Interaction of advanced glycation end products (AGE) with AGE receptors induces several cellular phenomena potentially relating to diabetic complications. Five AGE receptors identified so far are RAGE (receptor for AGE), galectin-3, 80K-H, OST-48, and SRA (macrophage scavenger receptor class A types I and II). Since SRA is known to belong to the class A scavenger receptor family, and the scavenger receptor collectively represents a family of multitigand lipoprotein receptors, it is possible that CD36, although belonging to the class B scavenger receptor family, can recognize AGE proteins as ligands. This was tested at the cellular level in this study using Chinese hamster ovary (CHO) cells overexpressing human CD36 (CD36-CHO cells). Cellular expression of CD36 was confirmed by immunoblotting and immunofluorescent microscopy using anti-CD36 antibody. Upon incubation at 37 °C, 125I-AGE-bovine serum albumin (AGE-BSA) and 125I-oxidized low density lipoprotein (LDL), an authentic ligand for CD36, were endocytosed in a dose-dependent fashion and underwent lysosomal degradation by CD36-CHO cells, but not wild-type CHO cells. In binding experiments at 4 °C, 125I-AGE-BSA exhibited specific and saturable binding to CD36-CHO cells (Kd = 5.6 μg/ml). The endocytic uptake of 125I-AGE-BSA by these cells was inhibited by 50% by oxidized LDL and by 60% by FA6-152, an anti-CD36 antibody inhibiting cellular binding of oxidized LDL. Our results indicate that CD36 expressed by these cells mediates the endocytic uptake and subsequent intracellular degradation of AGE proteins. Since CD36 is one of the major oxidized LDL receptors and is up-regulated in macrophage- and smooth muscle cell-derived foam cells in human atherosclerotic lesions, these results suggest that, like oxidized LDL, AGE proteins generated in situ are recognized by CD36, which might contribute to the pathogenesis of diabetic macrovascular complications.

In the Maillard reaction, proteins react with glucose to form Schiff base and Amadori products. Upon long-term incubation, these early products are converted to advanced glycation end products (AGE), which are characterized physicochemically by fluorescence, brown color, and intra- or intermolecular cross-linking (1, 2) and biologically by specific recognition by AGE receptors. Previous immunological demonstration of AGE in several human tissues suggests that AGE may be involved in aging processes, diabetic complications, and atherosclerosis (3–11).

The physiological significance of AGE has mainly been examined from the perspective of AGE structure(s) expressed in vivo and AGE-binding proteins or AGE receptors, through which AGE are believed to elicit several biological phenomena in monocytes/macrophages (12–17), endothelial cells (18, 19), and mesangial cells (20, 21). Cellular interactions with AGE proteins are known to induce several biological responses, not only endocytic uptake and degradation, but also induction of cytokines and growth factors, which are likely linked to the development of diabetic vascular complications (9). These responses are thought to be mediated by AGE receptors, which include RAGE (receptor for AGE) (22, 23), galectin-3 (24), SRA (macrophage scavenger receptor class A types I and II) (28, 29), 80K-H (25, 26), and OST-48 (25, 26). We have recently shown that SRA, which is known as a receptor for oxidized low density lipoprotein (Ox-LDL) (27, 28), mediates the endocytic uptake and degradation of AGE-BSA by macrophages (28, 29). Since foam cells in the early phase of atherosclerosis are derived from monocyte/macrophages, in which SRA is highly expressed (30, 31), SRA may also play an important role as an AGE receptor in the early stages of atherosclerosis.

In contrast, although functionally related to SRA, the class B receptors differ significantly in structure. CD36, the defining member of this class, is a highly glycosylated, single chain 88-kDa protein that binds Ox-LDL, fatty acids, anionic phospholipids (including phosphatidylinositol and phosphatidylserine), and the proteins collagen and thrombospondin (32–36). As a result of the broad ligand specificity of CD36, multiple roles for this protein have been proposed. In vitro and in vivo studies indicate that CD36 mediates a significant proportion of binding and internalization of Ox-LDL by tissue-differentiated macrophages (37–39). Antibodies to CD36 have been shown to block up to 50% of the binding of Ox-LDL to, as well as its endocytic uptake by, normal monocyte-derived macrophages (37, 38). In monocyte/macrophages from donors with a human...
polymorphism associated with lack of CD36 expression (Naka), the capacity to bind and internalize Ox-LDL and the capacity to accumulate cholesteryl esters were reduced to 50% compared with those obtained from normal subjects (39). Similarly, the cell association of Ox-LDL at 37 °C with, and its cell binding at 4 °C to, peritoneal macrophages obtained from CD36 null mice were reduced to 37 and 53–60%, respectively, compared with wild-type macrophages (54). Furthermore, CD36 is expressed in monocytes/macrophages in the core of atherosclerotic plaques (40). Considered together, these studies suggest that the role of CD36 in atherogenic processes might be different from that of SRA.

This study was conducted to test whether AGE proteins could be recognized as ligands by CD36. The results obtained from cellular experiments using Chinese hamster ovary (CHO) cells overexpressing human CD36 (CD36-CHO cells) demonstrated a potential novel role for CD36 in the pathogenesis of AGE-induced diabetic macrovascular complications.
CD36 as an AGE Receptor

EXPERIMENTAL PROCEDURES

Chemicals and Materials—Penicillin G (1650 IU/mg), streptomycin sulfate (750 IU/mg), Dulbecco’s modified Eagle’s medium, and Ham’s F-12 medium were purchased from Life Technologies, Inc. Na125I (3.7 GBq/ml) was purchased from Amersham Pharmacia Biotech. FA6-152 (mouse anti-human CD36 monoclonal antibody) was purchased from Immunotech. MOC25 (mouse anti-human CD36 monoclonal antibody) was kindly provided by Dr. G. A. Jamieson (American Red Cross). MOPC21 (mouse IgG) was purchased from Sigma. Other chemicals were of the best grade available from commercial sources.

Ligand Preparation and Induction—AGE-BSA was prepared as described previously (41). Briefly, 2.0 g of BSA was dissolved in 10 ml of 0.5 M sodium phosphate buffer (pH 7.4) with 3.0 g of d-glucose. Each sample was sterilized by ultrafiltration, incubated at 37 °C for 40 weeks, and dialyzed against phosphate-buffered saline (PBS) (pH 7.4). The extent of lysine modification was 71% for AGE-BSA. The N-carboxymethyllysine content of AGE-BSA was 7.7 mol/mol of BSA (42). Human LDL (d = 1.019–1.063 g/ml) was isolated from platelet-rich plasma using NycoPrep® 1.063. The cells were collected and solubilized in lysis buffer (20 mM Tris and 150 mM NaCl) (pH 7.4). The electrophoretic mobilities of AGE-BSA preparations toward the anode were 1.4 times higher than those of unmodified BSA. Under identical conditions, Ox-LDL showed a similar increase in electrophoretic mobilities. Thus, the modification of BSA with glucose is associated with a significant increase in the net negative charge (43).

Cell Culture and Isolation of a Transfected Cell Line—CHO-K1 cells were maintained at 37 °C in medium A (Ham’s F-12 medium supplemented with 100 units/ml penicillin and 100 units/ml streptomycin) containing 10% fetal calf serum. The cDNA of human CD36 was amplified from a human placenta cDNA library by polymerase chain reaction using the following primers: sense, 5′-CTCAGAAGCTTAAAGATGCCGTACAGCTG-3′; and antisense, 5′-GCGAACGCAGCTGCACTGCTTAAG-3′. The amplified human CD36 cDNA was cloned into pCMV (Invitrogen), sequenced, and transfected into CHO-K1 cells by the electroporation method using a Gene-Pulser (Bio-Rad). To select CD36-positive colonies, cells were cultured in medium B (medium A supplemented with 10% fetal calf serum and 0.5 mg/ml G418). Positive clones (CD36-CHO cells) detected by immunofluorescence microscopy with anti-CD36 monoclonal antibody PA6-152 were selected and maintained as a stock culture in medium B, which selects the cells against cells that spontaneously lose expression of CD36.

Immunoblotting—Human platelets (a positive control) were prepared using Nycodenz® (Nycoderm Pharma AS, Oslo, Norway). Blood obtained from healthy volunteer who had not received any medication for at least 1 week was anticoagulated with 100 U/ml heparin. Platelet-rich plasma was prepared by centrifugation at 600 × g for 15 min at room temperature. Washed platelets were then isolated from platelet-rich plasma using Nycodenz® 1.063. The cells were collected and solubilized in lysis buffer (20 mM Tris and 150 mM NaCl) (pH 7.4) (Tris-buffered saline containing 2% Triton X-100, 100 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin A, and 1 μM aprotinin). Samples (50 μg of protein/lane) were subjected to SDS-polyacrylamide gel electrophoresis (45) and then electrotransferred onto a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20 (Tris-buffered saline/Tween) for 2 h. The membrane was then incubated with a 1:1000 dilution of mouse anti-CD36 monoclonal antibody (MOC25) in Tris-buffered saline/Tween for 1 h and washed with the same buffer three times. Immunoreactive bands were detected by incubation with a 1:2000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (H + L) (Zymed Laboratories Inc., South San Francisco, CA) for 45 min, followed by addition of ECL reagent.

Immunofluorescence Microscopy—For CD36 immunofluorescence microscopy, cells were cultured in a 4-well LAB-Tek® chamber slide (Nalge Nunc International, Naperville, IL) for 50 min, washed three times with 0.01 % PBS (pH 7.4), fixed with 3.7% paraformaldehyde at 4 °C for 20 min, and washed again with PBS. The cells were then blocked with 5% BSA at room temperature and incubated with anti-CD36 antibody (FA6-152) diluted 1:100 in PBS containing 1% BSA for 1 h, followed by sequential incubation with FluoroLink Cy2-labeled goat anti-mouse IgG (3.7 GBq/ml) (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) diluted 1:1000, and Alexa Fluor 647 (Invitrogen) (1 μM) (final concentration of 2.0 μg/ml) or fluorescein isothiocyanate-conjugated mouse IgG, final concentration of 2.0 μg/ml at 4 °C for 30 min and assayed on a confocal laser scanning microscope (FLUOVIEW, Olympus, Tokyo, Japan).

Immunofluorescent Flow Cytometry—Immunofluorescent flow cytometric analysis was performed using a mouse monoclonal antibody against CD36 (FA6-152). Cells (1 × 105 cells in 200 μl) were incubated with 125I-Ox-LDL (3.7 GBq/ml) and Ox-LDL labeled with 125I by IODO-GEN (Pierce), and Ox-LDL was labeled as described by McFarlane (44) to specific radioactivities of 850 and 400 cpn/mg, respectively.

FIG. 2. Endocytic uptake of 125I-Ox-LDL by CD36-CHO cells. Cells were incubated for 5 h in 0.5 ml of medium C with increasing concentrations of 125I-Ox-LDL in the presence (■) or absence (○) of 20-fold excess amounts of the unlabeled ligands. The amounts of cell-associated 125I-Ox-LDL (upper panels) and its degradation (lower panels) were determined as described under “Experimental Procedures.” The specific cell association and degradation (▲) were plotted after correcting for nonspecific cell association and degradation. Data represent the means of three separate experiments. Error bars represent S.D.

Cellular Assays—Except for the binding study, all cellular experiments were performed at 37 °C in a humidified atmosphere of 5% CO2 in air. Untransfected CHO cells were cultured in medium A containing 10% fetal calf serum, and CD36-CHO cells were cultured in medium B. Cells (8 × 104) were seeded in a 24-well plate and cultured for 2 days in 1.0 ml of medium B (for CD36-CHO cells) or medium A containing 10% fetal calf serum (for untransfected CHO cells), which was then replaced by medium C (Dulbecco’s modified Eagle’s medium containing 3% BSA). After culture for 1 h, each well received 0.5 ml of medium C containing various concentrations of 125I-Ox-LDL or 125I-AGE-BSA in the presence or absence of 20-fold excess amounts of the unlabeled ligand to be tested. After incubation for the indicated times, the medium was taken from each well, and soluble radioactivity in trichloroacetic acid was determined as an index of cellular degradation as described previously (3). After the cells were washed three times with 1 ml of medium C and then three more times with PBS, they were lysed with 1 ml of 0.1 N NaOH for 30 min at 37 °C, and the cell-associated radioactivity and cellular proteins were determined with the BCA protein assay reagent (Bio-Rad). For the binding study, cells that had been seeded in each well as described above were incubated for 90 min at 4 °C in 0.5 ml of
CD36 as an AGE Receptor

RESULTS

Overexpression of Human CD36 in CHO Cells Leads to Endocytic Uptake of \(^{125}\text{I}\)-Ox-LDL—CD36-CHO cells, which stably express human CD36, were prepared as described above. To confirm overexpression of CD36, we performed Western blotting with anti-CD36 monoclonal antibody (MI25). Human platelets, which express a significant amount of CD36, were used as a positive control. Wild-type CHO cells did not yield any bands, whereas CD36-CHO cells showed a distinctive band (Fig. 1A). We performed further flow cytometric analysis and immunofluorescence microscopy with anti-CD36 monoclonal antibody (FA6-152). Human CD36 was expressed on the cell surface of CD36-CHO cells, but not wild-type CHO cells (Fig. 1B and C). Control IgG showed no immunofluorescence (Fig. 1C). To determine whether CD36 expressed by these cells was functional or not, the cell association and endocytic degradation of Ox-LDL by CD36-CHO cells were compared with untransfected wild-type CHO cells. The specific cell association of \(^{125}\text{I}\)-Ox-LDL with, and its specific degradation by, CD36-CHO cells exhibited a dose-dependent saturation pattern, with specific association showing a maximal level of 175 ng/mg of cell protein; the apparent \(K_d\) for cell association was 0.7 μg/ml, and the maximal ligand degradation was 52.6 ng/mg of cell protein (Fig. 2). The specific degradation of \(^{125}\text{I}\)-Ox-LDL reached a plateau at >175 ng/mg of cell protein; the apparent \(K_d\) for degradation was 0.7 μg/ml, and the maximal ligand degradation was 232.6 ng/mg of cell protein (Fig. 2). Parallel experiments in untransfected cells (Fig. 2) and mock-transfected CHO cells showed that the cell association of \(^{125}\text{I}\)-Ox-LDL and its endocytic degradation occurred at a negligible level (data not shown). These results indicate that CD36 expressed on CD36-CHO cells serves as a receptor for Ox-LDL.

Endocytic Uptake and Degradation of \(^{125}\text{I}\)-AGE-BSA by CD36-CHO Cells—We determined the endocytic uptake and degradation of \(^{125}\text{I}\)-AGE-BSA by CD36-CHO cells at 37 °C. The amount of cell association of \(^{125}\text{I}\)-AGE-BSA with CD36-CHO cells increased in a dose-dependent manner and was almost competed away by a 20-fold excess of unlabeled AGE-BSA (Fig. 3). The specific cell association exhibited a dose-dependent saturation pattern with a plateau level of 140 ng/mg of cell protein, an apparent \(K_d\) of 4.1 μg/ml, and a maximal ligand association of 215.1 ng/mg of cell protein. This level was >3.5-fold higher than that of specific cell association with wild-type CHO cells, which showed only a slight cell association for AGE-BSA (Fig. 3). The degradation of \(^{125}\text{I}\)-AGE-BSA by the transfected cells was also significant. The specific degradation was increased dose-dependently, with a plateau level of 38 ng/mg of cell protein; the apparent \(K_d\) for degradation was 3.9 μg/ml, and the maximal ligand degradation was 52.6 ng/mg of cell protein (Fig. 3). In sharp contrast, untransfected cells did not degrade \(^{125}\text{I}\)-AGE-BSA at all under the same conditions (Fig. 3). Furthermore, mock-transfected CHO cells also demonstrated no endocytic degradation of \(^{125}\text{I}\)-AGE-BSA (data not shown). This result disclosed a novel function of CD36 as a receptor for AGE-BSA.

Binding of \(^{125}\text{I}\)-AGE-BSA to CD36-CHO Cells—We attempted to determine the cellular binding of \(^{125}\text{I}\)-AGE-BSA to CD36-CHO cells at 4 °C. The total binding of \(^{125}\text{I}\)-AGE-BSA was inhibited by 80% by an excess amount of unlabeled AGE-BSA. The specific binding, obtained by subtracting the nonspecific binding from the total binding, yielded a saturation pattern. Scatchard analysis of this specific binding disclosed a binding site with an apparent \(K_d\) of 5.6 μg/ml and maximal surface binding of 130 ng/mg of cell protein, indicating that
CD36-CHO cells possess a high affinity binding site for AGE-BSA (Fig. 4). The $K_d$ value of Ox-LDL for CD36 was 3.1 mg/ml (data not shown).

**Effect of Ox-LDL on Endocytic Uptake of $^{125}$I-AGE-BSA by CD36-CHO Cells**—To examine whether the recognition site for AGE-BSA in CD36-CHO cells was identical to that for Ox-LDL, we studied the effect of Ox-LDL on the cellular binding of $^{125}$I-AGE-BSA to CD36-CHO cells at 4 °C. The cellular binding of $^{125}$I-AGE-BSA to CD36-CHO cells was effectively (60%) replaced by unlabeled AGE-BSA, whereas unlabeled Ox-LDL had a slightly weaker effect (30%). Unlabeled LDL (used as a negative control) was ineffective in suppressing the cellular binding of AGE-BSA (Fig. 5A). We further studied the effect of Ox-LDL on the endocytic uptake of $^{125}$I-AGE-BSA by CD36-CHO cells at 37 °C. The cell association of $^{125}$I-AGE-BSA to CD36-CHO cells was effectively (>80%) replaced by unlabeled AGE-BSA, whereas unlabeled Ox-LDL had a slightly weaker effect (<40%). Unlabeled LDL (the negative control) was ineffective in suppressing the cell association of AGE-BSA (Fig. 5B). Similarly, the endocytic degradation of $^{125}$I-AGE-BSA at 37 °C was inhibited almost completely by the presence of an excess amount of unlabeled AGE-BSA, whereas unlabeled Ox-
CD36 as an AGE Receptor

LDL had a slightly weaker effect (<55%) (Fig. 5C). Unlabeled LDL was also ineffective in suppressing the degradation of AGE-BSA by CD36-CHO cells (Fig. 5C).

Effect of Anti-CD36 Antibody on Endocytic Uptake of 125I-AGE-BSA by CD36-CHO Cells—To determine the effects of anti-CD36 monoclonal antibody (FA6-152) on the endocytic uptake of 125I-Ox-LDL by CD36-CHO cells, we first confirmed the capacity of this antibody to inhibit CD36-mediated cellular binding of Ox-LDL at 4 °C. As shown in Fig. 6A, the cellular binding of 125I-Ox-LDL to CD36-CHO cells was inhibited by addition of anti-CD36 antibody (>60%). The extent of inhibition by anti-CD36 antibody was almost the same as with unlabeled Ox-LDL (>68%), whereas nonimmune IgG had no effect on this process. We further studied the effect of anti-CD36 antibody on the cell association of 125I-Ox-LDL with CD36-CHO cells at 37 °C. The cell association of 125I-Ox-LDL with CD36-CHO cells was also effectively (>70%) inhibited by anti-CD36 antibody. The extent of inhibition by anti-CD36 antibody was almost the same as with unlabeled Ox-LDL (>70%), whereas nonimmune IgG had no effect on this process (Fig. 6B). It is therefore likely that the specific cell association of 125I-Ox-LDL with these cells was completely inhibited by anti-CD36 antibody. This antibody was equally effective against the endocytic degradation of 125I-Ox-LDL at 37 °C; the total endocytic degradation of 125I-Ox-LDL by CD36-CHO cells was effectively inhibited by the antibody (>65%) and by unlabeled Ox-LDL (68%) (Fig. 6C). These results suggest that anti-CD36 antibody (FA6-152) recognizes a portion of CD36 that serves as a binding site for Ox-LDL in CD36-CHO cells. Under identical experimental conditions, we examined the effect of the same antibody on the cellular binding of 125I-AGE-BSA to, and endocytic uptake by, CD36-CHO cells at 4 °C. As shown in Fig. 7A, the cellular binding of 125I-AGE-BSA was inhibited by unlabeled AGE-BSA by >60%, but only by 25% by the antibody. Similarly, the antibody could replace 40% of the total cell association of 125I-AGE-BSA at 37 °C, whereas the inhibition was more dominant when unlabeled AGE-BSA was used (Fig. 7B). This antibody was also equally effective against the endocytic degradation of 125I-AGE-BSA at 37 °C (Fig. 7C). These results indicate that a major part of the cell association as well as the subsequent endocytic degradation of AGE-BSA by CD36-CHO cells is mediated by CD36.

DISCUSSION

Our previous studies using CHO cells overexpressing the class A scavenger receptor (SRA) (29) and peritoneal macrophages prepared from SRA knockout mice (28) indicated that SRA is one of the major AGE receptors involved in the endocytic uptake of AGE proteins by macrophages or macrophage-derived cells. The present study provided additional and novel information that CD36, a member of the class B scavenger receptor family, also serves as an AGE receptor in vivo and therefore might participate in the pathogenesis of diabetic microvascular complications.

CD36 was identified as an Ox-LDL receptor by Endemann et al. (32). Nozaki et al. (39) demonstrated that the endocytic uptake of Ox-LDL by monocyte-derived macrophages obtained from CD36-deficient patients was reduced by ~50% compared with that by macrophages prepared from normal subjects and that the subsequent Ox-LDL-induced accumulation of cholesterol esters was also reduced in CD36-deficient macrophages. Furthermore, the experiments using CD36 null mice demonstrated that the cell association of Ox-LDL with peritoneal macrophages obtained from CD36 null mice was reduced by 63% compared with macrophages prepared from wild-type mice (54). Therefore, it is generally accepted that both CD36 and SRA are major Ox-LDL receptors in macrophages. A recent report by Nakata et al. (40) revealed a difference in the histo-
logical distribution pattern between CD36 and SR-A. CD36 is highly expressed in macrophage-derived foam cells in the core of atherosclerotic plaques, whereas SRA-positive macrophage-derived foam cells tend to localize in the periphery of atherosclerotic lesions, suggesting that CD36 might play a different role compared with SRA in the formation of atherosclerotic plaques. The immunohistochemical study by Kume et al. (11) using a monoclonal antibody against N\(^\text{\epsilon}\)-carboxymethyllysine, a major AGE structure, demonstrated that, in the early stage of atherosclerotic lesions (diffuse intimal thickening and fatty streaks), AGE proteins are localized in macrophage-derived foam cells, whereas in the advanced stage (atherosclerotic plaques), AGE proteins are also observed in vascular smooth muscle cell-derived foam cells and the extracellular matrix. Other immunohistochemical studies with antibodies against different AGE structures demonstrated similar findings (47–49), suggesting that AGE proteins generated extracellularly are actively endocytosed by monocyte-derived macrophages via SRA and CD36 or both, with subsequent intralysosomal accumulation (intracellularly). In addition, since the interaction of AGE proteins with these cells is known to induce cellular responses such as secretion of cytokines, it is likely that CD36 may contribute to the formation of atherosclerotic lesions as a major AGE receptor.

RAGE is known as the main AGE receptor expressed in microvascular endothelial cells, where the interaction of AGE proteins with RAGE leads to enhancement of angiogenesis through induction of the vascular endothelial growth factor (50). A recent report demonstrated, however, that CD36 is also expressed in microvascular endothelial cells and plays a role in apoptosis-dependent neovascularization as a thrombospondin-1 receptor (51). Therefore, it is possible that CD36 is also involved in diabetic microangiopathy as an AGE receptor. Further studies are required to determine the relative contributions of CD36 and RAGE to the in vivo pathogenesis.

A competition study using anti-CD36 monoclonal antibody (52) indicated that the binding site of CD36 for Ox-LDL resides in the region corresponding to amino acids 155–183 of its extracellular domain. Anti-CD36 monoclonal antibody (FA6-152) was reported to compete with Ox-LDL for binding to CD36 (53). Specific endocytic uptake of \(^{125}\)I-Ox-LDL by CD36-CHO cells was completely inhibited by FA6-152 under our experimental conditions (Fig. 6), confirming the above notion that FA6-152 could recognize the binding domain of CD36 for Ox-LDL. Under these conditions, the endocytic degradation of \(^{125}\)I-AGE-BSA by CD36-CHO cells was inhibited by FA6-152 to a slight but significant degree (40%) and also by Ox-LDL (50%), an authentic ligand for CD36. These results clearly indicate that the binding domain of CD36 for AGE-BSA might overlap to some extent with the binding domain of CD36 for Ox-LDL, suggesting that binding of AGE ligands to CD36 might occur in or near this region.

CD36 is known as a multifunctional molecule, recognizing many ligands of different structures, including Ox-LDL (32, 39, 52, 54), apoptotic cells (55, 56), thrombospondin-1 (57, 58), and long chain fatty acids (54, 59–61). These ligands have, in common, a negatively charged nature, raising the possibility that CD36 recognizes the negative charge of AGE proteins (42). Further studies are needed to determine the AGE structure(s) required for recognition by CD36.

It was reported that peroxisome proliferator-activated receptor-\(\gamma\) modulates the expression of CD36 in macrophages (62–64). Recently, Iwashima et al. (65) demonstrated that AGE/BSA induces the expression and activation of peroxisome proliferator-activated receptor-\(\gamma\) in cultured mesangial cells, raising the possibility that CD36 could be up-regulated by AGE proteins through the activation of peroxisome proliferator-activated receptor-\(\gamma\). In addition, CD36 was reported to be involved in signal transduction. First, CD36 is physically associated with Src family kinases in human platelets (66, 67). Second, CD36 modulates signal transduction via the activation of Src family kinases in human platelets (66, 67). Because of the physical and functional association of CD36 with tyrosine kinases or nuclear factor-\(\kappa\)B, the interaction of AGE proteins with CD36 could modulate signal transduction.

In addition to CD36, SR-BI (scavenger receptor class B type I) also belongs to the class B scavenger receptor family (69). The ligand specificity of SR-BI is essentially identical to that of CD36, except that both SR-BI and CD36 bind high density lipoprotein, but only SR-BI efficiently mediates the cellular uptake of cholesteryl esters from high density lipoprotein particles (70, 71). It would be an interesting issue to determine whether SR-BI also serves as a receptor for AGE proteins.

To our knowledge, there are no reports to date on the association of CD36 with the pathogenesis of diabetes or diabetic complications. Recent results obtained in SHR/Ncrj rats indirectly suggested that a major function of CD36 is as a potential recognition and activation of peroxisome proliferator-activated receptor-\(\gamma\) for insulin resistance (72). Although a role for CD36 in insulin resistance was claimed in another recent study (73), experiments in CD36 null mice provided direct functional evidence that CD36 is involved in fatty acid uptake by adipocytes (54). Interestingly, CD36 null mice exhibit hypoglycemia (the mechanism is not clear), suggesting a functional role for CD36 in maintenance of plasma glucose (54), which raises the possibility that CD36 might be linked to the development of diabetic conditions. Our finding that CD36 recognizes and endocytoses AGE proteins as ligands suggests the possible involvement of CD36 in diabetic macrovascular complications.

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CD36, a Member of the Class B Scavenger Receptor Family, as a Receptor for Advanced Glycation End Products
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