Oxidized LDL Increases and Interferon-γ Decreases Expression of CD36 in Human Monocyte–Derived Macrophages

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Abstract—CD36 is a glycoprotein with an M_r of 88 kDa that is expressed on platelets, monocytes/macrophages, capillary endothelial cells, and adipocytes. We previously demonstrated that CD36 is involved in the uptake of oxidized low density lipoprotein (OxLDL) by using CD36-deficient macrophages (*J Clin Invest*. 1995;96:1859). However, the regulation of CD36 expression in human monocyte–derived macrophages has not been fully elucidated. The current study attempted to clarify the effect of OxLDL and cytokines, both of which are present in atherosclerotic lesions and may play an important role in atherogenesis, on the expression of CD36. A cell enzyme-linked immunosorbent assay and flow cytometry were used to detect CD36 protein. A ribonuclease protection assay was used to measure CD36 mRNA in human monocyte–derived macrophages. The expression of CD36 was increased during the differentiation of monocytes to macrophages. Incubation of macrophages with 25 μg/mL OxLDL for 24 hours increased the level of CD36 protein by 56% and that of CD36 mRNA by 58%. Lysophosphatidylcholine did not affect the expression of CD36. The effects of OxLDL were demonstrated in macrophages that had already differentiated to the point where CD36 expression was almost maximal. Interferon-γ (IFN-γ) reduced the expression of CD36 in a dose-dependent manner. A concentration of 1000 U/mL IFN-γ significantly reduced the expression of CD36 protein by 57% and that of CD36 mRNA by 30%. In conclusion, CD36 may be important in the formation of foam cells by induction through its ligand (OxLDL). Moreover, some local factors, such as IFN-γ, may suppress CD36 expression on macrophages in human atherosclerotic lesions. **(*Arterioscler Thromb Vasc Biol*. 1998;18:1350-1357.)**

Key Words: CD36 ■ oxidized LDL receptor ■ monocyte-derived macrophages ■ scavenger receptor ■ interferon-γ

Human monocyte–derived macrophages are thought to be involved in the formation of foam cells by scavenging modified LDL.[1–6] In the early stages of atherosclerosis, monocytes are thought to attach to the walls of the artery and to differentiate into macrophages. They take up oxidized LDL (OxLDL), which is present in atherosclerotic arterial walls in vivo, and then become foam cells. Kodama et al[7,8] identified and cloned one of the modified LDL receptors, the scavenger receptor (types I and II), which is believed to be a receptor for OxLDL in human macrophages. However, some investigators suggested that OxLDL receptor(s), other than the scavenger receptor, may be present. Other receptors have been reported to bind OxLDL, including CD36,[9] SR-BI,[10] FcγRII,[11] and a 94- to 97-kDa protein.[12] Of these newly detected OxLDL receptors, CD36 is thought to be one of the major receptors in monocyte-derived macrophages.

CD36 is a glycoprotein with an M_r of 88 kDa. CD36 is expressed on platelets, monocytes, macrophages, capillary endothelial cells, and adipocytes.[13] CD36 has been proposed to be a receptor for thrombospondin,[14] collagen,[15] *Plasmodium falciparum*–infected erythrocytes,[16,17] and long-chain fatty acids.[18] However, the physiological function of CD36 has not been elucidated. Endemann et al[9] suggested that CD36 may also be a receptor for OxLDL in mice and THP-1 cells. In our recent study, through a comparison of monocyte-derived macrophages from CD36-deficient subjects with those from normal subjects, we demonstrated that CD36 is a major receptor for OxLDL and is involved in ≈50% of OxLDL uptake.[19] The accumulation of cholesteryl ester after incubation for 24 hours with OxLDL was reduced by 40% in macrophages from CD36-deficient subjects compared with those from normal subjects. CD36 is also highly expressed in lipid-laden macrophages in the core of atherosclerotic lesions in the human aorta (A.N. et al, unpublished data, 1997).

Because these findings indicate that CD36 may be one of the major receptors for OxLDL in vivo that is involved in

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atherogenesis, it is important to identify factors that may influence the expression of CD36 in human macrophages. In localized atherosclerotic lesions, many kinds of cells and factors combine to form a complex network. Many of these network components may be involved in the regulation of CD36. In particular, the T lymphocytes and macrophages that have invaded the subendothelial space secrete a variety of cytokines and growth factors. To clarify the regulation of foam cell formation in human atherosclerotic lesions, we investigated the regulation of CD36 by the ligand, OxLDL, and by several cytokines involved in atherogenesis.

Methods

Human Monocyte–Derived Macrophages

Buffy coats from the blood cells of healthy donors were obtained from the Osaka Red Cross Blood Center. Mononuclear cells were isolated by density gradient centrifugation by using lymphocyte-separation solution (Nacalai Tesque) and were suspended in RPMI-1640 medium supplemented with 10% human type AB serum and antibiotics. This medium was designated as the culture medium. Mononuclear cells were plated in 10-cm cell culture dishes (Falcon Labware; Becton, Dickinson and Co) and incubated for 1 hour at 37°C. Nonadherent cells were then removed by washing the dishes twice with PBS (150 mmol/L NaCl and 10 mmol/L phosphate buffer, pH 7.2), and the remaining adherent cells were grown in culture medium. The medium was replaced every 3 days. Cell viability determined by trypan blue exclusion was >95% in all experiments. Freshly isolated mononuclear cells from the whole blood of healthy volunteers were used in the experiments on cell differentiation.

Isolation and Modification of LDL

LDL (d=1.019 to 1.063 g/mL) was isolated from the sera of normolipidemic healthy volunteers by ultracentrifugation according to the method of Havel et al.[20] LDL was acetylated with repeated additions of acetic anhydride. OxLDL was prepared by incubating LDL with 5 μmol/L $CuSO_4$ for 24 hours at 37°C. Native LDL and modified LDL used in the bioassays were extensively dialyzed against PBS and sterilized before use. The extent of oxidative modification of LDL was evaluated by agarose gel electrophoresis. We measured the endotoxin concentration in the OxLDL preparation by using an ELISA kit, and the concentration was <60 pg/mL, which is below the level that has any effect on biological activity.

Cytokines and Chemicals

Lysophosphatidylcholine (lyso-PC) and sodium oleate were purchased from Wako Pure Chemical Co. Recombinant human interferon-γ (IFN-γ) was generously provided by Biogen (Cambridge, Mass/Shionogi, Osaka, Japan). Recombinant human platelet-derived growth factor BB chain was purchased from Gibco BRL, and tumor necrosis factor-α was from Endogen. Recombinant human interleukin-1β was purchased from Oncogene Science.

Cell ELISA

Human mononuclear cells were isolated as described above. Cells were plated on a 96-well plate (Falcon Labware) at a density of 2×10^6 cells per well in RPMI-1640 containing 10% human type AB serum. After a 4-hour incubation at 37°C in 5% CO_2, nonadherent cells were removed. To investigate CD36 expression during the differentiation of macrophages, mononuclear cells were separated from the same individual blood sample and cultured for 1, 2, 5, 7, 10, and 14 days. To investigate the effects of OxLDLs, macrophages were cultured for 7 days before conducting the assay. In brief, cells were washed twice with PBS and fixed in 4% paraformaldehyde for 20 minutes at 4°C. Mouse monoclonal antibodies against human CD36 (FA6-152; Cosmo Bio) and mouse IgG_1 (negative control) were used as primary antibodies and diluted 500-fold. Biotinylated anti-mouse IgG_1 was used as the second antibody and was diluted 1000-fold. Expression of cell surface antigen was determined by staining with biotin/avidin (Vectastain ABC kit, Vector Laboratories) by using 3,3′-diaminobenzidine as the substrate. The reaction was stopped by the addition of 4N H_2SO_4 and quantified by measuring the absorbance at 490 nm with a microplate ELISA reader (Bio-Rad Laboratories).

Immunocytochemistry

Expression of CD36 protein on the cell surface was analyzed by using anti-human CD36 antibody (FA6-152, 0.2 μg/mL) and a labeled streptavidin/biotin (LSAB) kit (Dako Co).[21] Human monocyte–derived macrophages were plated into 8-well slide chambers (Nunc) and incubated. After fixation with 4% paraformaldehyde for 10 minutes, the cells were washed in PBS containing 1% BSA, air dried for 30 minutes, and washed in Tris-buffered saline (pH 7.6). Cells were incubated for 5 minutes with 0.5% normal goat serum. They were subsequently incubated with a 1:200 dilution of FA6-152 antibody for 20 minutes at room temperature and then washed in Tris-buffered saline. Finally, the cells were incubated for 10 minutes with a 1:200 dilution of biotinylated goat anti-rabbit IgG by using the LSAB–alkaline phosphatase system. New fuchsin was used as the chromogen. Sections were counterstained with hematoxylin and eosin. As a negative control, the primary antibody was replaced with normal mouse IgG_1 (Dako Co).

Immunofluorescence Flow Cytometry

Immunofluorescence flow cytometric analysis (FACS) was performed by using FITC-conjugated mouse monoclonal antibodies against human CD36 (OKM5, Ortho Diagnostic Systems, and FA6-152). Human monocyte–derived macrophages were incubated for 24 hours at 37°C in a medium containing various concentrations of OxLDL. After incubation, the cells were washed twice with PBS, gently removed from the dish with a rubber scraper, dispensed in PBS, and immediately fixed with 1% paraformaldehyde for 20 minutes at 4°C. After being washed with PBS, 2×10^5 cells in 50 μL PBS were incubated with FITC-conjugated monoclonal antibodies for 30 minutes at 4°C. The cells were then washed twice before being assayed with a FACScan flow cytometer (Becton Dickinson).[22] Data were analyzed by using the Cell Quest software program. Cell debris was excluded by an appropriate 2-dimensional gating method. Specific fluorescence intensity was calculated by subtracting the FITC-IgG_1 intensity (negative control) from the mean fluorescence intensity of the FITC-labeled monoclonal antibodies.

Ribonuclease Protection Assay

The cDNA corresponding to nucleotides 517 to 948 of the human CD36 gene was cloned into a pGEM-T vector (Promega) and linearized by *Eco*RI.[23] An antisense RNA, transcribed by using T7 RNA polymerase in the presence of [^{32}P]UTP (3000 Ci/mmol, New England Nuclear), was used as a probe for the ribonuclease protection assay. Radiolabeled antisense RNA for human β-actin was used as an internal standard. Twenty micrograms of total RNA from human monocyte–derived macrophages was hybridized with 4×10^4 cpm of each probe at 42°C overnight. Annealed products were digested with ribonuclease T1 at 37°C for 30 minutes. The protected fragments were precipitated and electrophoresed on a 6% polyacrylamide/urea gel. The results were analyzed by autoradiography and quantified by laser densitometric scanning (FUJIX BAS 2000).

Protein Measurement

Protein concentrations were determined by using the method of Lowry et al,[24] with BSA as the standard.

Statistics

Data are presented as mean±SD. Differences were analyzed by Student's unpaired t test. A level of $P<0.05$ was accepted as statistically significant.

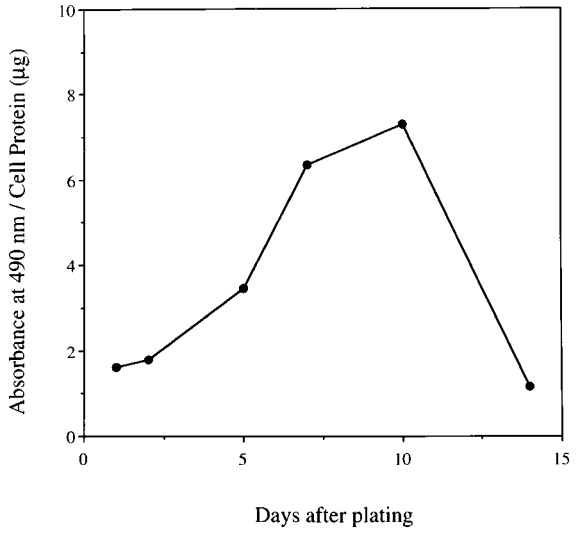

Figure 1. Induction of CD36 expression on the surface of macrophages as detected by ELISA. Mononuclear cells were freshly isolated from whole blood of the same volunteer and separated each day. They were plated at a density of 2×10^6 cells in 96-well plates. Cells plated after 1, 2, 5, 7, 10, and 14 days were assayed by ELISA. Assay was performed by using the avidin-biotin system with monoclonal antibody FA6-152. Mouse IgG_1 antibody was used as a negative control. Specific absorbance at 490 nm was calculated by subtracting mean absorbance of a negative control from that of FA6-152. Triplicate measurements of cell protein from unstained wells were performed.

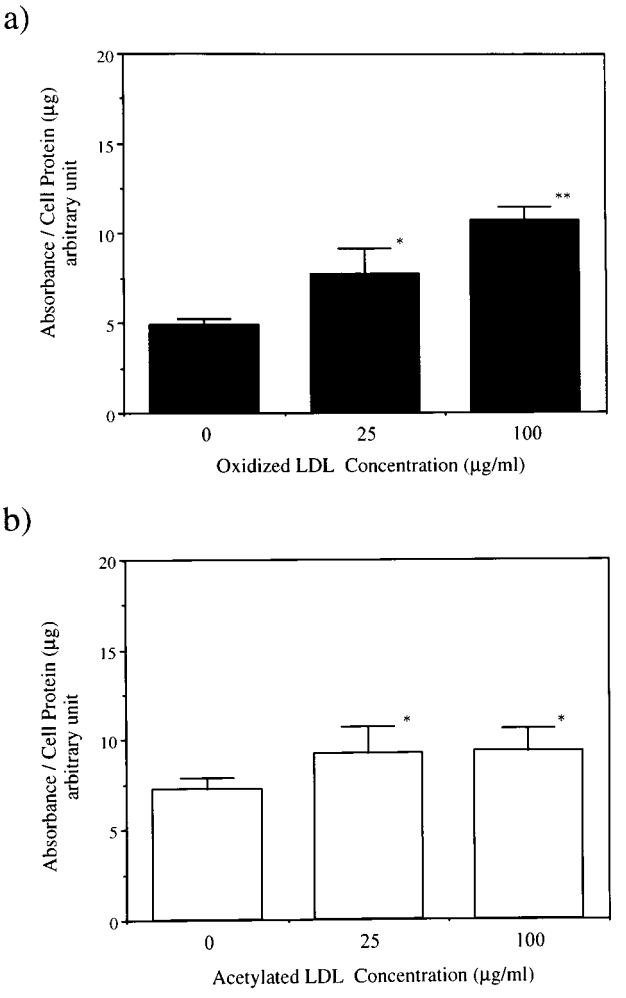

Figure 2. Effects of OxLDL and acetylated LDL on expression of CD36, as detected by ELISA. Mononuclear cells were plated at a density of $2x10^6$ cells per well in 96-well plates. Seven days after plating, cells were incubated for 24 hours with 0, 25, or 100 μg/mL OxLDL (a) or acetylated LDL (b) at 37°C, and assays were performed. Cell ELISA was performed with the avidin-biotin system, with monoclonal antibody FA6-152. Mouse IgG_1 antibody was used as a negative control. Specific absorbance was calculated by subtracting mean absorbance of negative control from that of FA6-152. Triplicate measurements of cell protein from unstained wells were performed. Data represent mean of 6 wells ±SD. $^*P<0.05$, $^{**}P<0.01$ versus control (without addition of modified LDL).

Results

CD36 Expression During Differentiation of Macrophages

To evaluate the changes in CD36 expression on the cell surface during the differentiation of monocytes to macrophages, monocytes were cultured from days 0 to 14. Monocytes were prepared on different days from the same subject and assayed on the same day. The morphological changes in monocytes after attachment to the culture dishes were examined by immunocytochemistry (data not shown). The cells became larger and spindle shaped throughout the course of the experiment. The monocytes gradually differentiated into macrophages, and the expression of CD36 increased during differentiation. Figure 1 shows the expression of CD36 on the cell surface. Data are expressed as the absorbance at 490 nm per microgram of cell protein. Cell surface CD36 was induced during the incubation, reached a peak on day 10, and began to decrease by day 14.

Effects of OxLDL on CD36 Expression in Macrophages

We next examined the effects of OxLDL on the expression of CD36 in macrophages. Figure 2a shows the CD36 expression in macrophages after a 24-hour incubation with and without OxLDL. Macrophages that were incubated in medium containing 25 or 100 μg/mL OxLDL showed a 1.5- and a 2-fold, respectively, higher expression of CD36 than did cells incubated in medium without OxLDL. Amounts of 25 and 100 μg/mL acetylated LDL also induced CD36 expression (Figure 2b); however, the induction was less than that observed with OxLDL.

FACS analysis was also performed to determine the expression of cell surface CD36. Figure 3 shows the log fluorescence of FITC-labeled FA6-152, a monoclonal antibody against CD36, after a 72-hour incubation with and without OxLDL. There was a basal expression of CD36 on the macrophages incubated in medium without OxLDL. The addition of OxLDL at a concentration of 25 μg/mL induced a shift of the fluorescence peak to the right, suggesting an enhanced expression of CD36 by OxLDL. The size and shape of the macrophages treated with OxLDL were not affected by the increase in CD36 expression, as determined by immunocytochemical analysis (Figure 4).

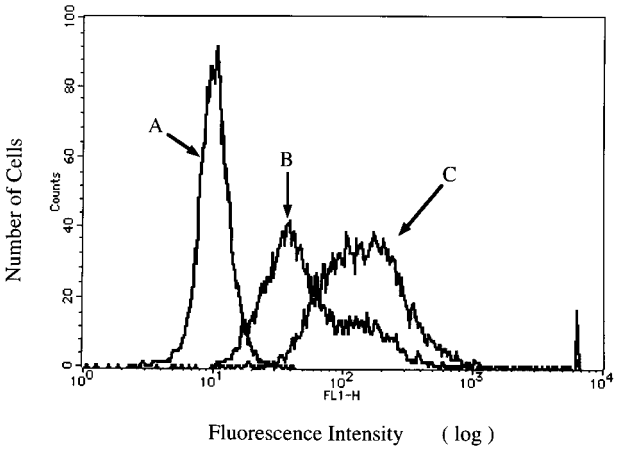

Figure 3. Induction of CD36 expression on macrophages by OxLDL, as detected by FACS. Human monocyte–derived macrophages (cultured for 7 days after plating) were incubated for 72 hours at 37°C after addition of medium with and without 25 μg/mL OxLDL. FITC-labeled FA6-152 was used for detection of cell surface CD36. Peak A, incubated without OxLDL and stained with FITC-labeled mouse IgG_1. Peak B, incubated without OxLDL and stained with FITC-labeled FA6-152. Peak C, incubated with 25 μg/mL OxLDL and stained with FITC-labeled FA6-152.

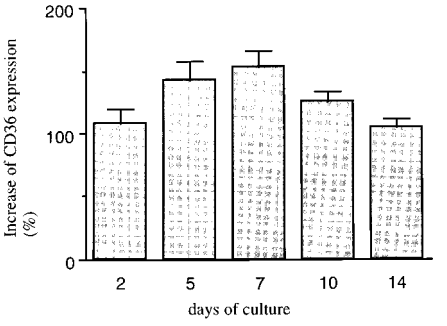

Figure 5. Comparison of effects of OxLDL on CD36 expression in macrophages cultured for varying periods. Expression of CD36 in macrophages incubated with 50 μg/mL OxLDL was compared with that in macrophages incubated without OxLDL on each day of culture by using FACS analysis. Level of CD36 expression in macrophages incubated without OxLDL was adjusted to 100% on each day. Data represent mean±SD of 6 wells.

Next, we compared the magnitude of the effects of OxLDL on CD36 expression of macrophages at an earlier time (day 2), at a later time (days 5, 7, and 10), and on day 14, after CD36 expression had declined. The effect of OxLDL on CD36 expression in macrophages on days 2 and 14 was minimal, and the maximal increase in CD36 expression was observed in macrophages on days 5 and 7. The expression of CD36 on macrophages incubated with OxLDL was increased by 53% of that on control macrophages cultured without OxLDL, suggesting that the effects of OxLDL were demonstrated in macrophages that had already differentiated to the point where CD36 expression was almost maximal (Figure 5).

The ribonuclease protection assay was performed to clarify the mechanism for the increased expression of CD36 protein by OxLDL (Figure 6). The data were normalized to the β-actin signal and presented as arbitrary units. CD36 mRNA was increased in a dose-dependent manner.

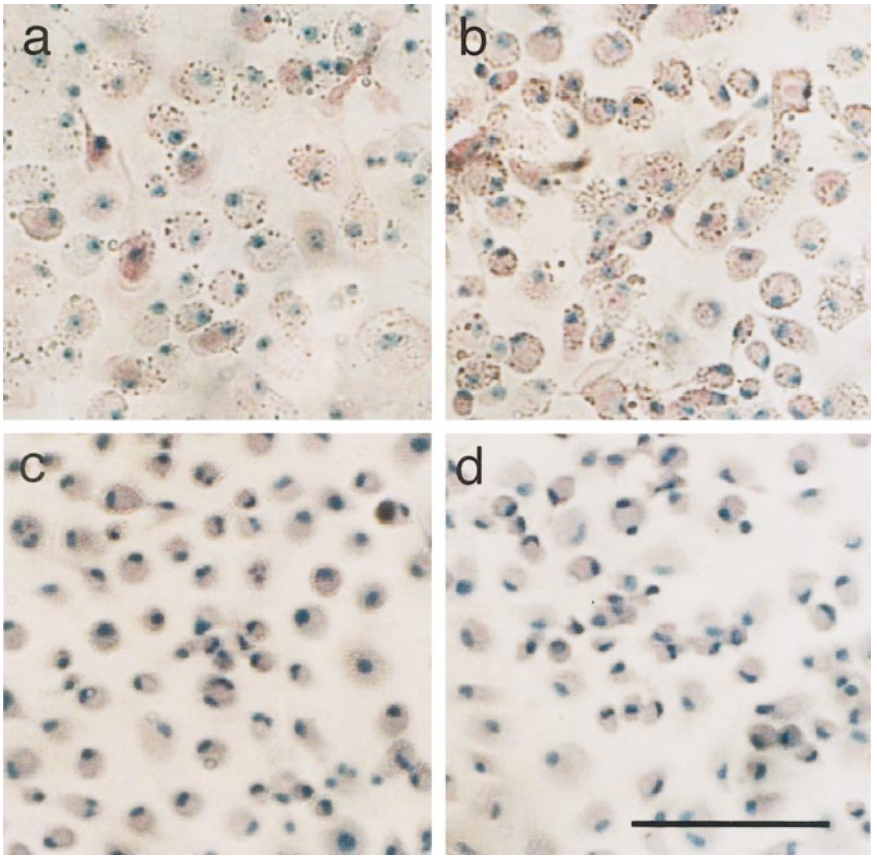

Figure 4. Immunohistochemical expression of CD36 in macrophages incubated with OxLDL and without OxLDL for 24 hours. CD36 expression was detected by LSAB method with FA6-152 as described in Methods. Human macrophages were grown for 7 days. Bar represents 100 μm. a, CD36 staining in macrophages incubated without OxLDL; b, CD36 staining in macrophages incubated with 25 μg/mL OxLDL; c, negative control staining, with nonimmune mouse IgG_1 in macrophages incubated without OxLDL; and d, negative control staining with nonimmune mouse IgG_1 in macrophages incubated with 25 μg/mL OxLDL.

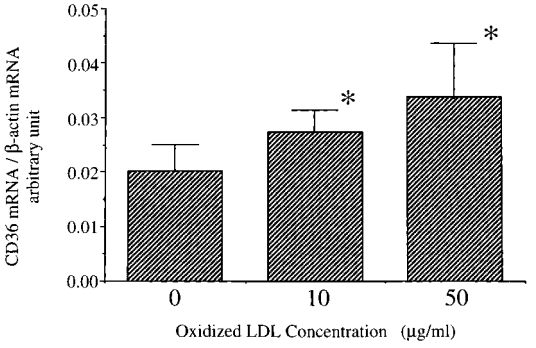

Figure 6. Induction of CD36 mRNA expression by OxLDL in macrophages. Bar graph shows expression of CD36 mRNA in macrophages, as detected by ribonuclease protection assay. Total RNA (2.0 μg) from human monocyte–derived macrophages incubated with and without OxLDL (0, 10, and 50 μg/mL) for 24 hours was hybridized with ^{32}P-labeled human CD36 cRNA. Annealed materials were digested with RNase A and T1. Protected fragments were analyzed by electrophoresis on 6% polyacrylamide/urea gel. Data were normalized to the β-actin signal and presented as arbitrary units. Data represent mean±SD of triplicate experiments. *$P<0.05$ compared with control values (without addition of OxLDL).

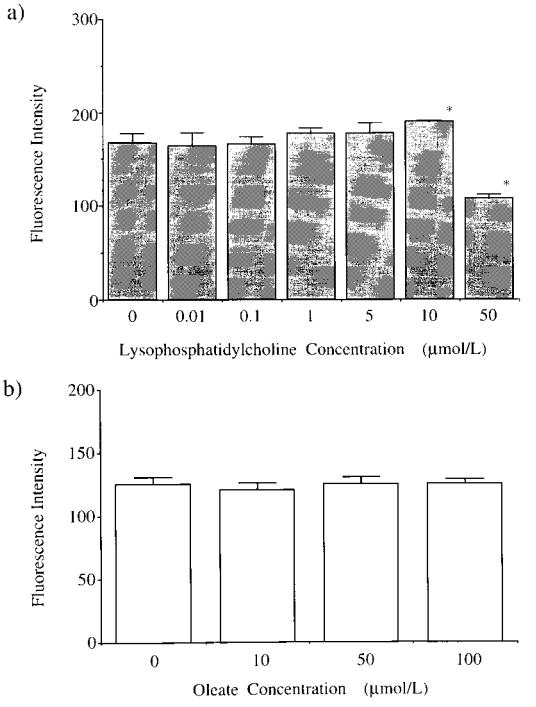

Figure 7. Effect of lyso-PC and oleate on expression of CD36 on macrophages, as detected by FACS. Monocyte-derived macrophages, cultured for 7 days, were incubated with indicated concentrations of lyso-PC (a) and sodium oleate (b) in RPMI medium for 24 hours. Lyso-PC was dissolved in ethanol solution, and final concentration of ethanol in medium was up to 0.1%, which did not affect cell viability. Sodium oleate was directly dissolved in RPMI culture medium, according to the method of Nicholson et al.[35] FITC-labeled FA6-152 was used for detection of cell surface CD36. Data represent mean±SD of triplicate experiments. *$P<0.01$ compared with control values (without lyso-PC).

The effects of lyso-PC, a biologically active components of OxLDL, on CD36 expression was investigated by flow cytometry. As shown in Figure 7a, lyso-PC slightly increased the expression of CD36 on the cell surface at a concentration of 10 μmol/L. However, the increase was not sufficient to account for the effect of OxLDL. At a concentration of 50 μmol/L, CD36 expression was decreased. We also evaluated whether oleate, reported to be another ligand for CD36, could induce CD36 expression. As shown in Figure 7b, sodium oleate did not affect the expression of CD36.

To investigate the mechanism for the upregulation of CD36 by OxLDL, we studied the effect of 10 nmol/L phorbol 12-myristate 13-acetate (PMA) on the expression of CD36. However, no significant effect on CD36 expression was observed, although both OxLDL and PMA increased the expression of the major histocompatibility complex class II antigen (HLA-DR) on macrophages (data not shown). We also investigated the effect of the anti-CD36 antibody, OKM5 (4 μg/mL), on the expression of CD36 on macrophages, and could not find any effect.

Effects of IFN-γ and Other Cytokines on CD36 Expression

We evaluated the effect on CD36 expression of IFN-γ, which is known to reduce the expression of scavenger receptors on human monocyte–derived macrophages. Different concentrations of IFN-γ were added to the culture medium, and the macrophages were incubated for 24 hours. The amount of CD36 was evaluated by the cell ELISA method (Figure 8). IFN-γ significantly suppressed the expression of cell surface CD36 in a dose-dependent manner. The abundance of CD36 mRNA, as measured by the ribonuclease protection assay, also decreased after a 24-hour incubation with 1000 U/mL IFN-γ (Figure 9). Other cytokines involved in the development of atherosclerosis, such as the platelet-derived growth factor BB chain, tumor necrosis factor-α, and interleukin-1β, had no significant effect on the expression of CD36 on macrophages (data not shown).

Discussion

Our previous study suggested that CD36 is a major receptor for OxLDL in human monocyte–derived macrophages.[19] We also found that CD36 is expressed in the intima of atherosclerotic lesions but not in that of aortic walls without atherosclerosis in vivo (A.N. et al, unpublished observations, 1997). It is, therefore, important to identify the factors that affect the expression of CD36 in human macrophages to prevent the progression of atherosclerosis in human arterial walls. Yesner et al[25] reported the regulation of CD36 expression in monocytes in response to several cytokines, and Huh et al[26] reported regulation of macrophage CD36 by macrophage colony stimulating factor. The effects of fatty acids on CD36 have also been evaluated.[22] Expression of CD36 in heart was increased in mice fed a high-fat diet.[27] Michelson et al[28] demonstrated that thrombin activation increases the expression of CD36 on the surface of platelets. Recently, Han et al[29] reported that native and modified LDLs increased the functional expression of CD36 by using J774 murine macrophages. However, the regulation of CD36 in human monocyte–derived macrophages has not been fully elucidated.

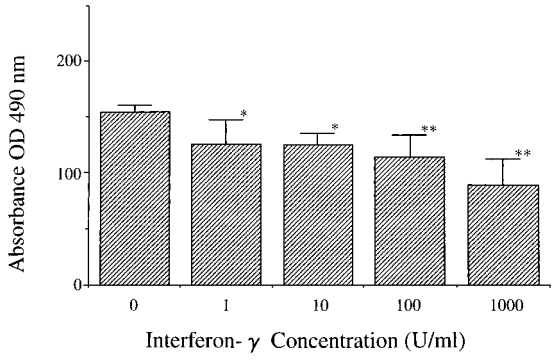

Figure 8. Effects of IFN-γ on expression of CD36, as detected by ELISA. Seven days after plating, monocyte-derived macrophages were incubated for 24 hours with 1, 10, 100, and 1000 U/mL IFN-γ at 37°C, and then assays were performed. Quantitative measurement was performed at an optical density of 490 nm. Data represent mean of 6 wells ±SD. *$P<0.05$, **$P<0.01$ versus control (without IFN-γ).

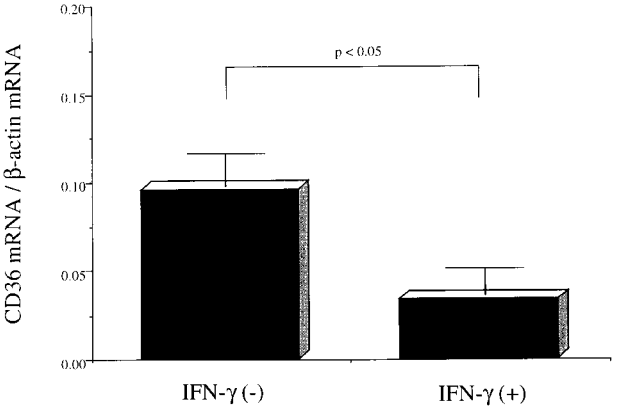

Figure 9. Suppression of CD36 mRNA expression by IFN-γ in macrophages. Expression of CD36 mRNA in macrophages was detected by ribonuclease protection assay. Total RNA (10 μg/mL) from human monocyte–derived macrophages incubated with and without IFN-γ (1000 U/mL) for 24 hours was assayed by the method as described previously. Data were expressed after normalization to β-actin signal and are presented as arbitrary units. Data represent mean±SD of triplicate experiments. *$P<0.05$ compared with control values (without addition of IFN-γ).

A novel finding in the current study is that OxLDL itself increased the expression of CD36 in monocyte-derived macrophages. This phenomenon may be specific for CD36, because there have been no reports of lipoprotein receptors being upregulated by the ligand itself. Although we did not investigate the intracellular levels of CD36, we found that the amount of CD36 mRNA was increased after the addition of OxLDL. These data suggest that upregulation of CD36 by OxLDL may occur at the transcriptional level.

We initially hypothesized that lyso-PC in OxLDL preparations might upregulate the expression of CD36, because recent studies revealed that some of the biochemical activities of OxLDL in atherogenesis depend on lyso-PC. Lyso-PC affects arterial endothelial cells and increases cell surface expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1.[30] In addition, Sakai et al[31] reported that lyso-PC has a mitogenic effect on macrophages. However, in our study, the addition of lyso-PC to the culture medium failed to significantly induce macrophage CD36 expression, suggesting that lyso-PC may not contribute to the upregulation of CD36 by OxLDL.

Collagen or CD36-specific antibodies have been shown to activate platelets.[32] OKM5, a monoclonal antibody to CD36, activates platelets and induces an oxidative burst in both monocytes and platelets.[33] Signal transduction through CD36 is thought to cause these actions. Huang et al[34] showed that protein tyrosine kinases of the *src* gene family are tightly associated with the CD36 molecule and exhibit autophosphorylation activity in vitro. Oleic acid is reported to be a ligand of CD36.[35] Because it is possible that binding of a ligand to its receptor may induce CD36 expression, we evaluated the effect of oleate on the expression of CD36 in human macrophages, but no significant effect was found. We also could not detect induction of CD36 mRNA in macrophages after the addition of OKM5 (data not shown). These data suggest that the stimulation of CD36 production by OxLDL may not be the result of signal transduction.

The effect of OxLDL on macrophage differentiation may be of concern in the upregulation of CD36 by OxLDL. Huh et al[26] demonstrated that the expression of CD36 increased during differentiation of monocytes to macrophages. We obtained similar results, except that the induction of macrophage CD36 expression was observed on day 10 as the cells underwent differentiation, although induction was observed on day 4 in the study by Huh et al. Although we cannot clearly explain the difference, because our experimental conditions seem to resemble the method used by Huh et al, there are still several differences in the procedures. Different conditions of culturing the macrophages and different sources of macrophages may lead to different effects on the activation of macrophages and may result in such a discrepancy. Frostegard et al[36] reported that OxLDL increased the amount of a cell surface differentiation marker, HLA-DR. However, in the current study, although both OxLDL and PMA induced HLA-DR antigen, PMA did not increase CD36 expression on the cell surface, suggesting that the protein kinase C pathway may not be involved in the mechanism of upregulation of CD36 by OxLDL.

The upregulation of CD36 by OxLDL, as shown in this study, may have some role in the development of atherosclerotic lesions. Although the mechanism of this upregulation has not been clarified in this study, cell differentiation and accumulation of cholesteryl ester in macrophages may both be involved, because acetylated LDL also induced CD36 expression to some extent. Because the effect of OxLDL exceeded that of acetylated LDL, factors that affect the differentiation of macrophages are thought to be more important than the accumulation of cholesteryl ester. These findings suggest that there exists a positive-feedback mechanism of CD36 expression during the differentiation process and by the uptake of OxLDL in the aortic wall. This would eventually accelerate the formation of foam cells, leading to the development of atheromatous plaques.

We next examined the regulatory factors for macrophage CD36 expression. Of the cytokines involved in atheromatous plaque formation, IFN-γ is believed to be mainly produced by

activated lymphocytes. The effects of IFN-γ on the various cell types involved in atherogenesis are controversial, because IFN-γ is known to have complex effects on the proliferation of vascular smooth muscle cells in vitro.[37] Geng and Hansson[38] showed that IFN-γ reduces the uptake of acetylated LDL and the accumulation of cholesteryl ester in human macrophages via scavenger receptors (types I and II), although the mechanisms were not well clarified. They also showed that IFN-γ inhibits the accumulation of cholesteryl ester in macrophages and suppresses foam cell formation in vitro. Our findings indicate that IFN-γ markedly suppressed CD36 expression in human monocyte–derived macrophages and reduced CD36 mRNA levels.

The structure of the human CD36 molecule has been demonstrated by Armesilla and Vega.[39] The 5′-promoter region of the CD36 gene contains 2 parts of putative consensus sequences for IFN-γ regulatory elements, indicating a mechanism for CD36 downregulation by IFN-γ. Although different effects were observed in keratinocytes,[36] dermal microvascular endothelial cells,[37] and monocytes,[25] IFN-γ is thought to directly affect these encoding regions, thus suppressing CD36 expression in human monocyte–derived macrophages.

In summary, we identified 2 new factors that affect the regulation of CD36 and that may be important in the formation of atherosclerotic plaques. OxLDL and IFN-γ are both present in human atherosclerotic lesions,[40] and these factors may interact on the monocyte-derived macrophage to regulate foam cell formation in vivo.

Acknowledgments

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