Toll-Like Receptor 2 Mediates Apolipoprotein CIII–Induced Monocyte Activation

Akio Kawakami, Mizuko Osaka, Masanori Aikawa, Satoshi Uematsu, Shizuo Akira, Peter Libby, Kentaro Shimokado, Frank M. Sacks, Masayuki Yoshida

Abstract—Apolipoprotein (apo)CIII predicts risk for coronary heart disease. We recently reported that apoCIII directly activates human monocytes. Recent evidence indicates that toll-like receptor (TLR)2 can contribute to atherogenesis through transduction of inflammatory signals. Here, we tested the hypothesis that apoCIII activates human monocytoid THP-1 cells through TLR2. ApoCIII induced the association of TLR2 with myeloid differentiation factor 88, activated nuclear factor (NF)-κB in THP-1 cells, and increased their adhesion to human umbilical vein endothelial cells (HUVECs). Anti-TLR2 blocking antibody, but not anti-TLR4 blocking antibody or isotype-matched IgG, inhibited these processes (P <0.05). ApoCIII bound with high affinity to human recombinant TLR2 protein and showed a significantly higher (P <0.05) and saturable binding to 293 cells overexpressing human TLR2 than to parental 293 cells with no endogenous TLR2. Overexpression of TLR2 in 293 cells augmented apoCIII-induced NF-κB activation and β1 integrin expression, processes inhibited by anti-apoCIII antibody as well as anti-TLR2 antibody. Exposure of peripheral blood monocytes isolated from C57BL/6 (wild-type) mice to apoCIII activated their NF-κB and increased their adhesiveness to HUVECs. In contrast, apoCIII did not activate monocytes from TLR2-deficient mice. Finally, intravenous administration to C57BL/6 mice of apoCIII-rich very-low-density lipoprotein (VLDL), but not of apoCIII-deficient VLDL, activated monocytes and increased their adhesiveness to HUVECs, processes attenuated by anti-TLR2 or anti-apoCIII antibody. ApoCIII-rich VLDL did not activate monocytes from TLR2-deficient mice. In conclusion, apoCIII activated monocytes at least partly through a TLR2-dependent pathway. The present study identifies a novel mechanism for proinflammatory and proatherogenic effects of apoCIII and a role for TLR2 in atherosclerosis induced by atherogenic lipoproteins. (Circ Res. 2008;103:1402-1409.)

Key Words: apolipoprotein ■ atherosclerosis ■ inflammation ■ monocyte ■ Toll-like receptor

Toll-like receptors (TLRs) contribute importantly to neutrophil-mediated responses during inflammation. Exogenous ligands such as lipopolysaccharide (LPS) and peptidoglycan, as well as endogenous factors produced on stress or cell damage, eg, heat shock proteins, activate these pattern-recognition receptors. The recent identification of mammalian TLRs as principal sensors of the innate immune system provides a mechanistic link among infection, inflammation, and atherosclerosis.1 Moreover, activation of TLRs by endogenous ligands can provoke sterile inflammation linked to atherogenesis susceptibility.2-3 Among TLRs, TLR2 and TLR4 may particularly in the inflammatory response and in atherosclerosis. Recent studies in fat-fed mice have shown that TLR4, TLR2, and myeloid differentiation factor 88 (MyD88) contribute to atherosclerotic plaque accumulation induced by hyperlipidemia.1,4 Michelsen et al found that a loss of TLR4 or its adaptor protein MyD88 reduces disease severity in atherosclerosis-prone apolipoprotein (apo)E-deficient mice.4 Mullick et al1 reported that, in the absence of any known exogenous TLR2 agonist, complete deficiency of TLR2 in LDL receptor–deficient mice reduced atherosclerosis, whereas loss of TLR2 expression in bone marrow–derived cells did not have an impact on disease. These results suggest that unknown endogenous TLR agonists impact atherosclerotic disease. Although the mechanism(s) by which TLRs contribute to atherogenesis remains obscure, we and others previously reported that TLR2 and TLR4 play a role in the enhancement of monocyte–endothelial interaction, a crucial step throughout atherogenesis.5-7

Plasma levels of apoCIII independently predicts risk for coronary heart disease.8 We recently demonstrated that apoB lipoproteins that contain multiple copies of apoCIII, but not apoCIII-deficient apoB lipoproteins, induce human monocyte adhesion to vascular endothelial cells (ECs)9,10 via a protein
kinase (PKC)α- and nuclear factor (NF)-κB-dependent mechanism.11 Interestingly, apoCIII alone also had similar effects on vascular cells, suggesting that these effects of apoCIII-containing apolipoproteins are mediated by apoCIII rather than by either apolipoproteins or lipids in very-low-density lipoprotein (VLDL) or LDL and not by apoB/E receptors on monocytes. We thus hypothesized that TLRs mediate apoCIII signaling and contribute to proinflammatory properties for apoCIII. The present study determined whether TLR2 or TLR4 participates in apoCIII-induced activation of monocytes and their adhesion to ECs in vitro and in vivo.

Materials and Methods

Animals and Cells

Seven-week-old male C57BL/6 (wild-type) mice (Oriental Yeast, Tokyo, Japan) or TLR2-deficient mice consumed a standard diet (CLEA Japan, Tokyo, Japan). Food and water were provided ad libitum. Human peripheral blood monocytes were collected under a protocol approved by the Human Research Committee of the Brigham and Women’s Hospital and were cultured as described previously.12 Human umbilical vein endothelial cells (HUVECs), Brigham and Women’s Hospital and were cultured as described protocol approved by the Human Research Committee of the

Figure 1. TLR2 mediates apoCIII-induced THP-1 cell activation. A through D, THP-1 cells were pretreated with indicated antibodies (µg/mL) for 30 minutes and then incubated with or without apoCIII (100 µg/mL) for 8 hours (A and D) or 2 hours (B and C). mem indicates the membrane fraction of the cell lysates (B). E, THP-1 cells were incubated with or without apoCIII (100 µg/mL) for 2 hours. *P<0.01 vs apoCIII (−)/antibodies (−), #P<0.05 vs apoCIII (+)/antibodies (−). IB and IP indicate immunoblottting and immunoprecipitation, respectively. Blots represent 4 to 6 independent experiments using apoCIII from 4 to 6 different donors that yielded similar results (B through E).

Reagents

Human apoC, -CII, -CIII, and -E were purchased from Academy Biomedical (Houston, Tex). They were purified from human plasma using HPLC and immunoaffinity column chromatography. Their purity was >99.0%, as determined by SDS-PAGE. Endotoxin levels in apolipoproteins measured using a chromogenic Limulus amebocyte lysate test (Associates of Cape Cod, East Falmouth, Mass) were <0.03 endotoxin unit (EU)/mL. Free fatty acid levels in apolipoproteins determined enzymatically were <20 nmol/L. Antibodies used in the present study were as follows: anti-β1 integrin, anti-MYD88, anti-Rac1, anti-NF-κB p65, fluorescein isothiocyanate (FITC)-conjugated NF-κB p65, anti-CD14, and anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, Calif); anti–PKCα (BD Biosciences, San Jose, Calif); anti–apoCIII (Academy Biomedical); anti–TLR2 and anti–TLR4 (Imgenex, Sun Diego, Calif); and anti–NF-κB p65 (pS276) (Rockland, Gilbertsville, Pa). Polymyxin B, peptidoglycan (Staphylococcus aureus), and LPS (Escherichia coli O26:B6) were purchased from Sigma.

Static Adhesion Assay

HUVECs seeded on 1% gelatin-coated 96-well culture plates were maintained for 2 days to allow the formation of a confluent monolayer, and stimulated with interleukin 1β (Genzyme, Cambridge, Mass) at 10 U/mL for 4 hours before adhesion assay. After THP-1 cells or freshly isolated mice peripheral blood monocytes were incubated with or without apoCIII or reagents as indicated, cells were labeled with BCECF-AM (Calbiochem, La Jolla, Calif), placed on HUVEC monolayers at 1×10⁵ per well, and allowed to adhere for 10 minutes. After nonadherent cells were removed by washing gently twice with RPMI-1640, the fluorescent intensities of adherent cells in 6 wells were measured by CytoFluor II (Perceptive Biosystems, Framingham, Mass) with 485-nm excitation and 530-nm emission. The ratio of fluorescence intensity of the adherent cells to that of the total cells applied to the well was expressed as leukocyte adhesion (percentage). Cell viability after incubation with
lipoproteins and reagents was examined by staining with 0.25% trypan blue solution.

**Immunoblotting and Immunoprecipitation**

Total cell lysates and the membrane fraction of the indicated cells were prepared as described previously. An equal amount of protein was subjected to 12% SDS-PAGE and transferred to poly(vinylidene difluoride) membrane. Immunoreactive proteins in the membrane were detected using indicated antibodies with an enhanced chemiluminescence (ECL) plus (Amersham Biosciences, Piscataway, NJ). Activation of PKC was examined by detecting the membrane-bound protein that translocated from cytosol fraction.

For immunoprecipitation, a cell lysate from THP-1 cells was incubated with anti-TLR2 antibody. Then, 50 L of anti-IgG affinity gel (MP Biomedicals, Solon, Ohio) was added for an additional 60 minutes, after which the immunocomplexes were collected and resuspended in SDS-PAGE sample buffer for immunoprecipitation as described previously.

**Protein-Binding Studies**

Ninety-six-well tissue culture plates were coated with or without recombinant TLR2/Fc chimera protein, TLR4/Fc chimera protein (R&D Systems, Minneapolis, Minn) at 2 µg per well. ApoCIII proteins were labeled with FITC using EZ-Label FITC protein-labeling kit according to the instructions of the manufacturer (Pierce, Rockford, Ill). After 96-well tissue culture plates were blocked with the albumin (Sigma), FITC-labeled apoCIII (100 µg/mL) was added to 96-well plates and incubated for 10 minutes at 4°C. Some experiments included nonlabeled apoCIII or other potential competitors. After extensive washing, FITC associated with cells was measured using a chromogenic Limulus amebocyte lysate test (Associates of Cape Cod) were <0.03 EU/mL.

**In Vivo Stimulation**

For in vivo stimulation, the mice were injected intravenously with VLDL preparations (500 µg apoB per mouse) from femoral veins. After 2 hours, monocytes were isolated as described above for immunoblotting or immunofluorescence microscopy. For static adhesion assay, they were isolated 6 hours after VLDL injection.

**Lipoprotein Preparation**

Blood was drawn in tubes containing EDTA from newly diagnosed 18 hypertriglyceridemic patients at 12-hours fasting. The study was approved by the Institutional Review Board of the Tokyo Medical and Dental University. The subjects had no other serious diseases and had not taken cardiovascular medications, lipid-lowering medications, pharmacological doses of antioxidants, or estrogen for more than 14 days. ApoCIII-rich VLDL (VLDL CIII) and apoCIII-deficient VLDL (VLDL CIII) were isolated from plasma as described previously. Endotoxin levels in the lipoprotein fractions measured using a chromogenic Limulus amebocyte lysate test (Associates of Cape Cod) were <0.03 EU/mL.

**In Vivo Stimulation**

For in vivo stimulation, the mice were injected intravenously with VLDL preparations (500 µg apoB per mouse) from femoral veins. After 2 hours, monocytes were isolated as described above for immunoblotting or immunofluorescence microscopy. For static adhesion assay, they were isolated 6 hours after VLDL injection.

**Immunofluorescence Microscopy**

For NF-κB p65 staining, isolated mouse monocytes were fixed with 4% formaldehyde for 30 minutes and then washed with 0.05% Triton X-100 for 5 minutes. The cells were incubated with FITC-conjugated NF-κB p65 antibody (1:200) for 45 minutes. The cells were rinsed and mounted onto slides, then analyzed and imaged using a fluorescent microscope (Olympus) with a 400-fold magnification.
Statistical Analysis
Results are given as the means±SD. Data were analyzed using unpaired t test or 2-way ANOVA, with a value of P<0.05 considered significant.

Results
Involvement of TLR2 in ApoCIII-Induced THP-1 Cell Activation
ApoCIII induces the adhesion of THP-1 cells to vascular endothelial cells through activation of PKCα and NF-κB.11 We first examined whether these processes involve TLR2 or TLR4 using blocking antibodies to these receptors. Anti-TLR2 antibody treatment of THP-1 cells inhibited apoCIII-induced THP-1 cell adhesion in a concentration-dependent manner. Neither anti-TLR4 nor an irrelevant isotype-matched IgG had minimal effects on these processes (data not shown). We also reported that apoCIII increases β1 integrin expression in THP-1 cells.8 Anti-TLR2 antibody decreased apoCIII-induced β1 integrin expression in a concentration-dependent manner (Figure 1D). Recent studies showed that CD14 physically interacts with TLR2 and facilitates ligand binding. Miller et al reported that minimally modified LDL binds to macrophage CD14.17 We thus examined the involvement of CD14 in apoCIII-induced monocyte activation. Anti-CD14 binding blocking antibody had minimal effect on apoCIII-induced NF-κB activation (data not shown). The signaling pathways arise from the intracytoplasmic TIR (Toll/interleukin-1 receptor) domain, conserved in all TLRs.18 Recent studies also reported that Rac1 mediates TLR2-induced NF-κB activation independently of MyD88.19 ApoCIII stimulation of THP-1 cells induced the association of TLR2 with MyD88 but not Rac1 (Figure 1E).

We also performed additional experiments using known ligands for TLRs to validate the efficacy and specificity of these antibodies. Anti-TLR2 antibody and anti-TLR4 antibody inhibited NF-κB in THP-1 cells (Figure I, A, in the online data supplement, available at http://circres.ahajournals.org), a process activated by either the TLR2 ligand peptidoglycan (Staphylococcus aureus) or the TLR4 ligand LPS (Escherichia coli). Adding the LPS antagonist polymyxin B to the culture media did not inhibit apoCIII-triggered NF-κB activation (supplemental Figure I, B). In contrast, anti-apoCIII antibody abolished apoCIII-induced NF-κB activation and THP-1 cell adhesion (supplemental Figure I, C and D). Thus, LPS contamination did not account for apoCIII proinflammatory activity, as determined by the Limulus amebocyte lysate test, absence of inhibition by anti-TLR4 antibody, and insensitivity to polymyxin B. These results suggest that observed effects depended on apoCIII itself and were mediated, at least in part, by a TLR2- and MyD88-dependent pathway.

ApoCIII also promoted PKCα activation and NF-κB activation in human peripheral blood monocytes and induced their adhesion to HUVECs.9 Anti-TLR2 antibody but not anti-TLR4 antibody attenuated these processes (Figure 2A through 2C).

Association of ApoCIII With TLR2
We then examined the binding of FITC-labeled apoCIII with TLR2 protein–coated plastic plates. ApoCIII bound to TLR2 protein with significantly higher affinity than albumin or TLR4 protein (Figure 3A). Pretreatment of apoCIII with anti-apoCIII blocking antibody abolished apoCIII binding (Figure 3B). Excess nonlabeled apoCIII, but not apoCI, apoCII, or apoE (data not shown), competed for binding of FITC-labeled apoCIII with TLR2 protein (Figure 3C). Anti-TLR2 antibody inhibited the association of apoCIII with
Precipitation of TLR2 (Figure 3C). Interestingly, peptidoglycan failed to compete with apoCIII binding to TLR2 protein (Figure 3C).

Association of ApoCIII With Human TLR2-Transfected 293 Cells

We further examined the association of apoCIII with TLR2 in situ using human TLR2-transfected 293 cells (hTLR2-293 cells). ApoCIII showed significantly higher affinity with hTLR2-293 cells compared with parental 293 cells that did not express TLR2 (Figure 4A and 4B). ApoCIII showed saturable binding with hTLR2-293 cells over 100 μg/mL (Figure 4B). In line with the results shown in Figure 3, anti-TLR2 antibody and nonlabeled apoCIII inhibited the association of apoCIII with hTLR2-293 cells (Figure 4A and 4C). Peptidoglycan did not compete with apoCIII binding, suggesting the apoCIII binding to a region of TLR2 distinct from the peptidoglycan-binding site (Figure 4C). We then tested whether overexpression of TLR2 functionally enhances the response to apoCIII treatment. Baseline levels of NF-κB activation and β1 integrin expression did not change between parental 293 cells and hTLR2-293 cells. Although apoCIII induced NF-κB activation and β1 integrin expression in 293 cells that did not express TLRs or CD14, apoCIII effects were further enhanced in hTLR2-293 cells (Figure 4D and 4E). Anti-TLR2 antibody but not anti-TLR4 antibody inhibited apoCIII-induced NF-κB activation in hTLR2-293 cells (supplemental Figure II, A). The NF-κB inhibitor SN50 but not a control scrambled peptide inhibited apoCIII-induced β1 integrin expression in hTLR2-293 cells (supplemental Figure II, B), without affecting apoCIII-binding to hTLR2-293 cells (data not shown). These results indicate that TLR2 not only increases the binding of apoCIII but also functions as the modulator of apoCIII-induced proinflammatory signal transduction, whereas some apoCIII has the ability to activate 293 cells without TLR4 or CD14.

Involvement of TLR2 in ApoCIII-Induced Mouse Peripheral Blood Monocyte Activation

We further used monocytes isolated from C57BL/6 (wild-type) mice or TLR2-deficient mice to examine the mechanism of proinflammatory action of apoCIII ex vivo. Our validation studies demonstrated that LPS activated NF-κB in TLR2-deficient mouse monocytes, as well as wild-type mouse monocytes. However, peptidoglycan failed to activate NF-κB in TLR2-deficient mouse monocytes (supplemental Figure III). Although apoCIII significantly promoted PKCα activation and NF-κB activation in wild-type mouse monocytes, their activation was not significant in TLR2-deficient

Figure 4. Binding of apoCIII with human TLR2-transfected 293 cells. A, FITC-labeled apoCIII (100 μg/mL) was placed on cultured 293 cells or hTLR2-293 cells for 10 minutes. Then, FITC-labeled apoCIII was observed with a fluorescent microscope. In some experiments, hTLR2-293 cells were pretreated with anti-TLR2 antibody (50 μg/mL) for 30 minutes. Images are representative of 3 separate experiments. B, FITC-labeled apoCIII was placed on cultured 293 cells or hTLR2-293 cells for 10 minutes at indicated concentrations. *P<0.05, **P<0.01 vs 293 cells. C, FITC-labeled apoCIII (100 μg/mL) was placed on cultured hTLR2-293 cells for 10 minutes in the presence of an excess amount of nonlabeled apoCIII (self) (30-fold), indicated antibodies (50 μg/mL), or peptidoglycan (10 μg/mL). *P<0.05 vs (−) D and E. Cultured 293 cells or hTLR2-293 cells were incubated with FITC-labeled apoCIII (100 μg/mL) for 2 hours (D) or 8 hours (E). *P<0.05 vs apoCIII (−) 293 cells, **P<0.01 vs apoCIII (−) hTLR2-293 cells, †P<0.05 vs apoCIII (+) 293 cells. IB indicates immunoblotting. Blots represent 5 independent experiments using apoCIII from 5 different donors that yielded similar results.

1406 Circulation Research December 5, 2008
This article was retracted in February 2012

Kawakami et al  ApoCIII Activates Monocytes Through TLR2  1407

We tested whether TLR2 is crucial for apoCIII-rich VLDL (VLDL CIII⁺)-dependent monocyte activation in vivo. Monocytes were isolated from C57BL/6 (wild-type) mice after intravenous administration of VLDL preparations. VLDL CIII⁺ treatment, but not apoCIII-deficient VLDL (VLDL CIII⁻) treatment, significantly increased the adhesiveness of monocytes to HUVECs. VLDL CIII⁺ pretreated with anti-apoCIII antibody before administration showed decreased effects on monocyte adhesion (Figure 6A). Pretreatment of the mice with anti-TLR2 antibody but not anti-TLR4 antibody attenuated VLDL CIII⁺-induced monocyte adhesion. Isotype-matched IgG did not affect monocyte adhesion (Figure 6B).

We further assessed the activation of monocytes from TLR2-deficient mice, as well as those from wild-type mice. VLDL CIII⁺ induced phosphorylation of NF-κB p65 of wild-type mouse monocytes but not TLR2-deficient mouse monocytes (Figure 6C). VLDL CIII⁻ did not induce phosphorylation of NF-κB p65 of wild-type mouse monocytes or TLR2-deficient mouse monocytes (data not shown). We also assessed NF-κB activation by its nuclear translocation. VLDL CIII⁺ treatment induced the translocation of NF-κB p65 from cytosol to nuclei in wild-type mouse monocytes, as demonstrated by immunofluorescence microscopy. In contrast, VLDL CIII⁻ had minimal effect on NF-κB p65 nuclear translocation in TLR2-deficient mouse monocytes (Figure 6D). TLR2-deficient mouse monocytes also showed less incremental adhesiveness to HUVECs induced by VLDL CIII⁺ compared with wild-type mouse monocytes (Figure 6E). Taken together, these results suggest that the TLR2 signaling pathway plays a role in the effect of apoCIII or VLDL CIII⁺ in the activation of monocytes in vivo.

Discussion

We recently reported that VLDL CIII⁺ activated β₁ integrin through PKCa in THP-1 cells and increased their adhesion to ECs under static or flow conditions.⁹ VLDL CIII⁺ also induced vascular EC activation and increased adhesion molecule expression.¹⁰ Interestingly, in both studies, apoCIII alone as well as VLDL CIII⁺ activated these cells suggesting that distinct signaling pathways that do not involve apoB/E receptors mediate the direct proinflammatory and proatherogenic effects of apoCIII. The present study implicates TLR2 in apoCIII-induced monocyte activation. Blocking antibody to TLR2 or genetic inactivation of TLR2 reduced the response of human or mouse monocytes exposed to apoCIII or apoCIII-rich VLDL. Overexpression of TLR2 in 293 cells not only increased the binding of apoCIII to the cells but also augmented apoCIII-induced NF-κB activation and β₁ integrin expression. Thus, cells that express TLR2 or conditions that increase expression of TLR2 may exhibit enhanced response to apoCIII, although this study does not exclude a TLR2-independent process, because apoCIII activated NF-κB in 293 cells that did not express TLRs. Our results indicate direct interaction of apoCIII with TLR2. Several studies have described endogenous ligands for TLRs. Recent reports suggest that endogenous unidentified ligands for TLR2 contribute to atherogenesis.¹⁴ The present results raise the possibility that apoCIII in apoB lipoproteins can serve as an endogenous TLR2 ligand.

MyD88 plays a crucial role in the signaling pathway downstream of the TLRs.²⁰ However, recent studies reported a MyD88-independent pathway. Several studies showed the involvement of a Rac1-dependent pathway in TLR2-mediated NF-κB activation of THP-1 and in 293 cells.¹⁹ Harokopakis et al reported that TLR2 mediated monocyte adhesion and transmigration via Rac1- and phosphatidylinositol 3-kinase-mediated signaling in response to Porphyromonas gingivalis fimbriae.⁶ In the present study, apoCIII stimulation recruited MyD88 to associate with TLR2 protein, whereas the association with Rac1 was not prominent, and apoCIII did not activate phosphatidylinositol 3-kinase (data not shown). These results suggest that apoCIII exerts proadhesive effects through a distinct signaling pathway that involves TLR2 and MyD88.

In the previous study, pertussis toxin (PTX), a specific Gi/o protein inhibitor inhibited apoCIII-induced PKCa and NF-κB activation. Heterotrimeric G proteins couple with various
types of membrane receptors, and their Gα subunit mediates signal transduction, including PKC activities.21–23 We previously showed that apoCIII-rich remnant lipoproteins activated PKC in rat smooth muscle cells and that PTX inhibited PKC activation,15 suggesting that specific components of VLDL or VLDL remnants interact with PTX-sensitive G protein or its membrane receptors. Recent studies showed that G proteins mediate TLR signaling in several cell types,24–26 supporting our previous and present studies. Because the crosstalk between G protein coupled receptor and TLR signaling pathways exists,26 addressing the detailed role of PTX-sensitive G protein in apoCIII-induced responses requires further investigations.

ApoCIII mediates the activation of mouse monocytes triggered by VLDL CIII and their adhesion to HUVECs, as suggested by experiments using anti-apoCIII antibody and reconstituted VLDL CIII.9 The present study supports a pivotal role for TLR2 in these processes. Notably, apoCIII on VLDL particles correlates with a high lipid content and additional apolipoprotein content.27 Thus, these other (lipid) components that stimulate TLR2 may augment the effects of apoCIII-rich VLDL.

In conclusion, this study demonstrated that the TLR2 signaling pathway participates in the proinflammatory action of apoCIII, alone or in association with VLDL, inducing NF-κB activation and β3 integrin expression in monocytes and their adhesion to ECs. This pathway may contribute to the diverse inflammatory responses to apoCIII and the link between apoCIII levels and adverse clinical outcomes and may further support the involvement of TLR2 in atherogenesis induced by dyslipidemia. Our observations shed new light on the molecular pathways that link dyslipidemia, inflammation, atherosclerosis, and cardiovascular events.

Acknowledgments
We thank Makoto Harada for the technical assistance.

Sources of Funding
This study was supported by grants from the Ono Medical Research Foundation, Takeda Science Foundation, Mitsukoshi Health and Welfare Foundation, Uehara Memorial Foundation, and Japan Research Promotion Society for Cardiovascular Diseases (Sakakibara Memorial Research grant) (to A.K.); Ministry of Education, Science, Sports and Culture of Japan grant 18590805, a grant from the Ono Research Foundation (to M.Y.); and National Heart, Lung, and Blood Institute grant R01 HL 34636 (to P.L.).

Disclosures
None.

References
Toll-Like Receptor 2 Mediates Apolipoprotein CIII–Induced Monocyte Activation: Retracted
Akio Kawakami, Mizuko Osaka, Masanori Aikawa, Satoshi Uematsu, Shizuo Akira, Peter Libby, Kentaro Shimokado, Frank M. Sacks and Masayuki Yoshida

_Circ Res._ 2008;103:1402-1409; originally published online October 30, 2008; doi: 10.1161/CIRCRESAHA.108.178426

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/103/12/1402

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2008/10/30/CIRCRESAHA.108.178426.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/
Notice of Retraction

The authors of the following article have requested that it be retracted from publication in Circulation Research:


The corresponding author, Dr Akio Kawakami, admitted to the editors to improperly handling the collection and presentation of data in this article such that the authors can no longer verify the authenticity and accuracy of the data presented. These errors include, but may not be limited to, the blots in Figure 2A, Figure 4D, and Online Figure III originating from unrelated experiments of the corresponding author, and the incorrect reporting of “n” in Figures 5 and 6, which are less than indicated. As such, data in those figures are not verifiable.

All co-authors involved in this study other than the corresponding author, Dr Kawakami, had no knowledge of any scientific impropriety related to the collection, analysis, or presentation of data in this article. Dr Kawakami takes full responsibility for this.
(A) THP-1 cells were pretreated with indicated antibodies (50 µg/mL), and then incubated with peptidoglycan (PGN) (10 µg/ml) or LPS (200 ng/ml) for 2 hours. (B) THP-1 cells were incubated with apoCIII (100 µg/mL) for 2 hours in the presence or absence of polymyxin B (PMB) (0.5 µg/ml). (C, D) THP-1 cells were incubated with apoCIII(100 µg/mL) for 2 hours (C) or 8 hours (D). In some experiments, apoCIII was pretreated with indicated antibodies (50 µg/mL) for 30 minutes. *p<0.05 vs. apoCIII(-), #p<0.05 vs. apoCIII alone. Blots represent 3 independent experiments using apoCIII from 3 different donors that provided similar results (B, C).
Online Figure II

(A) Cultured hTLR2-293 cells were incubated with FITC-labeled apoCIII (100 µg/mL) for 2 hours. In some experiments, the cells were pretreated with indicated antibodies (50 µg/mL) for 30 minutes. *p<0.01 vs. apoCIII(-)/antibodies(-), #p<0.05 vs. apoCIII(+)/antibodies(-). (B) Cultured hTLR2-293 cells were incubated with FITC-labeled apoCIII (100 µg/mL) for 8 hours. In some experiments, the cells were pretreated with NF-κB inhibitor SN50 or control scrambled peptide (CP) (20 µmol/mL) for 30 minutes. *p<0.01 vs. apoCIII(-)/inhibitors(-), #p<0.05 vs. apoCIII(+)/inhibitors(-). Blots represent 4 independent experiments using apoCIII from 4 different donors that provided similar results.
Monocytes isolated from indicated mice were incubated with peptidoglycan (PGN) (10 µg/ml) or LPS (200 ng/ml) for 2 hours. *p<0.01 vs. PGN(-) or LPS(-)/C57BL/6, #p<0.01 vs. LPS(-)/TLR2 deficient. Blots represent 4 independent experiments that provided similar results.