Atorvastatin Attenuates Remnant Lipoprotein-Induced Monocyte Adhesion to Vascular Endothelium Under Flow Conditions

Akio Kawakami, Akira Tanaka, Katsuyuki Nakajima, Kentaro Shimokado, Masayuki Yoshida

**Abstract**—Remnant lipoproteins have been reported to play a causative role in atherogenesis. We investigated the effect of remnant-like lipoprotein particles (RLPs) on monocyte-endothelial interaction and their potential regulation by atorvastatin. Monocytic U937 cells were incubated with RLPs isolated from hypertriglyceridemia subjects and their adhesion to human umbilical vein endothelial cells (HUVECs) was examined under flow conditions. Incubation of U937 cells with 15 μg protein/mL RLPs increased their adhesion to HUVECs activated with IL-1β (untreated: 6.8 ± 1.6 cells/HPF versus RLP: 16.2 ± 3.3 cells/HPF, P < 0.05). Flow cytometric analysis revealed that incubation with RLPs increased expression levels of CD11a, CD18, and CD49d in U937 cells. Moreover, RLP-induced RhoA activation as well as FAK activation was seen in U937 cells, and RLP-induced RhoA activation seemed to be involved with PKC-dependent signaling. To explore the effect of atorvastatin on RLP-induced U937 cell adhesion to HUVECs, U937 cells were incubated with RLPs in the presence of atorvastatin. Pretreatment of U937 cells with 10 μmol/L atorvastatin significantly decreased RLP-induced U937 cell adhesion to activated HUVECs (RLP 15.2 ± 1.5 cells/HPF versus atorvastatin RLP 10.2 ± 1.0 cells/HPF; P < 0.05) and decreased the enhanced integrin expression in RLP-treated U937 cells. Atorvastatin also inhibited RLP-induced RhoA activation and FAK activation in U937 cells. In summary, RLPs induced monocyte adhesion to vascular endothelium by sequential activation of PKC, RhoA, FAK, and integrins, indicating a role of remnant lipoproteins in vascular inflammation during atherogenesis. Atorvastatin attenuated this enhanced monocyte adhesion to HUVECs, suggesting an antiinflammatory role for this compound. (*Circ Res. 2002;91: 263-271.*)

**Key Words:** remnant-like lipoprotein particles • monocyte adhesion • atherosclerosis • 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitor

Hypercholesterolemia is regarded as a major risk factor of ischemic heart diseases, and modified low-density lipoprotein (LDL) has been reported to contribute to early atherosclerotic lesion formation. However, recent studies have reported that elevated serum triglyceride was observed in patients with coronary artery disease without marked hypercholesterolemia, and that serum triglyceride may be a risk factor of ischemic heart disease. Remnant lipoproteins, produced by hydrolysis of chylomicron (CM), and very low-density lipoprotein (VLDL) are considered to be atherogenic triglyceride-rich lipoproteins (TRLs). Indeed, clinical studies have revealed that remnant lipoproteins are closely related to atherosclerosis, independent of LDL. Remnant lipoproteins play a causative role in atherogenesis, as a previous report has shown that remnant-like lipoprotein particles (RLPs) induced vascular smooth muscle cell proliferation. However, the cellular mechanisms of RLPs in atherogenesis have not been fully elucidated.

Monocyte adhesion to vascular endothelium plays an important role in atherogenesis and another report showed that RLPs increased the expression levels of adhesion molecules on endothelial cells. In the present study, we investigated the direct effects of RLPs on monocytes and subsequent monocyte-endothelial interactions under flow conditions, along with the mechanisms involved in cell surface integrin expression, actin cytoskeleton, and inside-out signal transduction.

3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, or statin, has been suggested to have beneficial effects for the prevention of atherosclerosis, independent of its LDL-cholesterol lowering effect. We recently found that statin modulated the actin cytoskeleton, downregulated integrins in monocytes, and decreased monocyte adhe-
sion to endothelial cells. Thus, in the present study, we attempted to determine whether atorvastatin modifies the effect of RLPs on monocytes.

Materials and Methods

Cell Culture and Reagents

U937 and THP-1 cell lines obtained from American Type Culture Collection, Manassas, Va, and human umbilical vein endothelial cells (HUVECs) isolated from normal-term umbilical cords were cultured as described previously. C3 exoenzyme was obtained from Wako, Japan. Human recombinant apolipoprotein (apo) E, mevalonic acid, and cytochalasin D were obtained from Sigma-Aldrich, Japan. Calphostin C and phorbol-12-myristate-13-acetate (PMA) were obtained from Calbiochem, Germany. FITC-conjugated phalloidin was obtained from Molecular Probes. Atorvastatin calcium hydrate, monocalcium bis [(3R,5R)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenylcarbamoyl-1H-pyrol-1-yl]-3,5-dihydroxyheptanoate] trihydrate was a gift from Pfizer Inc, Groton, Conn. An RLP-C Kit was a gift from Japan Immunoresearch Laboratories, Tokyo, Japan. Antibodies used in the present study were as follows: mouse anti-CD11a monoclonal antibody (clone 38, Ancell Corp), mouse anti-CD11b monoclonal antibody (clone 44, YLEM, Italy), mouse anti-CD18 monoclonal antibody (clone MEM48, Southern Biotechnology Associates), mouse anti-CD49d monoclonal antibody (clone A4-PUJ1, Upstate Biotechnology), mouse anti-L-selectin monoclonal antibody (clone MEM48, Southern Biotechnology Associates), mouse anti-focal adhesion kinase (FAK) polyclonal and anti-phosphorylated (p)FAK (397Y) polyclonal antibodies (Bio-source), mouse anti-β1-integrin antibody (HUTS21) (PharMingen), mouse anti-β1-integrin antibody (7B4R) (Santa Cruz Biotechnology), mouse anti-PKCα, β, γ, - δ, ε monoclonal antibodies (New England Biolabs), and HRP-conjugated goat anti-mouse IgG and FITC-conjugated goat anti-mouse IgG antibodies (Cal-tag). To examine cell viability, U937 cells were stained with a 0.25% trypan blue solution after incubation with RLPs or atorvastatin.

Lipoprotein Preparation

EDTA plasma was obtained from 24 patients with hypertriglyceridemia who showed an elevated RLP-cholesterol concentration (>0.19 mmol cholesterol/L [7.5 mg cholesterol/dL]). 4 hours after their breakfast [8 kcal/kg standard weight (carbohydrate 62%; fat 17%; protein 21%)]. They had no cardiovascular diseases or diabetes and had not taken carbohydrate medicines or antioxidants. The protocol of this study complies with the guidelines for the conduct of research involving human subjects by the Committee on Human Research at the Tokyo Medical and Dental University. Total TRLs (d<1.006) was isolated by density gradient ultracentrifugation from plasma samples. RLPs were isolated from plasma samples using an RLP-C Kit, as described previously. TRLs (total TRL) and RLPs were then dialyzed overnight against 5 liters of PBS containing 50 μmol/L EDTA (pH 7.4), and then sterilized using a 0.22-μm filter unit (Millipore). The protein concentration of lipoprotein fractions was determined by a modified method of Lowry et al. The lipid fraction was extracted from purified lipoproteins (TRL and RLP) or U937 cells using chloroform and methanol, dried under N2 gas, and stored in dimethylosulfoxide before use. Degraded RLPs were prepared by repeated freezing and thawing. Trypsinized (tryp-) RLPs, devoid of immunochromically detectable apo E, were prepared as described previously. The cholesterol patterns of TRLs and RLPs were selectively detected by sensitive high-performance liquid chromatography (HPLC). Ten microgram proteins of TRLs and RLPs were analyzed by SDS-PAGE in a 5% to 20% linear gradient gel (Funakoshi), and visualized with a silver stain reagent (Daiichi, Japan). Lipid compositions of the lipoproteins (TRL and RLP) or U937 cell lipid extracts were measured enzymatically by SRL, Japan.

Monocyte Adhesion Assay

The protocols of the adhesion assay under static and flow conditions have been previously described in detail. For the flow assays, HUVEC monolayers were stimulated with 10 U/mL IL-1β (Genzyme) for 4 hours on coverslips and then positioned in a flow chamber mounted on an inverted microscope (IX70, Olympus, Japan). HUVEC monolayers were perfused for 5 minutes with perfusion medium, and then U937 cells (1×106/mL) were drawn through the chamber with a syringe pump (PHD2000, Harvard Apparatus) for 10 minutes at a controlled flow rate to generate a shear stress of 1.0 dyne/cm². The entire period of perfusion was recorded on videotape, and then transferred to a personal computer for image analysis to determine the number of rolling and adherent U937 cells on HUVEC monolayers in 10 randomly selected 20× microscope fields.

Integrin Expression in U937 Cells

U937 cells (1×106/mL) were treated with the indicated primary antibodies for 45 minutes on ice, washed twice with RPMI-1640 and 5% FCS, and incubated with FITC-conjugated goat anti-mouse antibody. Fluorescent intensity was analyzed using a FACS Caliber (Becton-Dickinson).

Quantitation of Filamentous Actin in U937 Cells

Filamentous actin (F-actin) in U937 cells was quantitated as described previously. In brief, U937 cells (1×106/mL) were fixed with 1% paraformaldehyde for 5 minutes, permeabilized with 0.1% Triton X-100 for 60 seconds, and incubated with FITC-conjugated phalloidin for 60 minutes. Fluorescent intensity of the U937 cells was quantitated using a fluorescent plate reader and also observed using a fluorescent microscope.

Translocation of RhoA and PKC in U937 Cells

To examine the translocation of RhoA and PKC from the cytosol to the membrane, membrane and total cell lysates of U937 cells (1×106/mL) were prepared as described previously. An equal amount of protein (10 μg) from each fraction was subjected to 12.5% SDS-PAGE, and then Western blotting analysis was performed using anti-RhoA monoclonal antibody and monoclonal antibodies to the indicated PKC isoforms. Immuneoreactive RhoA and PKC protein was detected with an enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech).

Phosphorylation of FAK in U937 Cells

A total cell lysate of U937 cells (1×106/mL) was prepared as described above, and Western blotting analysis was performed using anti-pFAK(397Y) and anti-FAK antibodies. Immuneoreactive pFAK and FAK proteins were detected with an ECL kit.

Antisense Treatment

FAK antisense oligonucleotides (5'-ATAATCCAGCTTGAAACCAAG-3'), sense oligonucleotides (5'-CTTGGTACAAGCTGGATTAT-3'), and mismatch sense oligonucleotides (5'-ATAATCCAGCTTGAAACCAAG-3'), selected from the human FAK gene (GenBank accession No. L13616), were synthesized with a phosphorothioate modification by Sawady Technology, Japan. THP-1 cells were suspended in 6-well plates (2×105/well) for 24 hours, and 750 μL of OptiMem (Life Technologies) containing 12 μL of Lipofectin (Life Technologies)/2 μg oligonucleotide was added. After 5 hours, 1500 μL of culture medium was added to THP-1 cells. An adhesion assay using transfected THP-1 cells was performed 24 hours after transfection.

Statistical Analysis

Results are presented as mean±SD. Data were analyzed using analysis of variance (ANOVA), with a value of P<0.05 considered significant.
Results

Characterization of Lipoproteins

An SDS-PAGE analysis showed that the TRLs consisted of particles containing apo E and apo B-100. RLPs were found to be enriched in apo E, and contained apo B-100 as well as a small amount of apo B-48. Apo A-I was not detected (Figure 1A). HPLC analysis showed that RLPs consisted mainly of lipoproteins with particle sizes in the range of VLDL and some in the range of CM. TRLs consisted of lipoproteins with particle sizes in the typical range of VLDL (Figure 1B). Taken together, the prepared RLPs consisted of VLDL remnants and a small amount of CM remnants, as reported previously, while TRLs were composed of nascent VLDL. SDS-PAGE analysis showed that tryp-RLPs had a trace of apo E (data not shown). HPLC showed that degraded RLPs had lost peaks of CM- and VLDL-cholesterol (data not shown). As shown in Table 1, lipid compositions were not significantly different between TRLs and RLPs.

RLPs Induce U937 Cell Adhesion to HUVECs

U937 cells were incubated with various concentrations of TRLs or RLPs for 48 hours, after which static adhesion assays were performed. Adhesion of RLP-treated U937 cells to HUVECs was significantly increased in a concentration-dependent manner (Figure 2A). The effect was observed with as little as 15 μg protein/mL, which corresponded to 7.5 mg cholesterol/dL, and with this concentration, the adhesion of U937 cells was significantly increased under static conditions with as little as 18 hours of preincubation (data not shown). In contrast, incubation with TRLs had no effect on the adhesion of U937 cells to HUVECs (data not shown). Thus, we chose to incubate U937 cells with 15 μg protein/mL RLPs for 18 hours in the following experiments.

Because RLPs consist of several lipid components and apolipoproteins, we considered it important to elucidate which of these components play a primary role in enhanced U937 cell adhesion. As shown in Figure 2B, RLP lipid extract, degraded RLPs, and tryp-RLPs all failed to induce a comparable level of U937 cell adhesion to HUVECs as native RLPs. Incubation with apo E, the most abundant apolipoprotein found in RLPs, also did not affect U937 cell adhesion.

The effect of RLPs on monocyte-endothelial interaction was next examined under flow conditions (shear stress of 1.0 dyne/cm²). When U937 cells were incubated with RLPs, adhesion of U937 cells to activated HUVECs was significantly increased compared with medium alone (control) (Figure 2C). In contrast, the number of rolling U937 cells on activated HUVECs was not significantly affected by RLPs. Incubation with TRLs did not affect either the rolling or adhesion of U937 cells (Figure 2C).

The effect of atorvastatin on RLP-induced monocyte-endothelial interaction was also examined. U937 cells were pretreated with various concentrations of atorvastatin for 48 hours before the addition of RLPs. RLP-induced U937 cell adhesion to activated HUVECs was reduced in a concentration-dependent manner with up to 10 μmol/L atorvastatin. The inhibitory effect of atorvastatin was abrogated by the coincubation with 10 μmol/L mevalonic acid, which metabolically bypasses the effect of atorvastatin (Figure 2D). With this concentration, RLP-induced U937 cell adhesion was reduced after 24 hours of preincubation and reached a plateau after 48 hours (data not shown). Similar results were obtained under static conditions (data not shown). Thus, we chose to pretreat U937 cells with 10 μmol/L atorvastatin for 48 hours in the following experiments.

Lipid Content of U937 Cells Treated With RLPs

To evaluate the potential role of lipid uptake by U937 cells in the observed adhesive interaction, U937 cells were incubated...
as described in Figure 2, and then intracellular cholesterol ester (CE) and triglyceride (TG) concentrations were measured (Table 2). When U937 cells were treated with RLPs, these lipid concentrations were significantly increased as compared with the medium alone (control). Further, lipid concentrations of U937 cells treated with degraded RLPs and tryp-RLPs were significantly lower than those treated with native RLPs. Pretreatment of U937 cells with atorvastatin (atr/RLP) did not significantly alter the increase of CE and TG in RLP-treated U937 cells.

### Table 2. Lipid Content in U937 Cells Treated With Lipoproteins

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TRL</th>
<th>RLP</th>
<th>tryp-RLP</th>
<th>Degraded RLP</th>
<th>atr/RLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>10.0±2.3</td>
<td>35.2±4.6*</td>
<td>98.5±16.1**</td>
<td>40.5±9.9***</td>
<td>30.2±13.2***</td>
<td>90.0±34.1</td>
</tr>
<tr>
<td>CE</td>
<td>0.5±0.2</td>
<td>2.5±1.2*</td>
<td>6.7±1.4**</td>
<td>2.8±1.8**</td>
<td>2.1±0.8**</td>
<td>4.5±1.5</td>
</tr>
</tbody>
</table>

CE indicates cholesterol ester; TRL, triglyceride-rich lipoproteins; RLP, remnant-like lipoprotein particles; and atr, atorvastatin.

Data are from 5 separate experiments. Values (μg/mg cell protein) are presented as mean±SD.

*P<0.05 vs control; **P<0.01 vs control; ##P<0.05 vs RLP; ###P<0.01 vs RLP.

**RLPs Induce Integrin Expression on U937 Cells**

To elucidate the molecular mechanism(s) of RLP-induced U937 cell adhesion, U937 cell surface integrin expression was examined by flow cytometric analysis. As shown in Figure 3A, when U937 cells were incubated with RLPs, the expressions of CD11a, CD18, and CD49d were increased, and when pretreated with atorvastatin, the expression of RLP-induced integrin expression was attenuated. In contrast, the expressions of CD11b and L-selectin remained relatively low at baseline, were not affected by RLPs or atorvastatin (Figure 3A). Further, TRLs did
A

CD11a
CD11b
CD18
CD49d
L-selectin

control

RLP

RLP+str

B

0 6 12 18 24 30 36 42 48
Adherent cells (%)

50

RLP

RLP+atrac

Figure 3. Effects of RLPs and atorvastatin on integrin expression in U937 cells. A, U937 cells (1 × 10^6/mL) were incubated in the presence of 15 μg protein/mL RLPs (RLP) or medium alone (control) for 18 hours, or pretreated with 10 μmol/L atorvastatin (RLP+str) for 48 hours before incubation with RLPs. Expression levels of integrins in U937 cells were analyzed by a flow cytometric analysis using monoclonal antibodies to CD11a, CD11b, CD18, CD49d, and L-selectin for each condition. Five thousands cells were analyzed. Data are representative of 4 separate experiments. B, Effect of antibodies to integrins on RLP-induced U937 cell adhesion under static conditions. RLP-treated U937 cells (2 × 10^6/mL) were incubated with antibodies to the indicated integrins for 45 minutes and a static adhesion assay to HUVEC monolayers was performed. Data are from 4 separate experiments. **P < 0.01 vs control; #P < 0.05 vs RLP.

not have any effect on integrin expression (data not shown). To examine whether the integrins were functionally correlated with RLP-induced U937 cell adhesion, RLP-treated U937 cells were incubated in the presence of antibodies to the integrins for 45 minutes and static adhesion assays were performed. Treatment with the anti-CD49d antibody significantly decreased RLP-induced U937 cell adhesion. Treatment with the anti-CD11a and anti-CD18 antibodies also reduced U937 cell adhesion, though less significantly (Figure 3B).

RLPs Induce Actin Polymerization in U937 Cells

The effect of RLPs on actin cytoskeleton organization in U937 cells was examined by detecting F-actin. Observation under a fluorescent microscope showed morphologically abundant F-actin in RLP-treated U937 cells (Figure 4A), whereas TRLs had no effect on the actin cytoskeleton (data not shown). When U937 cells were preincubated with atorvastatin, the increase in F-actin induced by RLPs was significantly smaller as compared with RLPs alone (Figure 4A). RLP-induced U937 cell adhesion was significantly reduced after 10 minutes of pretreatment with 1 μg/mL cytochalasin D, a specific inhibitor of actin polymerization (Figure 4C).

RLPs Induce RhoA Activation in U937 Cells

The effect of RLPs on RhoA activation in U937 cells was investigated by examining the translocation of RhoA from the cytoplasm to the membrane. Western blotting analysis revealed that the expression level of RhoA protein in the membrane fraction was significantly increased after incubation with RLPs (Figure 4B); however, it was not affected by TRLs (data not shown). In contrast, when U937 cells were pretreated with atorvastatin, RLP-induced RhoA translocation in U937 cells was significantly inhibited (Figure 4B). RLP-induced U937 cell adhesion was significantly reduced by 48 hours of pretreatment with 30 μg/mL C3 exoenzyme, a specific inhibitor of RhoA (Figure 4C).

RLPs Induce PKC Activation in U937 Cells

The effect of RLPs on PKC activation in U937 cells was also investigated. Western blotting analysis using antibodies to pFAK(397Y) revealed that activated FAK was significantly increased after incubation with RLPs (Figure 5A). When U937 cells were pretreated with C3 exoenzyme or cytochalasin D, RLP-induced FAK activation was significantly decreased. Further, pretreatment with atorvastatin also caused a similar reduction of FAK activation (Figure 5A). Incub with TRLs did not affect FAK activation in U937 cells (data not shown). To examine the functional significance of FAK in monocyte-endothelial interaction, we performed a static adhesion assay using THP-1 cells transfected with FAK antisense oligonucleotides to inhibit the FAK function. FAK protein expression in THP-1 cells transfected with FAK antisense was significantly reduced (Figure 5B), and the amount of adhesion to HUVECs was also greatly reduced as compared with those transfected with sense or mismatch sense oligonucleotides, whereas RLP treatment failed to induce adhesion to HUVECs in FAK antisense–transfected THP-1 cells (Figure 5C). To investigate the involvement of FAK in integrin activation, Western blotting analysis was performed using HUTS21 to detect the activation-dependent epitope of β1-integrin in THP-1 cells transfected with FAK sense or FAK antisense. Activated β1-integrin was significantly increased after incubation with RLPs in THP-1 cells transfected with FAK sense, but not with FAK antisense (Figure 5D).

RLPs Induce PKC Activation in U937 Cells

The involvement of PKC in RLP-induced U937 cell adhesion was investigated. To monitor PKC activation, the translocation of PKC from the cytoplasm to the membrane was examined. Western blotting analysis revealed that the expression levels of PKCα, PKCβ, and PKCδ proteins in the
membrane were significantly increased after incubation with RLPs, although they were not affected by C3 exoenzyme (Figure 6A). PKC activation was not induced by incubation with TRLs (data not shown) and pretreatment of U937 cells with atorvastatin did not affect RLP-induced PKC activation (data not shown). When U937 cells were pretreated with 5 μmol/L PMA for 18 hours, to deplete intracellular active PKC, or for 18 hours with 2.5 μmol/L calphostin C, a specific PKC inhibitor, RLP-induced RhoA activation and U937 cell adhesion were significantly inhibited (Figures 6B and 6C).

Discussion

We investigated the effects of remnant lipoproteins on the adhesion of monocytes to vascular endothelium under flow conditions. Incubation of U937 cells, a monocytic cell line, with pathophysiological concentrations of RLPs (>0.19 mmol cholesterol/L (7.5 mg cholesterol/dL)) significantly increased their adhesion to HUVECs under flow conditions. The level of laminar shear stress (1.0 dyne/cm²) adopted for the present study has been observed physiologically at the points of bifurcation in large vessels, which are known to be atherosclerosis prone sites. In contrast, TRLs, which mainly consist of nascent VLDL, had little effect on monocyte-endothelial interaction. These results indicate that elevated levels of remnant lipoproteins may have a causative role in the development of atherosclerosis through monocyte recruitment on vascular endothelium.

We demonstrated that the surface expression levels of integrins (CD11a, CD18, and CD49d) in U937 cells were increased after incubation with RLPs; however, the relatively modest quantitative upregulation of these integrins might not be adequate for the observed induction of U937 cell adhesion to HUVECs by RLPs. Therefore, we investigated the effects of RLPs on the relevant intracellular mechanism(s) of U937 cells that may modulate monocyte-endothelial interactions.

First, we examined the effects of RLPs on the actin cytoskeleton and RhoA of U937 cells, because the actin cytoskeleton anchored to adhesion sites is known to be correlated with monocyte adhesion to vascular endothelium and migration, and RhoA is one of the most important molecules regulating the actin cytoskeleton. The observed effects of RLPs on RhoA activation and cytoskeleton modulation shed light on a novel intracellular mechanism by which RLPs induce monocyte adhesion.

It is also known that FAK regulates cell adhesion and migration in various cell types, including nonadherent blood cells, by transferring signals to integrins at the cellular adhesion site. FAK is autophosphorylated at 397Y flanked by the β₁-integrin binding site, which results in its enhanced kinase activity. We showed that the phosphorylation of FAK at 397Y was increased after incubation with RLPs, although they were not affected by C3 exoenzyme (Figure 6A). PKC activation was not induced by incubation with TRLs (data not shown) and pretreatment of U937 cells with atorvastatin did not affect RLP-induced PKC activation (data not shown). When U937 cells were pretreated with 5 μmol/L PMA for 18 hours, to deplete intracellular active PKC, or for 18 hours with 2.5 μmol/L calphostin C, a specific PKC inhibitor, RLP-induced RhoA activation and U937 cell adhesion were significantly inhibited (Figures 6B and 6C).
Concerning the mechanism by which RLPs activated RhoA, we showed the involvement of PKC in this process. It has been reported that RhoA is regulated by several factors. Recently, PKC has been reported to activate RhoA by regulating guanine nucleotide dissociation inhibitor (GDI) phosphorylation in endothelial cells, and our data indicated that RLP-induced PKC activation resulted in RhoA activation in U937 cells. This novel pathway for enhancing adhesion to vascular endothelium may be operative in U937 cells.

Remnant lipoproteins have been reported to be taken up via LDL receptor families. In the present study, deprivation of apo E as well as the degradation of RLPs, which resulted in decreased lipid content in U937 cells, attenuated RLP-induced U937 cell adhesion. Though apo E itself failed to induce U937 cell adhesion, it may be necessary for the uptake of RLPs, as apo E not only serves as a ligand for LDL receptor families, but also interacts with cell surface LPL and heparan-sulfate proteoglycan (HSPG), facilitating cellular lipid uptake. These results indicate that the lipid components taken up as RLPs are responsible for their major stimulatory effects on U937 cell adhesion.

We also demonstrated that preincubation of monocytes with atorvastatin significantly decreased RLP-induced monocyte adhesion to HUVECs. As we have previously reported, the inhibitory effect of statin in monocyte adhesion involves a modulation of RhoA. In the present study, we found that atorvastatin attenuated FAK activation as well as the expression levels of integrins induced by RLP treatment. Interestingly, CE accumulation induced by RLPs was not significantly affected by atorvastatin pretreatment. Although we did not measure the extent of HMG-CoA reductase activity in U937 cells after atorvastatin treatment, the inhibition of HMG-CoA reductase activity by treatment with 10...
In summary, RLPS were found to induce the adhesion of monocytes to vascular endothelium, which may be one of the direct causative roles of remnant lipoproteins in atherogenesis. PKC activation, RhoA activation, actin polymerization, and FAK activation followed by the induction of integrin expression may also be involved in this process. Moreover, atorvastatin attenuated RLP-induced monocyte adhesion to vascular endothelium by modulating RhoA activity. From these findings, we concluded that this compound has potential clinical benefits for the treatment of patients with hypercholesterolemia as well as those with combined hyperlipidemia with elevated levels of remnant lipoproteins.

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