Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) supports cell adhesion to fibronectin

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Abstract Lectin-like oxidized lipoprotein receptor-1 (LOX-1) is a specific receptor for atherogenic oxidized low density lipoprotein (OxLDL) which belongs to the scavenger receptor family. In the present report, we show that LOX-1 can also support cell adhesion to fibronectin (FN) in a divalent cation-independent fashion. CHO-K1 cells stably expressing bovine LOX-1 (BLOX-1-CHO), but not untransfected CHO-K1 cells, can adhere to FN-coated plates, but not to collagen-coated plates, in the presence of EDTA. BLOX-1-CHO adhesion to FN-coated plates can also be suppressed by scavenger receptor ligands, such as OxLDL, polyinosinic acid (poly I), and dextran sulfate, but not by native LDL, acetylated LDL, polycytidylic acid (poly C), or chondroitin sulfate. Cultured bovine aortic endothelial cells can similarly adhere to FN-coated plates, which was inhibited by OxLDL, poly I, and dextran sulfate in the presence of EDTA. LOX-1 may play an important role in cell adhesion to FN in an integrin-independent manner.

Key words: Lectin-like oxidized low density lipoprotein receptor-1; Cell adhesion; Fibronectin

1. Introduction

Cell adhesion to extracellular matrix (ECM) proteins, such as fibronectin (FN), laminin, and collagen, appears to be involved in cell migration and maintenance of cellular functions. Integrins, including αvβ3 integrin, are known to play key roles in adhesion between cells and ECM in a divalent cation-dependent manner, recognizing RGDS sequence in the ECM proteins [1]. Other molecules, however, may also be involved in cell–ECM interactions.

Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) is a type II membrane glycoprotein, belonging to the scavenger receptor family molecules, which was initially cloned in cultured bovine aortic endothelial cells (BAEC) [2–4]. Like other scavenger receptors, LOX-1 has a wide spectrum of pathophysiological ligands, including oxidized low density lipoprotein (OxLDL) [5], aged/apoptotic cells [6], activated platelets [7], and bacteria [8]. LOX-1 expression can be dynamically modulated by a variety of biological stimuli, such as tumor necrosis factor-α [9], transforming growth factor-β [10], angiotensin II [11,12], OxLDL [13–15], and fluid shear stress [13]. LOX-1 expression is not confined to vascular endothelial cells, but macrophages [16,17] and vascular smooth muscle cell [10,18–20] can express this molecule. More importantly, LOX-1 is highly expressed by these cell types in atherosclerotic lesions of humans and rabbits at the various stages in vivo [21,22].

Some of the scavenger receptors, such as macrophage class A scavenger receptors [23–25] and CD36 [26–28], can adhere to ECM proteins, including type I collagen and thrombospondin. Therefore, we sought to determine if LOX-1 can bind certain ECM proteins. In the present study, we show that LOX-1 can selectively adhere to FN in a mechanism independent of divalent cations and integrins.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle medium (DMEM), and Ham's F12 medium were obtained from Nissui (Tokyo, Japan). Fetal calf serum (FCS) was purchased from Sanko Junyaku (Tokyo, Japan). Polyinosinic acid (poly I), polycytidylic acid (poly C), dextran sulfate, chondroitin sulfate and RGDS were from Sigma (St. Louis, MO, USA). LDL was isolated from human plasma by sequential ultracentrifugation, and oxidative modification of LDL was carried out in vitro by cupric ion as previously described [2,5].

2.2. Cell culture

BAEC were isolated from bovine aortas by scraping the inner surface with sterile glass coverslips, and cultured in DMEM containing 10% heat-inactivated FCS in an atmosphere of 95% air, 5% CO_2 at 37°C. Wild-type CHO-K1 cells were maintained in F12/10% FCS. CHO-K1 cells stably expressing bovine LOX-1 (BLOX-1-CHO) were maintained in F12/10% FCS supplemented with 10 μg/ml of blasticidin S (Funakoshi) as previously described [2,5].

2.3. Adhesion assay

Cells were detached from culture plates by incubation with culture medium containing 5 mM EDTA at 37°C for 5 min. For adhesion assay, cells were washed twice, suspended in culture medium with or without 5 mM EDTA at the cell density of 1×10^5 cells/ml, loaded onto 35 mm plates coated with bovine FN or type I collagen (Iwaki, Tokyo, Japan), which had been preincubated with the same media alone (without cells) for 15 min, and incubated at 37°C for 30 min to allow the loaded cells to adhere. Tissue culture plates without coating served as negative controls. In some experiments, adhesion assay was performed in the presence or absence of scavenger receptor ligands or an integrin ligand, RGDS. These reagents were preincubated with cells for 15 min before cells were loaded onto the assay plates, and used at concentrations of 50 μg/ml for native LDL and OxLDL, 200 μg/ml for dextran sulfate and chondroitin sulfate, 100 μg/ml for

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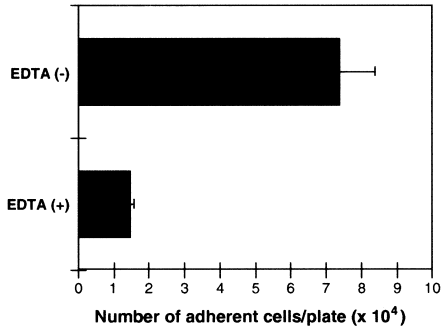

Fig. 1. Divalent cation-dependent and -independent adhesion of BAEC to FN-coated plates. BAEC, which are suspended in culture media containing 10% FCS with or without 5 mM EDTA, were loaded onto FN-coated culture plates. Numbers of cells adherent to FN-coated plates were determined by visual counting under phase-contrast microscopy. Values are means ± S.D. of three independent experiments.

poly I and poly C, and 1 mM for RGDS. Unbound cells were removed by washing three times with the assay medium. Numbers of cells adherent to FN-coated plates were counted under phase-contrast microscopy. For each well, the mean adherent cell number was calculated by counting adherent cells in three different microscopic fields under phase-contrast microscopy. All the experiments were performed in triplicate.

3. Results

3.1. Divalent cation-independent adhesion of BAEC to FN

Although most of BAEC adhesion to FN appears to depend upon divalent cations, approximately one fifth of BAEC adhesion to FN appears EDTA-insensitive (Fig. 1). This EDTA-resistant binding to FN was inhibited by OxLDL,

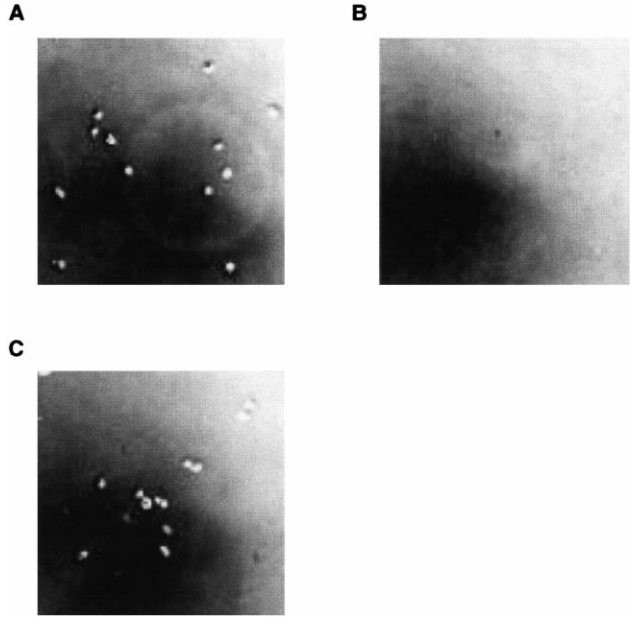

Fig. 2. Inhibition of BAEC adhesion to FN-coated plates by OxLDL. BAEC in culture medium containing 5 mM EDTA and 10% FCS were loaded onto FN-coated plates in the absence (A) or presence of OxLDL (B) or native LDL (C). Lipoproteins were preincubated with BAEC for 15 min and used at a concentration of 50 μg/ml. Representative photomicrographs are shown.

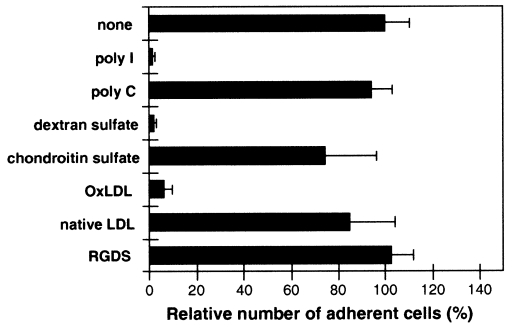

Fig. 3. Inhibition of BAEC adhesion to FN-coated plates by various scavenger receptor ligands. BAEC in culture medium containing 5 mM EDTA and 10% FCS were loaded onto FN-coated plates in the absence or presence of scavenger receptor ligands and the control compounds as indicated. Test reagents were preincubated with BAEC for 15 min and used at concentrations of 200 μg/ml for dextran sulfate and chondroitin sulfate, 100 μg/ml for poly I and poly C, 1 mM for RGDS. Numbers of cells adherent to FN-coated plates were counted under phase-contrast microscopy. Relative numbers of adherent cells are indicated. Values are means ± S.D. of three independent experiments.

but not by native LDL (Fig. 2), as well as scavenger receptor ligands, such as poly I and dextran sulfate, but not by poly C or chondroitin sulfate (Fig. 3). RGDS peptide, which blocks integrin-dependent adhesion to FN, did not suppress EDTA-resistant adhesion of BAEC to FN (Fig. 3). These results thus suggest that certain scavenger receptors, but not integrins, may be involved in EDTA-resistant cells adhesion to FN.

3.2. Divalent cation-independent adhesion of LOX-1 to FN

To explore the involvement of LOX-1 in divalent cation-independent cell adhesion to FN, we examined whether BLOX-1-CHO can adhere to FN-coated plates in the presence of EDTA. In the absence of EDTA, both BLOX-1-CHO and untransfected CHO-K1 cells were equally bound to FN-coated plates, probably depending upon integrin–FN interactions (Fig. 4). In the presence of EDTA, in contrast, BLOX-1-CHO, but not untransfected CHO-K1 cells, were bound to FN-coated plates, although the adherent cell numbers were reduced when compared to those without EDTA (Fig. 4).

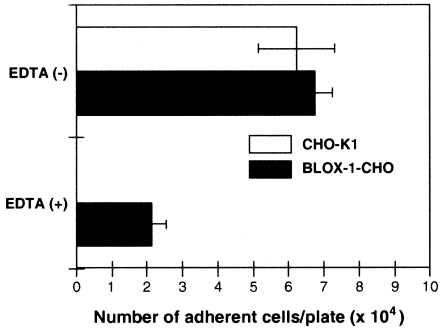

Fig. 4. Divalent cation-dependent and -independent adhesion of BLOX-1-CHO to FN-coated plates. BLOX-1-CHO (black bars) and untransfected CHO-K1 cells, which are suspended in culture media containing 10% FCS with or without 5 mM EDTA, were loaded onto FN-coated culture plates. Numbers of cells adherent to FN-coated plates were determined by visual counting under phase-contrast microscopy. Values are means ± S.D. of three independent experiments.

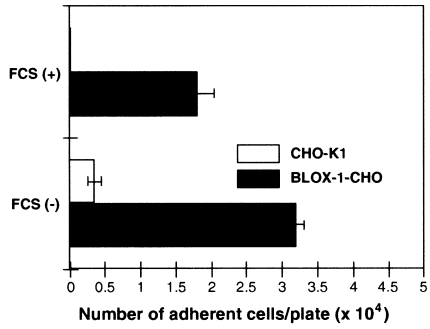

Fig. 5. Dependence upon serum factors in BLOX-1-CHO adhesion to FN-coated plates. BLOX-1-CHO (black bars) and untransfected CHO-K1 cells (white bars) suspended in culture medium containing 5 mM EDTA with or without 10% FCS were loaded onto FN-coated culture plates. Numbers of cells adherent to FN-coated plates were counted under phase-contrast microscopy. Values are means ± S.D. of three independent experiments.

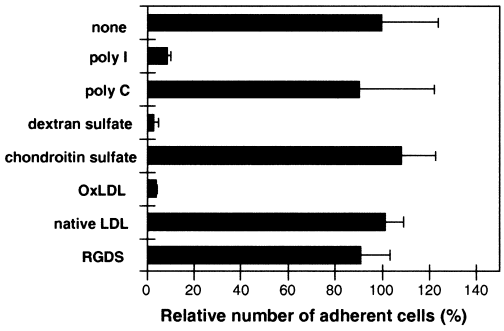

Fig. 7. Inhibitory effects of scavenger receptor ligands on BLOX-1-CHO adhesion to FN-coated plates. BLOX-1-CHO cells suspended in culture medium containing 5 mM EDTA and 10% FCS were loaded onto FN-coated plates in the presence or absence (none) of poly I, poly C, dextran sulfate, chondroitin sulfate, OxLDL, native LDL, or RGDS as indicated. Inhibitors were preincubated for 15 min and used at concentrations of 50 μg/ml for native LDL and OxLDL, 200 μg/ml for dextran sulfate and chondroitin sulfate, 100 μg/ml for poly I and poly C, and 1 mM for RGDS.

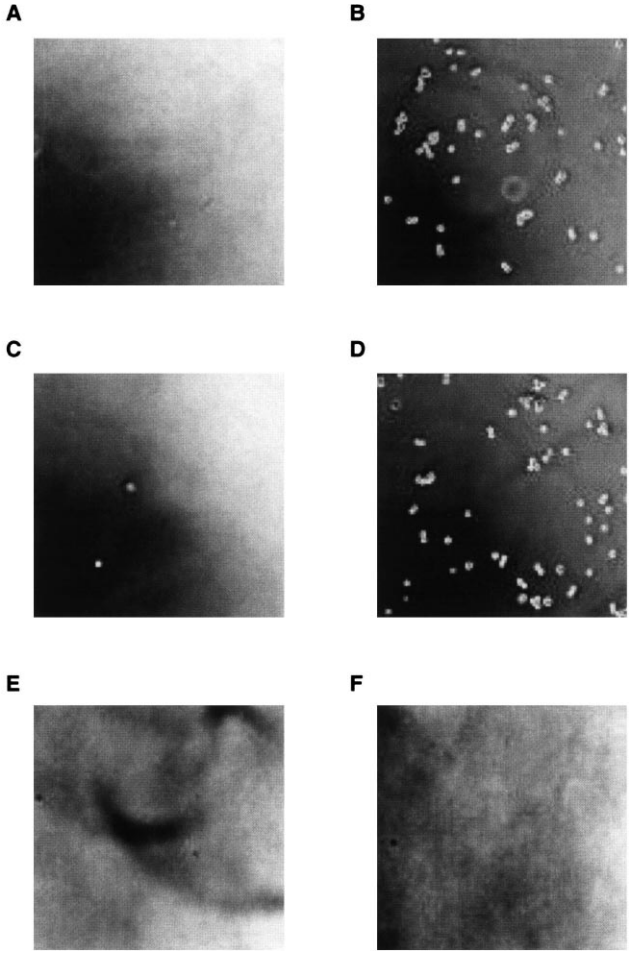

Fig. 6. Inhibition by OxLDL and various scavenger receptor ligands in BLOX-1-CHO adhesion to FN-coated plates. BLOX-1-CHO cells (B–F) suspended in culture medium containing 5 mM EDTA and 10% FCS were loaded onto FN-coated plates (A–D) in the absence (A, B) or presence of OxLDL (C), native LDL (D). Lipoproteins were preincubated for 15 min and used at a concentration of 50 μg/ml. Adhesion of untransfected CHO-K1 cells (A) to FN-coated plates, adhesion of BLOX-1-CHO cells to uncoated (E) or type I collagen-coated (F) plates served as negative controls. Photomicrographs were taken under phase-contrast microscopy (A–F).

CHO-K1 cells stably transfected with murine LOX-1 showed similar results (data not shown). In addition, COS-7 cells expressing LOX-1 similarly adhered to plates coated with human FN (Becton-Dickinson, Bedford, MA, USA; data not shown).

To examine whether serum factors are required in LOX-1–FN adhesion, adhesion assays were carried out in the presence or absence of FCS (Fig. 5). Adhesion of BLOX-1-CHO to FN-coated plates was rather slightly enhanced in the absence of FCS, suggesting that certain serum factors might rather suppress LOX-1–FN adhesion (Fig. 5).

3.3. Scavenger receptor ligands inhibit LOX-1–FN interactions

Negligible numbers of cells were bound to the culture plates without FN-coating (Fig. 6E) or type I collagen-coated plates (Fig. 6F) when compared to FN-coated plates (Fig. 6B) in the presence of EDTA. Untransfected CHO-K1 cells did not adhere to FN-coated plates (Fig. 6A). EDTA-resistant binding of LOX-1 to FN can be suppressed by OxLDL (Fig. 6C) but not by native LDL (Fig. 6D). Therefore, LOX-1–FN binding appears specific and FN and OxLDL share the same binding sites on LOX-1.

Previous studies have shown that scavenger receptor ligands, such as poly I and dextran sulfate, inhibited the binding of OxLDL to BLOX-1-CHO cells. We, therefore, examined whether these scavenger receptor ligands can inhibit the binding of LOX-1 to FN. As shown in Fig. 7, poly I and dextran sulfate inhibited the binding of BLOX-1-CHO cells to FN-coated plates. OxLDL also inhibited BLOX-1-CHO cell adhesion to FN as shown in Fig. 3C. In contrast, RGDS peptide, which is known to inhibit binding of integrins to FN, failed to show any inhibitory effects on LOX-1–FN adhesion, indicating that this binding is independent of integrins (Fig. 7). Neither poly C, chondroitin sulfate, nor native LDL inhibited the LOX-1–FN binding, indicating the specificity of the inhibitors (Fig. 7).

4. Discussion

Adhesion of vascular endothelial cells to ECM proteins appears to be involved in various aspects of vascular functions

including angiogenesis. Integrins have been shown to play key roles in endothelial–ECM adhesion; however, the present study demonstrated, for the first time, that LOX-1, a member of the scavenger receptor family molecules, can support adhesion to FN in a divalent cation-independent manner.

Binding of OxLDL to LOX-1 has been suppressed by scavenger receptor ligands, such as poly I and dextran sulfate [5]. As shown in this study, LOX-1 adhesion to FN was similarly suppressed by poly I and dextran sulfate. Furthermore, LOX-1–FN adhesion was inhibited by OxLDL. These results thus indicate that FN and OxLDL, as well as poly I and dextran sulfate, appear to share the common binding sites on the LOX-1 molecule.

Interactions between cells and ECM proteins appear to affect a variety of biological functions, including cell migration and invasion. In the vascular system, endothelial–ECM adhesion may play key roles in angiogenesis, in addition to ECM degradation. Interestingly, angiogenesis can be potently induced by hypoxia in vitro and in vivo, and LOX-1 expression can be drastically induced by hypoxia [29]. In addition, our previous studies with human atherosclerotic lesions revealed that LOX-1 was highly expressed by neovascular endothelial cells in the arterial intima [21]. These results suggest that hypoxia-induced LOX-1 and its interactions with ECM may play a role in the angiogenic processes, although this hypothesis remains to be proved.

In addition to vascular endothelial cells, LOX-1 is also expressed by macrophages and activated vascular smooth muscle cells; therefore, LOX-1 adhesion to ECM proteins might also be involved in migration and accumulation of these cell types in various pathophysiological settings including atherogenesis and inflammatory responses, although the roles of LOX-1 in vivo remain to be fully elucidated.

In summary, this report provides evidence, for the first time, that LOX-1, a member of the scavenger receptor family, can support cell adhesion to an ECM protein, FN. Further studies related to the overexpression and functional blocking of LOX-1 in a variety of pathophysiological settings in vivo may provide new insights into the roles of LOX-1.

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