Inhibition of Protein Kinase Cβ Prevents Foam Cell Formation by Reducing Scavenger Receptor A Expression in Human Macrophages

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Background—Low-density lipoprotein (LDL) uptake by monocyte-derived macrophages is a crucial step in foam cell formation and early atherosclerotic lesion. Increasing evidence supports the theory that activation of protein kinase Cβ (PKCβ) is involved in many mechanisms promoting atherosclerosis. Thus, we investigated whether inhibition of PKCβ prevents foam cell formation.

Methods and Results—The differentiation of human primary monocytes or the monocytic THP-1 cell line into monocyte-derived macrophages was induced by phorbol 12-myristate 13-acetate (PMA; 0.1 mmol/L), a potent activator of PKC. Incubation of monocyte-derived macrophages with Dil-modified LDL (acylated LDL and oxidized LDL, 10 μg/mL) led to lipoprotein uptake. Interestingly enough, the nonselective inhibitor of PKCβ, and PKCδ, LY379196 (5×10^{-7} to 10^{-5} mol/L), blunted LDL uptake in monocyte-derived macrophages as shown by flow cytometry. Specific siRNA-mediated knockdown of PKCβ exerted a similar effect. Furthermore, PMA alone and in the presence of modified LDL induced scavenger receptor A mRNA and protein expression, which was abolished by LY379196. CGP53353, a selective inhibitor of PKCδ, did not affect LDL uptake, nor did it prevent scavenger receptor A upregulation. Incubation of monocyte-derived macrophages with PMA/LDL increased PKCβ phosphorylation at the Thr-642 residue, which was blunted when exposed to CD68, a marker of activated macrophages, was not affected by LY379196. Moreover, LY379196 did not affect lipopolysaccharide-induced CD14 degradation, tumor necrosis factor-α release, or superoxide anion production, ruling out any effect of PKCβ inhibition on innate immunity.

Conclusions—Nonspecific inhibition of PKCβ prevents LDL uptake in macrophages. These findings suggest that PKCβ inhibitors may represent a novel class of antiatherosclerotic drugs.

Key Words: atherosclerosis ■ foam cells ■ protein kinase C ■ lipoproteins, LDL ■ macrophages ■ receptors, scavenger

Protein kinase C (PKC) comprises several structurally related serine/threonine kinases classified into 3 groups. The conventional or classic PKCs include PKCα, PKCβI, PKCβII, and PKCγ. These isoforms can be activated by Ca^{2+} and/or diacylglycerol, as well as by phorbol esters. The novel PKCδ, PKCe, and PKCθ also are activated by diacylglycerol and phorbol esters but are Ca^{2+} independent. The atypical PKCs, which include PKCζ and PKCc, are unresponsive to Ca^{2+}/diacylglycerol and phorbol esters. Particularly interesting is the modulation of PKC activity by phosphorylation of serine and threonine amino acid residues within its catalytic and regulatory domains. Both conventional and novel PKC isoforms can translocate to the membranous compartment of the cell to elicit biological actions in the presence of diacylglycerol, the de novo synthesis of which is increased by hyperglycemia. Indeed, the activation of PKC pathway, especially the PKCβ isorform, has been shown extensively to cause diabetic vascular dysfunction. Because nonspecific PKC inhibition is associated with lethal side effects, isof orm-specific inhibitors have been developed. The macrocyclic bis (indolyl) maleimides like ruboxistaurin (LY333531), LY379196, LY317615, and LY290181 are competitive inhibitors of ATP binding sites within the PKC molecule.

The advantage of macrocyclic bis (indolyl) maleimides is their high selectivity for PKCβ compared with other isoforms of PKC. Treatment of diabetic patients for 3 months with

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PKC412, a multitarget kinase inhibitor that also acts as a nonselective PKC inhibitor, resulted in significant gastrointestinal side effects such as nausea, vomiting, and diarrhea, as well as liver toxicity. On the contrary, a multicenter, randomized clinical trial with the selective PKCβ inhibitor ruboxistaurin in patients with diabetic retinopathy revealed that its oral administration was well tolerated and did not cause any adverse events in patients with diabetes at doses up to 16 mg twice daily for 28 days. Furthermore, in patients with type 1 and 2 diabetes and minimal retinopathy, retinal blood flow was increased by the PKCβ inhibitor in a dose-dependent fashion. Treatment for >1 year with ruboxistaurin has shown beneficial effects in delaying the progression of diabetic nephropathy. These studies provided the first demonstration that a PKCβ isoform–selective inhibitor can be used for long-term clinical treatment of diabetic microangiopathy with minimal side effects. However, increasing evidence suggests that activation of PKCβ is involved in many mechanisms promoting atherosclerosis. Interestingly, phorbol 12-myristate 13-acetate (PMA), a structural analogue of diacylglycerol, which is a natural activator of PKC, can trigger transformation of monocytes to macrophages. The accelerated atherosclerosis and chronic activation of PKCβ in vascular tissues of diabetic patients, including the retina, heart, aorta, and circulating monocytes, prompted us to hypothesize that, among all PKC isoforms, PKCβ could be involved in foam cell formation.

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The present study was designed to investigate whether selective pharmacological inhibition of PKCβ prevents modified LDL uptake and hence foam cell formation. Our findings unmask an antiatherosclerotic effect of PKCβ inhibitors, extending their application far beyond diabetic vascular complications.

**Methods**

**Cell Culture**

Peripheral blood mononuclear cells were isolated from healthy control subjects by density centrifugation in BD Vacutainer cell preparation tubes with sodium heparin (Becton Dickinson, Franklin Lakes, NJ) and further purified by magnetic-activated cell separation sorting with anti–human CD14 antibody (Miltenyi Biotec, Cologne, Germany) conjugated with magnetic beads. After density centrifugation, highly purified monocytes were recovered. Human monocyte purity was assessed by flow cytometry (FACSCanto, BD, Heidelberg, Germany) using FITC-conjugated anti–human CD14 antibody (Miltenyi Biotec). The human monocytic THP-1 cell line was obtained from the American Type Culture Collection (Rockville, MD). Monocytes were cultured in RPMI 1640 medium containing 25 mmol/L HEPES buffer (supplemented with 10% FCS, 1% L-glutamine 200 mmol/L, penicillin 100 U/mL, and streptomycin 100 μg/mL) in humidified air, 5% CO2 at 37°C. Freshly isolated human monocytes and THP-1 monocytes were differentiated into monocyte-derived macrophages (MDMs) in vitro by treatment with 0.1 μmol/L PMA (Calbiochem, Darmstadt, Germany) overnight in starvation medium, 0.5% FCS RPMI 1640. During starvation, the cells were exposed to LY379196 (10−8 mol/L), a nonselective inhibitor of both PKCβ isoforms, and CGP53353 (10−6 mol/L), a PKCβ-selective inhibitor, and then incubated for another 24 hours in the presence of 10 μg/mL Dil-labeled acetylated low-density lipoprotein (Dil-acLDL) and Dil-labeled oxidized LDL (Dil-oxLDL) (Intracel, Frederick, MD). Human MDMs also were pretreated with myristoylated cell-permeable myr-ϕPKC peptide (10−4 mol/L) based on the pseudosubstrate motif of PKCβ, which keeps the enzyme in an inactive state by interacting with the substrate binding site of PKCβ catalytic domain. In another set of experiments, MDMs were pretreated with the 2 PKCβ inhibitors or myr-ϕPKC and stimulated for 24 hours with 100 ng/mL lipopolysaccharide (Sigma, Buchs, Switzerland). Afterward, tumor necrosis factor-α (TNFα) levels were measured in cell supernatant by ELISA (R&D Systems, Minneapolis, Minn). To exclude cytotoxicity, a colorimetric assay for detection of lactate dehydrogenase in cell supernatant was performed according to the manufacturer’s recommendations (Roche, Basel, Switzerland).

**siRNA Transfection**

Transfections were performed with INTERFERin (Polyplus Transfection, Basel, Switzerland) according to the manufacturer’s instructions in human MDMs (THP-1 cell line). Commercially available human PKCβ- and GAPDH- (Santa Cruz, Heidelberg, Germany) specific siRNAs were used for transfecting. The MDMs were transfected after 24 hours of seeding. INTERFERin transfection reagent (8 μL) was added to 100 μL OptiMEM serum-free medium containing 80 nmol/L of each siRNA oligo, incubated for 10 minutes, and added to the 3-cm plate containing 2 mL medium. GAPDH siRNA was used as a negative control. The FITC-labeled (Santa Cruz) control siRNA A was used as a marker for transfection efficiency. Gene silencing was measured after 48 hours by Western blotting. The transfected cells were incubated for an additional 24 hours in the presence of 10 μg/mL Dil-acLDL (Intracel), and acLDL uptake was measured by flow cytometry. Another set of transfected MDMs were used for Western blotting.

**Real-Time Polymerase Chain Reaction**

Total RNA was extracted from MDMs (THP-1 cell line) with Trizol reagent (Invitrogen, Basel, Switzerland) according to the manufacturer’s recommendations. The total cellular RNA was converted to cDNA with Moloney murine leukemia virus reverse transcriptase and random hexamers (Amersham Bioscience, Otelfingen, Switzerland) in a final volume of 35 μL, using 4 μg cDNA according to the manufacturer’s recommendations. Real-time polymerase chain reaction (PCR) was performed in a MX3000P PCR cycler (Stratagene, La Jolla, Calif). All PCR experiments were performed in triplicate using the SYBR Green JumpStart kit provided by Sigma. Each reaction (25 μL) contained 2 μL cDNA, 1 pmol of each primer, 0.25 μL internal reference dye, and 12.5 μL JumpStart Taq ReadyMix (containing buffer, dNTPs, stabilizers, SYBR Green, Taq polymerase, and JumpStart Taq antibody). The following primers were used: for scavenger receptor A (SR-A): sense primer, 5′-CCAGGGACATTGGGAACTGCA-3′; antisense primer, 5′-CCAGTGGCACCTGATCTCCC-3′; and for human L28: sense primer, 5′-GCTGACTGAAGTGGTGT-3′; antisense primer, 5′-TGTCTTCTGCGATCATGGT-3′. The amplification program consisted of 1 cycle at 95°C for 10 minutes, followed by 40 cycles with a denaturing phase at 95°C for 30 seconds, an annealing phase of 1 minute at 60°C, and an elongation phase at 72°C for 1 minute. A melting curve analysis was performed after amplification to verify the accuracy of the amplification. For verification of the correct amplification, PCR products were analyzed on an ethidium bromide–stained 1% agarose gel. In each real-time PCR run for F3 and L28, a calibration curve was included that was generated from serial dilutions (2−2, 2−4, 2−6, 2×10−1, 2×100, 2×101, 2×102, and 2×103 copies per reaction for SR-A; 2×10−1, 2×100, 2×101, 2×102, 2×103, and 2×104 copies per reaction for L28) of purified amplions for SR-A and L28.

**Western Blotting**

Human MDMs, THP-1 cell line, and freshly isolated blood monocytes (when indicated) were washed twice with PBS and harvested in the extraction buffer (120 mmol/L sodium chloride, 50 mmol/L Tris, 20 mmol/L sodium fluoride, 1 mmol/L benzamidine, 1 mmol/L DTT, 1 mmol/L EDTA, 6 mmol/L EGTA, 15 mmol/L sodium pyrophosphate, 0.8 μg/mL leupeptin, 30 mmol/L p-nitrophenyl...
phosphate, 0.1 mmol/L phenyl-methane-sulfonyl fluoride, and 1% NP-40 for immunoblotting. All cell debris was removed by centrifugation (12 000g) for 10 minutes at 4°C. The protein extracts (20 μg) were treated with 5X Laemmli’s SDS-PAGE sample buffer (0.35 mol/L Tris-Cl, pH 6.8, 15% SDS, 56.5% glycerol, 0.0075% bromophenol blue), followed by heating at 99°C for 5 minutes, and then applied to 10% SDS-polyacrylamide gel for electrophoresis. The proteins were then transferred onto Immobilon-P filter papers (Millipore AG, Bedford, Mass) with a semidyrid transfer unit (Hoeffer Scientific, San Francisco, Calif). The membranes were then blocked by use of 5% skim milk in TBS-Tween buffer (0.1% Tween 20, pH 7.5) for 1 hour and incubated overnight with anti–human SR-A (1:500, TransGenic, Kobe, Japan), anti–human lectin-like oxLDL receptor 1 (LOX-1; 1:4000, R&D Systems, Bad Nauheim, Germany), anti–phospho-Thr-642 PKCδ2 (1:1000, BioSource, Nivelles, Belgium), anti–PKCβ1 (1:1000, Santa Cruz), and anti–CD14 (1:1000, Dako Cytomation, Baar, Switzerland) antibodies in 0.5% BSA PBS. Antigen detection was performed with an enhanced chemiluminescence detection system (Amersham Biosciences, Otelfingen, Switzerland).

**Measurements of Superoxide Anion Production**

Superoxide production in MDMs, both freshly isolated and from the THP-1 cell line, were subjected to flow cytometry to measure the amount of internalized LDL. Adherent and nonadherent cells were harvested by gentle scraping. Cells were then washed twice with PBS and resuspended in 0.2% BSA in PBS. Samples were analyzed with the FACScanto II flow cytometer and Flowjo software.

**Confocal Fluorescent Microscopy**

Human MDMs were washed once with PBS, fixed in 4% paraformaldehyde for 10 minutes, washed again with PBS, blocked with 0.1% NP-40 for 5 minutes, permeabilized with 0.2% Triton 100 for 7 minutes, and incubated overnight with anti–human SR-A (TransGenic), anti–PKCβ1 (BioSource), anti–PKCβ2 (BioSource), or anti–CD68 (Dako Cytomation) antibody in 0.2% BSA. Afterward, the cells were washed three times with PBS and incubated with secondary Alexa Fluor 488–labeled antibody (Molecular Probes, Eugene, Ore) in 0.2% BSA for 1 hour. Cells were counterstained with 4′, 6 diamidino-2-phenylindol (DAPI; Vector Laboratories, Burlingame, Calif) and analyzed with a Leica confocal laser microscope.

**Drugs**

LY379196 was provided by Eli Lilly (Indianapolis, Ind). CGP33535 was kindly provided by Dr Doriano Fabbro (Novartis Pharma AG, Basel, Switzerland). Calphostin C, PMA, and myr-IP3 were purchased from Calbiochem. Lipopolysaccharide was obtained from Sigma.

**Statistical Analysis**

Results are expressed as mean±SEM, and n indicates the number of experiments. Statistical evaluation of the data was performed with Student’s t test for simple comparisons between 2 values when appropriate. For multiple comparisons, results were analyzed by ANOVA followed by Fisher’s exact test. A value of P<0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Role of PKCβ in Mediating Human MDM Foam Cell Formation**

The differentiation of human primary monocytes and monocyctic THP-1 cell line into macrophages (MDMs) was induced by PMA (0.1 μmol/L). Incubation of human MDMs with DiI-acLDL (10 μg/mL) led to binding of acLDL to the plasma membrane and accumulation of lipoproteins into the cytoplasm as assessed by fluorescence microscopy (Figure 1A). The nonselective inhibitor of both PKCβ isozymes, LY379196 (10−6 mol/L), abolished acLDL uptake in MDMs as shown in Figure 1B. To identify the isof orm of PKCβ involved, human MDMs were exposed to selective antibodies against PKCβ1 and PKCβ2. PKCβ isozymes were localized in the nucleus and in the cytoplasm of MDMs, showing an activated state of the enzyme. The PKCβ1 isoform was present in the perinuclear patch and plasma membrane (Figure 1D).

Accordingly, LY379196 showed a dose-dependent decrease in both acLDL and oxLDL uptake in MDMs by flow...
cytometry (Figure 2A and 2B). In contrast, the selective inhibitor of PKCβ, CGP53353, did not exert any significant effect (data not shown).

**Effect of PKCβ Inhibition on SR-A Expression**
To delineate the molecular mechanism by which LY379196 blunted modified LDL uptake, MDM gene and protein expression of SR-A was determined. Quiescent cells did not express SR-A mRNA; stimulation with PMA (0.1 μmol/L) increased SR-A expression (Figure 3). A more pronounced upregulation of SR-A expression was achieved by the addition of acLDL (10 μg/mL) to the medium. SR-A mRNA expression was abolished by treatment with LY379196 (10^{-6} mol/L), whereas CGP53353 (10^{-6} mol/L) did not exert any inhibitory effect on PMA/acLDL-induced SR-A expression in human MDMs (Figure 3). In agreement with these results, SR-A protein expression was increased after exposure to acLDL as and oxLDL (Figure 4A and 4B). Treatment with LY379196, but not with CGP53353, totally abrogated PMA/acLDL-induced macrophage SR-A expression (Figure 4A). MDMs exposed to oxLDL in the presence of LY379196 (10^{-6} mol/L) showed similar results (Figure 4B). A myristoylated cell-permeable myr-ψPKC inhibitory peptide was used to confirm the modulatory role of PKCβ on SR-A expression. Like LY379196, the inhibitory peptide myr-ψPKC (10^{-4} mol/L) significantly blunted SR-A protein expression in human MDMs (Figure 4C).

In contrast to SR-A, LOX-1 protein expression did not change after exposure of MDMs to modified LDL. Furthermore, LY379196 (10^{-6} mol/L) did not exert any significant effect on LOX-1 expression (Figure 4D).
Effect of Silencing PKCβ on LDL Uptake and SR-A Expression

The transfection of PKCβ siRNA into MDMs resulted in reduced expression of the protein, whereas the GAPDH and mock controls did not exert any significant effect (Figure 5A). Using flow cytometry, we examined the Di-acLDL uptake by the transfected cells. PKCβ silencing reduced the uptake and blunted SR-A protein expression, reproducing the same effect as the pharmacological inhibitor LY379196 (Figure 5B and 5C).

Role of Thr-642 Phosphorylation in PKCβ1-Mediated SR-A Expression

Western blotting with an antibody against phosphorylated PKCβ, at a specific amino residue revealed that incubation of...
the cells with PMA/acLDL increased Thr-642 phosphorylation within the catalytic domain of this molecule (Figure 6). The inhibitor of both PKC\(\beta\) isoforms, LY379196 (10\(^{-6}\) mol/L), and the inhibitory peptide myr-\(\psi\)PKC (10\(^{-4}\) mol/L) blunted PMA/acLDL-induced Thr-642 phosphorylation (Figure 6), suggesting that phosphorylation of PKC\(\beta\) at Thr-642 may thus represent a selective regulatory mechanism for SR-A upregulation. Accordingly, the selective PKC\(\beta\) inhibitor, CGP53353 (10\(^{-6}\) mol/L), did not affect Thr-642 phosphorylation (Figure 6).

**Effect of PKC\(\beta\) Inhibition on Macrophage Activation and Functioning**

As shown by confocal microscopy, high levels of SR-A expression in MDMs stimulated with acLDL were blunted in the presence of LY379196 (Figure 7A and 7B). However, LY379196 did not exert any effect on the expression of CD68, a marker of macrophage activation (Figure 7C and 7D). In addition, human MDMs were exposed to lipopolysaccharide (100 ng/mL) to rule out an effect of PKC\(\beta\) inhibition on macrophage functioning in innate immunity. Lipopolysaccharide elicited degradation of CD14 and secretion of TNF\(\alpha\), crucial steps in the activation of innate immunity (Figure 8A and 8B). Interestingly enough, increasing concentrations of LY379196 did not inhibit either lipopolysaccharide-induced CD14 degradation (Figure 8A) or TNF\(\alpha\) release (Figure 8B). Treatment with LY379196 did not elicit any release of lactate dehydrogenase (data not shown), ruling out that its effects on human MDMs were due to cellular toxicity. In agreement with this finding, no apoptotic nuclei by DAPI staining were observed (Figure 7). Furthermore, the effect of LY379196 (10\(^{-6}\) mol/L) on the ability of macrophages to produce superoxide anion (O\(_2^−\)) was measured. No significant changes in O\(_2^−\) production were observed (Figure 8C). In addition, silencing of PKC\(\beta\) did not affect O\(_2^−\) production (data not shown).

**Discussion**

Accumulation of cholesterol-loaded foam cells in the arterial intima is a hallmark and key event of early atherogenesis.\(^{23}\) Circulating monocytes adhere to activated endothelial cells and transmigrate into the subintima to become tissue macrophages. On exposure to modified lipoproteins such as the oxLDL and acLDL, these macrophages become foam cells.\(^{24}\) Two receptors appear to be essential in foam cell formation and receptor-mediated binding/uptake of modified lipoproteins: CD36 and SR-A.\(^{25}\) Despite increasing evidence supporting that PKC is involved in many mechanisms promoting atherosclerosis,\(^{16}\) only a few studies have examined the role of PKC signaling in foam cell formation.\(^{20,26}\)

In this study, we demonstrated that inhibition of PKC\(\beta\) prevents uptake of modified LDL by reducing human MDM SR-A expression. Several lines of evidence support this conclusion. First, fluorescent-activated cell sorter analysis (Figure 6).
revealed that the nonselective inhibitor of PKC isoforms blunted modified LDL uptake of human MDMs. Second, silencing of PKCβ by siRNA transfection also reduced LDL uptake. Third, we observed a selective Thr-642 phosphorylation within the catalytic domain of PKCβ and an increase in SR-A mRNA and protein expression in human MDMs exposed to modified LDL. Fourth, both LY379196 and the inhibitory peptide myr-ϕPKC blunted phosphorylation of Thr-642 and upregulation of SR-A. In contrast, CGP53353, the selective inhibitor of PKCβ, did not exert any significant effect. Expression and function of macrophage SR-A play a crucial role in the pathogenesis of atherosclerosis.27,28 Accordingly, SR-A gene–deficient mice bred with atherosclerosis-prone ApoE<sup>−/−</sup> or LDLrec<sup>−/−</sup> mice have been found to develop less atherosclerosis.29 We recently showed that ApoE<sup>−/−</sup> mice simultaneously lacking c-Jun N-terminal kinase 2 (ApoE<sup>−/−</sup>/Jnk2<sup>−/−</sup> mice) developed less atherosclerosis than ApoE<sup>−/−</sup> mice.30 Macrophages lacking c-Jun N-terminal kinase 2 displayed markedly decreased phosphorylation of SR-A and hence suppressed foam cell formation.30 Thus, upstream signaling molecules that regulate the expression and function of SR-A may represent potential targets for therapeutic interventions.24 The present findings clearly indicate that SR-A expression in human MDMs is regulated by PKCβ. In contrast to SR-A, LOX-1 expression did not change after stimulation of MDMs with modified LDL. Furthermore, LY379196 did not exert any significant effect on LOX-1. Because SR-A expression was enhanced on modified LDL stimulation and not LOX-1, we conclude that SR-A might be the primary receptor for modified LDL uptake.

Several studies have strongly implicated activation of PKCβ in the pathogenesis of the vascular complications of diabetes.31 The synthesis of isoform-specific inhibitors for PKCβ has provided not only important insights into diabetic cardiovascular disease but also effective drugs against diabetic microvascular complications.15,32,33 Glucose-induced activation of PKCβ may lead to endothelial dysfunction by causing activation of vascular NADPH oxidase, endothelial nitric oxide synthase uncoupling, and reactive oxygen species production.6,34 Furthermore, high glucose enhances human macrophage LOX-1 expression via PKCβ activation.35 Treatment with a PKCβ inhibitor prevents impaired endothelium-dependent vasodilation caused by hyperglycemia.3 We demonstrated that selective inhibition of PKCβ<sub>2</sub> inhibits glucose-induced vascular cellular adhesion molecule-1 expression in human endothelial cells.7 Interestingly enough, our data unmask an antiatherosclerotic effect of PKCβ inhibitors even in the nondiabetic condition of hypercholes-
The application of PKC inhibitors further supports our conclusion. Indeed, on silencing of PKCβ, LDL uptake was blunted, SR-A expression was reduced, and hence foam cell formation was prevented. The molecular link between the PKCβ signaling pathway, SR-A upregulation, and uptake of modified LDL might involve Thr-642 phosphorylation within the catalytic domain of PKCβ1. Indeed, inhibition of PKCβ by either LY379196 or the inhibitory peptide myr-δPKC blunting PMA/aCLDL-induced Thr-642 phosphorylation abolished upregulation of SR-A and LDL uptake of human MDMs. In contrast, the selective inhibitor of PKCβ2, CGP33533, did not affect any of these events. According to our results, phosphorylation of PKCβ at Thr-642 represents a selective regulatory mechanism for SR-A upregulation and foam cell formation.

Of particular interest is the fact that in our study LY379196, as a drug targeting macrophages, prevented only foam cell formation without affecting macrophage host defense activity. Indeed, LY379196 blunted modified LDL uptake but did not affect the expression of CD68, a marker of macrophage activation. We also demonstrated that LY379196 did not inhibit lipopolysaccharide-induced CD14 degradation or TNFα release in human MDMs. Moreover, PKCβ knockdown or inhibition did not affect superoxide anion production. These findings rule out any effect of PKCβ inhibition on macrophage functioning in innate immunity. In agreement with our results, PKCβ-deficient mice have not been reported to present any impairment in macrophage activity. Although these mice show a reduced peritoneal population of B-1 lymphocytes, the absolute number of splenic B cells is similar to wild-type animals. Furthermore, the thymuses of PKCβ-deficient mice were of normal size and cellularity and contained CD4+CD8+ double-positive cells and CD4+ or CD8+ single-positive cells at normal ratios.

Earlier studies of the role of vascular PKCβ activation in diabetes were focused primarily on microvascular dysfunction. Indeed, PKCβ inhibitors are currently being tested in clinical trials with microvascular end points.12–14

Conclusions
The results of our study suggest a role for PKCβ in atherogenesis even in the nondiabetic condition and anticipate the application of PKCβ inhibitors as putative antiatherosclerotic drugs. However, before deciding whether PKCβ inhibitors deserve to be tested in clinical trials of atherosclerosis, animal models will help evolve our current suggestive in vitro evidence concerning a proatherosclerotic role of PKCβ signaling.

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Disclosures
None.

References
Low-density lipoprotein (LDL) uptake by monocyte-derived macrophages is a hallmark and key event of early atherosclerosis. On exposure to modified lipoproteins such as oxidized LDL and acetylated LDL, these macrophages become foam cells. Increasing evidence supports that activation of PKCβ is involved in many mechanisms promoting atherosclerosis. In this study, we demonstrate that inhibition of protein kinase Cβ (PKCβ) prevents uptake of modified LDL by reducing human monocyte-derived macrophage scavenger receptor A expression. Several studies have strongly implicated activation of PKCβ in the pathogenesis of the vascular complications of diabetes. The synthesis of isoform-specific inhibitors for PKCβ has provided not only important insights into diabetic cardiovascular disease but also effective drugs against its microvascular complications. Interestingly enough, our data unmask an antiatherosclerotic effect of PKCβ inhibitors even in the nondiabetic condition of hypercholesterolemia. Specific siRNA-mediated knockdown of PKCβ further supports our conclusion. Indeed, on silencing of PKCβ, LDL uptake was blunted, scavenger receptor A expression was reduced, and hence foam cell formation was prevented. Of particular interest is the fact that in our study the PKCβ inhibitor D844.798, as a drug targeting macrophages, prevents only foam cell formation without affecting macrophage host defense activity. Although PKCβ inhibitors are currently being tested in clinical trials with microvascular end points, the present findings suggest a role for PKCβ in atherogenesis even in the nondiabetic condition and anticipate the application of PKCβ inhibitors as putative antiatherosclerotic drugs.
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