressed as percent of total.

RhoPull-Down Assay
Rho pull-down assay was carried out with a Rho activation kit (Pierce) following the manufacturer’s protocol. In brief, total cell lysates of THP-1 cells (1×10^6) were incubated with 400 μg Rhotekin-RBD (Rho-binding domain) to collect the activated form of RhoA. The activated RhoA was detected with an anti-RhoA antibody.

Quantification of F-actin in THP-1 Cells
THP-1 cells were fixed with 3.7% formaldehyde in PBS, made permeable with 0.1% Triton-X100 in PBS, and stained with FITC-phalloidin. F-actin content was measured with CytoFlour II.

Statistical Analysis
Adhesion assay data are presented as mean±SEM. Data were analyzed using ANOVA, with a value of P<0.05 considered significant.

Results

VLDL CIII+ and LDL CIII+ Induce the Adhesion of THP-1 Cells and Human Peripheral Monocytes to ECs
Treatment with VLDL CIII+ or LDL CIII+, but not with VLDL CIII− or LDL CIII−, significantly increased the adhesion of THP-1 cells to ECs (Figure 1A). Adhesion of THP-1 cells to ECs increased in a concentration-dependent manner up to 100 μg apoB/mL (Figure 1B). Adhesion significantly increased as early as 4 hours after incubation and reached a

**Figure 2.** ApoCIII mediates THP-1 cell adhesion to ECs induced by VLDL CIII+ or LDL CIII+. A, THP-1 cells were incubated in the presence of indicated concentrations of apoCIII for 8 hours, and static adhesion assays were carried out. *P<0.05, **P<0.01 vs 0 μg/mL. B, THP-1 cells were incubated in the presence of indicated lipoproteins (100 μg apoB/mL) or PBS (control) for 8 hours, and static adhesion assays were carried out. The molecular ratio of apoCIII to apoB in lipoprotein preparations was calculated as in Methods. *P<0.01 vs control; #P<0.05 vs VLDL CIII+ or LDL CIII+. C, VLDL CIII+, LDL CIII+, or apoCIII was pretreated in the presence or absence of indicated antibodies (50 μg/mL) for 30 minutes; then, THP-1 cells were incubated in the presence of the treated or untreated VLDL CIII+, LDL CIII+ (100 μg apoB/mL), or apoCIII, or PBS (control) for 8 hours, and static adhesion assays were carried out. *P<0.01 vs control; #P<0.05 vs VLDL CIII+, LDL CIII+, or apoCIII without antibodies. Data are representative of 4 independent experiments with similar results. D, Linear regression between THP-1 cell adhesion under static conditions (%) and the molecular ratio of apoCIII to apoB. Data points represent adhesion assays of 12 VLDL CIII+ samples (●) and 11 LDL CIII+ samples (○).
maximum at 8 hours after incubation. Therefore, subsequent experiments incubated THP-1 cells with lipoproteins at 100 μg apoB/mL for 8 hours before the adhesion assay. VLDL CIII or LDL CIII also increased the adhesion of human peripheral blood monocytes to ECs (Figure 1C).

**ApoCIII Mediates VLDL CIII and LDL CIII–Induced THP-1 Cell Adhesion to ECs**

ApoCIII treatment of THP-1 cells (Figure 2A) or human peripheral blood monocytes (Figure 1C) significantly induced their adhesion to ECs, whereas apoCI, apoCII, and apoE had no effect (data not shown).

Next, we added apoCIII to VLDL CIII or LDL CIII and reisolated the VLDL and LDL by ultracentrifugation. The molecular ratios of apoCIII to apoB of VLDL CIII (VLDL CIII +apoCIII) and LDL CIII (LDL CIII +apoCIII), formed in vitro, were ~30% of native VLDL CIII and LDL CIII. VLDL CIII, formed in vitro, enhanced THP-1 cell adhesion, although to a lesser extent compared with native VLDL CIII. Reconstituted LDL CIII only slightly increased THP-1 cell adhesion, probably because of low apoCIII enrichment (molecular ratio of apoCIII to apoB, 2.7) (Figure 2B). Preincubation of VLDL CIII or LDL CIII with anti-apoCIII significantly reduced their effects on THP-1 cell adhesion. In contrast, anti-apoCI, anti-apoCII, and anti-apoE antibodies did not affect their ability to induce THP-1 cell adhesion. Furthermore, anti-apoCIII also completely inhibited apoCIII-induced THP-1 cell adhesion (Figure 2C). These results indicate that apoCIII in these lipoprotein fractions induces the enhancement of THP-1 cell adhesion. Adhesion of THP-1 cells incubated with VLDL CIII or LDL CIII significantly correlated with the molecular ratio of apoCIII/apoB of these lipoproteins (Figure 2D).

**VLDL CIII+, LDL CIII+, and ApoCIII Induce the Adhesion of THP-1 Cells to ECs Under Flow Conditions**

ApoCIII or VLDL CIII+ treatment of THP-1 cells significantly increased their adhesion to ECs at each level of fluid shear examined (Figure 3A). LDL CIII also significantly increased THP-1 cell adhesion, although to a lesser extent. VLDL CIII+ or LDL CIII+ did not affect THP-1 cell adhesion. Strikingly, THP-1 cells treated with apoCIII alone or in combination with VLDL or LDL showed a distinct function compared with control cells. Firm adhesion makes up 90% to 95% of the EC interactions of treated THP-1 cells, whereas rolling interactions were minimal (Figure 3B; see also the online Data Supplement for video clip). In contrast, ~40% of the interactions of control THP-1 cells not exposed to apoCIII were rolling, and 60% were firmly adhered.
Because an increase in leukocyte arrest under shear flow would require integrin activation,15,17 these data suggest that THP-1 cell integrins become activated after treatment with apoCIII.

**VLDL CIII** and **LDL CIII** Acti-vate β1-integrin in THP-1 Cells

In vivo18 and in vitro studies, including ours,11 have shown a major role for β1-integrin in firm adhesion of monocytes to vascular endothelium. We therefore examined whether VLDL CIII or LDL CIII affects the expression or activity of β1-integrin in THP-1 cells using flow cytometry. Treatment with VLDL CIII, LDL CIII, or apoCIII alone increased the active forms of β1-integrin in THP-1 cells with a slight increase in total β1-integrin expression, indicating that they induced β1-integrin activation (Figure 4A). VLDL CIII or LDL CIII had no effect. Binding-blocking β1-integrin antibody (JB1A) abolished THP-1 cell adhesion induced by VLDL CIII, LDL CIII, or apoCIII (Figure 4B). Pretreatment of ECs with anti–VCAM-1 antibody, but not anti–ICAM-1 antibody, reversed the effects of VLDL CIII, LDL CIII, and apoCIII on THP-1 cell adhesion (Figure 4C), suggesting that apoCIII induces THP-1 adhesion by inducing β1-integrin and its consequent binding to its cognate ligand VCAM-1.

**VLDL CIII** and **LDL CIII** Activate RhoA in THP-1 Cells

RhoA plays a crucial role in the migration and adhesion of monocytes by increasing the expression and/or binding affinity of cell surface integrins. Treatment of THP-1 cells with VLDL CIII, LDL CIII, or apoCIII caused RhoA translocation to the membrane, an indicator of activation (Figure 5A), and increased the active form of RhoA in THP-1 cells (Figure 5B), whereas VLDL or LDL CIII had no effect. Pretreatment with C3 exoenzyme, a specific RhoA inhibitor, partially inhibited β1-integrin activation and THP-1 cell adhesion induced by VLDL CIII, LDL CIII, or apoCIII (Figure 5C, 5D). ApoCIII and VLDL CIII or LDL CIII increased F-actin content in THP-1 cells, indicating rearrangement of actin cytoskeleton by RhoA (Figure 5E).
VLDL CIII⁺ and LDL CIII⁺ Activate PKC in THP-1 Cells

PKC regulates monocyte adhesion in cooperation with or independently of RhoA. PKCα protein in the membrane fraction increased substantially after incubation with VLDL CIII⁺ or LDL CIII⁺. PKCβ was slightly activated. LDL CIII⁺ also activated PKCδ. In contrast, VLDL CIII⁻ or LDL CIII⁻ had little effect on the PKC isoforms (Figure 6A). VLDL CIII⁺ or LDL CIII⁺ did not activate PKCζ (data not shown). ApoCIII alone also activated PKCα, and PKCβ to a lesser extent, but did not affect PKCδ. Thus, we examined whether PKCα mediates β1-integrin activation and, in turn, THP-1 cell adhesion to ECs. Pretreatment with Go6976, a selective PKCα inhibitor, inhibited β1-integrin activation induced by VLDL CIII⁺, LDL CIII⁺, or apoCIII (Figure 6B). Go6976 partially inhibited RhoA activation (Figure 6C). In contrast, C3 did not affect PKCα activation by these preparations (data not shown), indicating that RhoA activation is dependent on PKCα. In accord with PKC inhibition, Go6976 significantly inhibited THP-1 cell adhesion induced by VLDL CIII⁺, LDL CIII⁺, or apoCIII (Figure 6D). Anti-apoCIII antibody inhibited PKCα activation induced by VLDL CIII⁺, LDL CIII⁺, or apoCIII (Figure 6E). These results indicate that PKCα activation by VLDL CIII⁺ or LDL CIII⁺ depends on apoCIII in these particles.

Effects of Lipids of VLDL CIII⁺ and LDL CIII⁺ on PKC Activation and THP-1 Cell Adhesion to ECs

Lipids of VLDL CIII⁺ and LDL CIII⁺ had minimal effects on THP-1 cell adhesion (Figure 7A). Although LDL CIII⁺ lipid activated PKCδ in THP-1 cells (Figure 7B), rottlerin, a specific PKCδ inhibitor, did not reduce LDL CIII⁺-induced THP-1 cell adhesion (Figure 7C). Thus, PKCδ does not appear to mediate the effect of LDL CIII⁺ on THP-1 cell adhesion.

Effects of HDL CIII⁻ and HDL CIII⁻ on THP-1 Cell Adhesion to ECs

In static adhesion assays, HDL CIII⁻ at 500 µg cholesterol/mL significantly reduced THP-1 cell adhesion by 27% (P<0.01). HDL CIII⁻ did not reduce adhesion (Figure 8A). In flow
Discussion

ApoCIII independently predicts CHD.7,8 Excessive apoCIII on apoB lipoproteins delays their lipolysis2 and inhibits their uptake by normal, high-affinity receptors on hepatocytes.3 However, direct effects on vascular cells of apoB lipoproteins with apoCIII specifically or apoCIII itself remain untested. We found that VLDL or LDL particles that contain apoCIII, but not those that do not, increase the adhesion of mononuclear cells to ECs under both static and flow conditions. ApoCIII itself rather than other apolipoproteins or lipids in the particles caused this proadhesive effect. Proadhesive concentrations of apoCIII-containing lipoproteins, 50 to 100 μg apoB/mL, are well within the range found in fasting plasma, eg, 50 μg apoB/mL in normolipidemic persons and >100 μg apoB/mL in hypertriglyceridemic persons or those with CHD.8,19

Because apoCIII-containing lipoproteins also contain other apolipoproteins such as apoCII, apoCII, and apoE,20 as well as various lipids, we performed experiments that localized the proadhesive effect to apoCIII itself. First, apoCIII itself enhanced THP-1 cell adhesion in a concentration range of human plasma, ie, 20 to 100 μg/mL. ApoCII, apoCII, or apoE did not increase adhesion. Second, apoCIII added in vitro to VLDL that did not have apoCIII in vivo conferred a proadhesive property to the VLDL. Third, antibodies against apoCIII, but not against apoCII, apoCII, or apoE, decreased the proadhesive effect. Fourth, the number of apoCIII molecules per VLDL or LDL particle strongly correlated with extent of adhesion. Finally, lipids extracted from apoCIII-containing VLDL or LDL did not increase adhesion. Thus, apoCIII itself, but not other apolipoproteins that commonly cluster with apoCIII on apoB lipoproteins or lipoprotein lipids, mediates the enhanced THP-1 cell adhesion to ECs. The effect of LDL CIII tended to decrease THP-1 cell adhesion at a shear stress of 1.0 dyne/cm² (P=0.09) but not at lower shear rates. HDL CIII did not affect THP-1 cell adhesion under flow conditions (Figure 8B).

Figure 6. VLDL CIII and LDL CIII activate PKC in THP-1 cells. A, THP-1 cells were incubated in the presence of indicated lipoproteins (100 μg apoB/mL), apoCIII (100 μg/mL), or PBS (control) for 2 hours, and the membrane and total PKC isoform protein was detected with immunoblotting. B, THP-1 cells were pretreated in the presence or absence of Go6976 (Go) (5 nmol/L) for 30 minutes and then incubated in the presence of VLDL CIII, LDL CIII (100 μg apoB/mL), apoCIII (100 μg/mL), or PBS (control) for 8 hours. Then, cell surface–activated β1-integrin was detected by flow cytometry. C, THP-1 cells were pretreated in the presence or absence of Go6976 (5 nmol/L) for 30 minutes and then incubated in the presence of VLDL CIII, LDL CIII (100 μg apoB/mL), apoCIII (100 μg/mL), or PBS (control) for 2 hours. The membrane and total RhoA protein was detected with immunoblotting. D, THP-1 cells were pretreated in the presence or absence of VLDL CIII, LDL CIII (100 μg apoB/mL), apoCIII (100 μg/mL), or PBS (control) for 8 hours. *P<0.01 vs control; #P<0.05 vs VLDL CIII, LDL CIII, or apoCIII without Go6976. E, VLDL CIII, LDL CIII (100 μg apoB/mL), or apoCIII (100 μg/mL) was pretreated in the presence or absence of apoCIII antibody (50 g/mL) for 30 minutes; then, THP-1 cells were incubated in the presence of the treated or untreated VLDL CIII, LDL CIII (100 μg apoB/mL), or apoCIII, or PBS (control) for 2 hours. Data are representative of 4 independent experiments with similar results.

adhesion assays, HDL CIII tended to decrease THP-1 cell adhesion at a shear stress of 1.0 dyne/cm² (P=0.09) but not at lower shear rates. HDL CIII did not affect THP-1 cell adhesion under flow conditions (Figure 8B).
rolling phase. ApoCIII treatment strongly promoted firm adhesion. Thus, apoCIII may reduce THP-1 cell rolling time before firm adhesion to ECs, may increase the percentage of rolling interaction that converts to firm adhesion, or perhaps may eliminate the rolling phase in some interactions.

We then investigated the intracellular mechanism(s) that mediate THP-1 interactions with ECs. We previously reported that RLPs induced U937 cell adhesion via activation of β1-integrin\(^\text{11}\) and that inactivation of β1-integrin by atorvastatin\(^\text{11}\) or amlodipine\(^\text{21}\) reduced THP-1 cell adhesion to ECs. β1-Integrin is expressed on monocytes, on lymphocytes, and at a low level on neutrophils and supports firm adhesion to VCAM-1 on activated endothelium.\(^\text{17}\) Blockade of β1-integrin reduced monocyte adhesion and atherosclerotic lesion formation in apoE\(^{−/−}\) mice.\(^\text{18}\) The present study demonstrates that VLDL CIII\(^+\) and LDL CIII\(^+\), components of RLPs, activate β1-integrin in THP-1 cells. Binding-blocking antibodies to β1-integrin or VCAM-1 abolished the increment of THP-1 cell adhesion induced by VLDL CIII\(^+\), LDL CIII\(^+\), or apoCIII. On apoCIII stimulation, THP-1 cell interaction with ECs under flow shifted from rolling to a firm adhesion phenotype, consistent with the action of β1-integrin. These results indicate that β1-integrin and its cognate ligand VCAM-1 play a dominant role in this process.

PKC plays an important role in several mechanisms that promote atherosclerosis.\(^\text{22}\) PKC increases monocyte-endothelial interaction by modulating the expression and activation of integrins.\(^\text{23}\) The PKC pathway includes the activation of Rho family proteins.\(^\text{24}\) RhoA is one of the most important molecules regulating the actin cytoskeleton, integrins, and monocyte-endothelial interaction.\(^\text{16}\) The present study showed that RhoA activation by VLDL CIII\(^+\), LDL CIII\(^+\), or apoCIII itself depends on PKCα activation. However, the effects of inhibiting RhoA on THP-1 cell adhesion and β1-integrin activation by apoCIII were smaller than inhibiting PKCα. Thus, PKCα activation may activate PKCα-dependent and RhoA-dependent and -independent signal transduction, leading to adhesion.

How apoCIII activates PKCα in THP-1 cells remains unclear. PKCα, one of the conventional PKC isoforms, is
activated by Ca\textsuperscript{2+}, phospholipids, and diacylglycerol.22 However, little information exists on possible direct effects of apoCIII on signal transduction in vascular cells and other type of cells. A recent study reported that apoCIII induces apoptosis of pancreatic \( \beta \) cells by increasing intracellular Ca\textsuperscript{2+} concentration, a potential activator of PKCa. However, the study did not determine whether this led to the activation of PKC.25 The exact mechanism(s) for apoCIII-induced PKC activation in THP-1 cells require further investigation.

VLDL CIII\textsuperscript{1} and LDL CIII\textsuperscript{1} compared with VLDL CIII\textsuperscript{2} and LDL CIII\textsuperscript{2} are enriched with lipids and apolipoproteins.19 Lipids extracted from these fractions did not increase THP-1 cell adhesion or activate PKC\( \alpha \). Lipids from LDL CIII\textsuperscript{1} activated PKC\( \delta \), but this did not cause adhesion. Thus, the lipids in apoCIII-containing lipoproteins do not play a role in adhesion but may affect other processes in monocytic cells that are dependent on PKC\( \delta \).

ApoCIII also resides on HDL particles.5,7 In contrast to apoB lipoproteins with apoCIII, HDL with apoCIII did not increase THP-1 cell adhesion. Some HDL preparations can inhibit integrin and adhesion molecule expression in leukocytes and ECs and reduce their adhesive interaction.26,27 Our study did not determine whether this led to the activation of PKC. However, inhibition of adhesion by HDL apoCIII\textsuperscript{1} was more prominent under static than shear flow conditions. Because HDL apoCIII\textsuperscript{1} did not reduce adhesion, apoCIII may have counteracted potentially protective actions of other HDL components. Several studies reported that apoCIII in HDL associates with CHD at least in univariate analysis,28,29 and apoCIII in HDL has positive rather than inverse correlations with other risk factors such as VLDL and triglycerides.30 Whether apoCIII affects antiatherogenic properties of HDL requires further study.

In conclusion, VLDL and LDL that contain apoCIII increase THP-1 cell adhesion to ECs via PKC- and RhoA-mediated \( \beta \)-integrin activation. ApoCIII itself caused these potentially proatherogenic effects. These results indicate that elevated levels of VLDL CIII\textsuperscript{1} and LDL CIII\textsuperscript{1} contribute to monocyte recruitment on vascular endothelium and suggest that apoCIII promotes atherosclerosis not only by impairing the catabolism of apoB lipoproteins but also by these direct mechanisms on vascular wall cells. Many previous studies have focused on the roles of lipid moieties such as oxidized lipids in the atherogenicity of apoB lipoproteins. Our observations provide novel insights into a role for apoCIII as a distinct contributor to inflammation and atherosclerosis.

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Disclosures

None.

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ApoCIII is a protein present on a portion of VLDL, LDL, and HDL particles. ApoCIII is high in people with dyslipidemia and is an independent risk factor for CHD. ApoCIII impairs catabolism of VLDL and LDL, thereby causing hypertriglyceridemia. However, apoCIII may have a direct adverse effect on cells present in atherosclerosis. We studied whether apoCIII affects the interaction of circulating monocytes with ECs as an event in atherogenesis and plaque instability. We found that apoCIII, either as a component of VLDL or LDL or alone, increased the adhesion of human mononuclear cells to ECs. The mechanism is activation of PKCα, RhoA, and β₁-integrin in the monocytes. Thus, elevated levels of VLDL and LDL with apoCIII may contribute to monocyte recruitment on vascular endothelium. Interestingly, several epidemiological studies reported that apoCIII in HDL is associated with CHD. This suggests that apoCIII exerts atherogenic properties beyond its effect on apoB lipoprotein metabolism. HDL tends to inhibit monocyte-endothelial cell interactions. However, we found that HDL with apoCIII failed to inhibit monocyte-endothelial interaction, suggesting that high apoCIII content in HDL impairs its antiatherogenic property. Many previous studies have focused on the roles of lipid moieties such as oxidized lipids in the atherogenicity of VLDL and LDL. Our observations may provide novel insights into a role for apoCIII as a distinct contributor to inflammation and atherosclerosis and the potential clinical relevance of treatments that lower apoCIII for the prevention of atherosclerosis.
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