The class A scavenger receptor (SR-A) binds modified lipoproteins and has been implicated in cholesterol ester deposition in macrophages. The SR-A also contributes to cellular adhesion. Using SR-A+/+ and SR-A−/− murine macrophages, we found SR-A expression important for both divalent cation-dependent and -independent adhesion of macrophages to the human smooth muscle cell extracellular matrix. The SR-A mediated 65 and 85% of macrophage adhesion to the extracellular matrix in the presence and absence of serum, respectively. When EDTA was added to chelate divalent cations, the SR-A mediated 90 and 95% of the macrophage adhesion without and with serum, respectively. SR-A-mediated adhesion to the extracellular matrix was prevented by fucoidin, an SR-A antagonist. Biglycan and decorin, proteoglycans of the extracellular matrix, were identified as SR-A ligands. Compared with control cells, Chinese hamster ovary cells expressing the SR-A showed 5- and 6-fold greater cell association (binding and internalization) of 125I-decorin and -biglycan, respectively. In competition studies, unlabeled proteoglycan or fucoidin competed for binding of 125I-labeled decorin and -biglycan, and biglycan and decorin competed for the SR-A-mediated cell association and degradation of 125I-labeled acetylated LDL, a well characterized ligand for the SR-A. These results suggest that the SR-A could contribute to the adhesion of macrophages to the extracellular matrix of atherosclerotic plaques.

Macrophages express several scavenger receptors that bind modified lipoproteins (1–5). The cloning and identification of individual scavenger receptors have permitted a detailed assessment of their functions. The type I and type II class A scavenger receptors (SR-A) were the first to be cloned (6, 7). They both bind a wide variety of molecules, including certain chemically modified lipoproteins such as oxidized low density lipoproteins (LDL) and acetylated LDL (AcLDL), certain polysaccharides such as fucoidin and dextran sulfate, and polyanionucleotides such as poly I and poly G (1). In addition, the SR-A binds a limited number of native proteins. We recently showed that the α-secretase cleavage products of the three main isoforms of the amyloid precursor protein (APP695, APP751, and APP770) are ligands for the SR-A (8). The SR-A also binds the amyloid-β (Aβ) peptide (9), another enzymatic cleavage product of APP and a major component of senile plaques in Alzheimer’s disease (AD).

Macrophage scavenger receptors have been postulated to contribute to the internalization of modified lipoproteins, intracellular cholesterol ester accumulation, foam cell formation, and atherogenesis (10). Numerous studies have suggested that the SR-A is critically involved in the deposition of cholesterol in arterial wall macrophages during the development of atherosclerotic lesions. Elimination of SR-A expression in mice significantly reduces the uptake of modified LDL by peritoneal macrophages from these animals and inhibits atherogenesis (11–13). Although reduced atherosclerosis in SR-A−/− mice is widely assumed to result from reduced uptake of modified lipoproteins and reduced accumulation of cholesterol esters in macrophages of the arterial wall, this mechanism has not been proved experimentally. SR-A may, in fact, contribute to atherogenesis in ways distinct from the function of SR-A in the uptake of modified lipoproteins (14). The SR-A also may play an important role in immune response and in cell adhesion (11, 15–20). In fact, it has been suggested that the SR-A may interact with components of the subendothelial space, thereby contributing to the adhesion and retention of macrophages in the artery wall (11, 15–19).

To examine this possibility further, we tested the hypothesis that the SR-A contributes to the adhesion of cells to the extracellular matrix. We found that biglycan and decorin, components of the extracellular matrix, as well as aggrecan, a proteoglycan that is similar to versican (a prominent matrix component) are ligands of the SR-A and that the SR-A contributes significantly to the divalent cation-dependent and -independent adhesion of macrophages to extracellular matrix derived from both smooth muscle cells and endothelial cells.

**EXPERIMENTAL PROCEDURES**

Materials—Bovine aggrecan, bovine biglycan, bovine decorin, hyaluronan, and fucoidin were obtained from Sigma. Collagen IV was obtained from ICN Biomedicals (Costa Mesa, CA). PD-10 columns and Na115O4 were purchased from Amersham Biosciences. Dulbecco’s modified Eagle’s medium, F12 medium, RPMI 1640 medium, trypsin-EDTA solution, and penicillin-streptomycin solution were purchased from Invitrogen. Fetal bovine serum was obtained from Hyclone (Logan, UT). Tissue culture dishes and other plastic ware were obtained from Falcon.
Lipoproteins and Proteoglycans—Human LDL (d = 1.02–1.05 g/ml) were isolated from the plasma of normal fasted donors by sequential ultracentrifugation at 4 °C (21). The LDL were radiolabeled by the iodine monochloride method (22) to a specific activity of 150–350 cpm/ng of protein. AcLDL was prepared by treating LDL with acetic anhydride (23). Lipoproteins were dialyzed against 0.15 M NaCl and 0.01% EDTA, pH 7.2, before use. Aggrecan, biglycan, and decorin were labeled with 125I using iododec (Pierce) as recommended by the manufacturer. The specific activities were 200–400 cpm/ng.

Cell Release Assay—Control Chinese hamster ovary (CHO) cells were grown in 95% air/5% CO2 at 37 °C in Dulbecco’s modified Eagle’s medium/F12 medium containing 10% fetal bovine serum. CHO cells stably transfected to express the type I SR-A were grown in the same medium containing 400 μg/ml of G418. The preparation and properties of CHO cells stably expressing the SR-A have been described previously (24). The cells were grown to confluence in untreated 35- or 60-mm tissue culture plates or in plates coated with the extracellular matrix from human smooth muscle cells (HSMCs). The extracellular matrix was prepared as described below. The plates were washed three times with phosphate buffered saline (PBS) and incubated with 1 ml of trypsin-EDTA solution at room temperature on an orbital platform. Trypsin activity was stopped at the times indicated with 1 ml of serum-trypsin-EDTA solution at 37 °C. The cells were washed three times with PBS and dried. Thioglycollate-elicited peritoneal macrophages from mice (11) were obtained by peritoneal lavage, seeded in wells coated with extracellular matrix, incubated for 1.5 h at room temperature. The cells were then washed three times with PBS and dried. Thioglycollate-elicited peritoneal macrophages from mice (11) were obtained by peritoneal lavage, seeded in wells coated with extracellular matrix, incubated for 1.5 h at room temperature. The cells were then washed three times with PBS and dried. Thioglycollate-elicited peritoneal macrophages from mice (11) were obtained by peritoneal lavage, seeded in wells coated with extracellular matrix, incubated for 1.5 h at room temperature. The cells were then washed three times with PBS and dried. Thioglycollate-elicited peritoneal macrophages from mice (11) were obtained by peritoneal lavage, seeded in wells coated with extracellular matrix, incubated for 1.5 h at room temperature. The wells were then washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and then the nuclei in each image (a standard field) were captured using a program (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.

Cell Association and Degradation of Proteoglycans and Lipoproteins—Control CHO cells and CHO cells expressing the SR-A were grown in 12-well plates as indicated above, washed three times with serum-free medium, and incubated for 5 h at 37 °C in serum-free medium containing 125I-aggrecan, -biglycan, or -decorin (1 μg/ml) alone or with the indicated competitors. Competitors were added 30 min before the labeled ligand or after 1 h of incubation. The cells were then washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and then the nuclei in each image (a standard field) were captured using a program (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.

Cell Association and Degradation of Proteoglycans and Lipoproteins—Control CHO cells and CHO cells expressing the SR-A were grown in 12-well plates as indicated above, washed three times with serum-free medium, and incubated for 5 h at 37 °C in serum-free medium containing 125I-aggrecan, -biglycan, or -decorin (1 μg/ml) alone or with the indicated competitors. Competitors were added 30 min before the labeled ligand or after 1 h of incubation. The cells were then washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and then the nuclei in each image (a standard field) were captured using a program (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.

Cell Association and Degradation of Proteoglycans and Lipoproteins—Control CHO cells and CHO cells expressing the SR-A were grown in 12-well plates as indicated above, washed three times with serum-free medium, and incubated for 5 h at 37 °C in serum-free medium containing 125I-aggrecan, -biglycan, or -decorin (1 μg/ml) alone or with the indicated competitors. Competitors were added 30 min before the labeled ligand or after 1 h of incubation. The cells were then washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and then the nuclei in each image (a standard field) were captured using a program (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.

Cell Association and Degradation of Proteoglycans and Lipoproteins—Control CHO cells and CHO cells expressing the SR-A were grown in 12-well plates as indicated above, washed three times with serum-free medium, and incubated for 5 h at 37 °C in serum-free medium containing 125I-aggrecan, -biglycan, or -decorin (1 μg/ml) alone or with the indicated competitors. Competitors were added 30 min before the labeled ligand or after 1 h of incubation. The cells were then washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and then the nuclei in each image (a standard field) were captured using a program (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.

Cell Association and Degradation of Proteoglycans and Lipoproteins—Control CHO cells and CHO cells expressing the SR-A were grown in 12-well plates as indicated above, washed three times with serum-free medium, and incubated for 5 h at 37 °C in serum-free medium containing 125I-aggrecan, -biglycan, or -decorin (1 μg/ml) alone or with the indicated competitors. Competitors were added 30 min before the labeled ligand or after 1 h of incubation. The cells were then washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and then the nuclei in each image (a standard field) were captured using a program (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.
lular matrix component versican, is a ligand for the SR-A. SR-A-expressing cells showed an 8-fold greater cell association of 125I-labeled aggrecan than control cells (Fig. 3). To examine further the specificity of interaction of the proteoglycans with the SR-A, the cell association and degradation of aggrecan, biglycan, and decorin were tested in competition experiments with unlabeled proteoglycan and fucoidin (Fig. 4). A 10-fold molar excess of aggrecan or fucoidin competed for 80–90% of the cell association and 80–85% of the degradation of 125I-aggrecan. Biglycan or fucoidin (10-fold molar excess) also competed for 80–90% of the cell association and ~80% of the degradation of 125I-biglycan, whereas a 10-fold excess of decorin or fucoidin competed for only 50–60% of the cell association and ~50% of the degradation of 125I-decorin. Higher concentrations (as high as a 100-fold excess) of competitor did not result in a substantially higher competition.

Aggrecan, biglycan, and decorin also competed for the cell association and degradation of 125I-AcLDL by CHO cells expressing the SR-A (Fig. 5). Aggrecan, a high molecular weight proteoglycan, was the most effective competitor for the SR-A-mediated cell association and degradation of AcLDL, whereas biglycan was as effective as AcLDL and fucoidin (Fig. 5). Decorin was a less potent competitor.

We also examined the ability of hyaluronan and collagen IV, other components of extracellular matrix, to compete for the SR-A-mediated cell association and degradation of AcLDL. We found that hyaluronan and collagen IV did not compete with AcLDL even at concentrations of 100 μg/ml (data not shown).

**DISCUSSION**

This study shows that the SR-A contributes to the divalent cation-dependent and independent adhesion of macrophages to extracellular matrix from HSMC and endothelial cells in the presence and absence of serum. This adhesion was inhibited by the SR-A antagonist fucoidin. Expression of SR-A by CHO cells delayed the release of cells from tissue culture dishes and from HSMC extracellular matrix by trypsin. Furthermore, we identified soluble biglycan and decorin, proteoglycans of the extracellular matrix, as ligands for the SR-A. In competition experiments, biglycan competed as effectively as AcLDL and fucoidin for the SR-A-mediated cell association and degradation of 125I-AcLDL.

**FIG. 2.** The SR-A mediates macrophage adhesion to the extracellular matrix. Macrophages from SR-A−/− (white bars) and SR-A+/+ (black bars) mice were seeded in 96-well plates coated with extracellular matrix from HSMC, incubated for 1.5 h at 37 °C with or without serum and EDTA as indicated, washed with PBS, and fixed with 3% paraformaldehyde. Nuclei of adherent cells were counted after Sitox green staining. Adhesion of SR-A−/− macrophages was also quantitated in the presence of 100 μg/ml fucoidin (hatched bars). Results are the mean ± S.D. of five independent fields from triplicate wells of a representative experiment, in a series of three with similar results.

**FIG. 3.** Aggrecan, biglycan, and decorin bind to the SR-A. Control CHO cells (white bars) and CHO cells expressing the SR-A (black bars) were incubated for 5 h at 37 °C with 125I-aggrecan, 125I-biglycan, or 125I-decorin (1 μg/ml). The cells were washed and the cell association of labeled proteoglycans was determined. The results are expressed as the fold increase in cell association after subtraction of the nonspecific binding obtained in the presence of 10 μg/ml fucoidin. Results are the mean ± S.D. of three independent experiments performed in duplicate.

**FIG. 4.** Fucoidin and nonlabeled proteoglycans compete for the cell association and degradation of 125I-aggrecan, 125I-biglycan, or 125I-decorin by CHO cells expressing the SR-A. CHO cells expressing the SR-A were incubated for 5 h at 37 °C with 125I-aggrecan (A, B), 125I-biglycan (C, D), or 125I-decorin (E, F) (1 μg/ml) alone (white bars) or in the presence of unlabeled competitor (10 μg/ml) (black bars). The cells were washed and the cell association or degradation of labeled proteoglycans was determined. Results are the mean ± S.D. of three independent experiments performed in duplicate.
AcLDL. Decorin was less effective, suggesting that it has a lower affinity for the SR-A than biglycan or AcLDL. Unlabeled decorin or fucoidin competed only partially for binding of 125I-decorin to CHO cells expressing the SR-A. The binding of versican, another prominent component of the extracellular matrix, was not examined directly. However, we determined that aggrecan, a predominant proteoglycan in cartilage that is analogous to versican (28, 29), is a ligand for the SR-A and a better competitor for the cell association and degradation of AcLDL than either biglycan or decorin. In contrast to biglycan and decorin, the core proteins of both aggrecan and versican are extensively substituted with chondroitin sulfate (28).

Macrophages exhibit both Cu²⁺-dependent and -independent adhesion. Unlike other cell types, macrophages attach to tissue culture plastic in the absence of divalent cations. In the presence of EDTA, which chelates Ca²⁺ and Mg²⁺, macrophages lose their spread morphology but remain adherent. The first indication that the SR-A contributes to adhesion was the observation that a monoclonal antibody to the SR-A (2F8) totally blocked their spread morphology but not their adherence in the absence of serum, suggesting that a component of serum is necessary for this adhesion (15, 16). Studies using a different paradigm which suggested that the SR-A is partially responsible for the trypsin-resistant adhesion of macrophages to tissue culture plastic (16). In those studies, RAW 264 macrophages were gently trypsinized and washed in serum-containing medium before adhesion assays. Trypsin release after adhesion was not studied. Under those conditions, the SR-A was determined to account for 15–20% of the trypsin-resistant cell adhesion (16).

The contribution of the SR-A to the adhesion of macrophages in vivo has not been demonstrated. However, our data and those reported previously suggest a role for the SR-A in adhesion under both normal and pathological conditions in both the vasculature and the central nervous system. The SR-A is highly expressed on activated microglia in the vicinity of Aβ-containing senile plaques in brains of patients with AD (30). The SR-A binds and internalizes microaggregates of the 42-amino acid form of Aβ in vitro (9). In addition, we recently reported that the SR-A binds secreted forms of APP (8). The Aβ peptide and secreted APP are major constituents of senile plaques and cerebrovascular deposits in patients with AD and Down’s syndrome (31–33). The SR-A may contribute to the clearance of both Aβ and secreted APP, which are produced continuously in normal and AD brains (32–35). However, the SR-A also mediates the adhesion of microglia and human monocytes to β-amyloid fibril-coated surfaces, leading to secretion of reactive oxygen species and cell immobilization (36). The SR-A may, therefore, contribute to the adhesion of cells to the senile plaques and/or to cells expressing APP on their plasma membrane. In addition to Aβ and sAPP, AD plaques contain a wide variety of molecules, including extracellular matrix proteoglycans. It would be interesting to determine whether these molecules have a role in the interaction with microglia and in the progression of the disease.
The SR-A may also contribute to the adhesion of macrophages in atherosclerotic lesions. Expression of the SR-A contributes to the development of atherosclerosis. SR-A−/− mice had smaller atherosclerotic lesions than control mice (11–13). Because SR-A−/− macrophages exhibit a reduced uptake and degradation of modified LDL compared with wild-type macrophages (11, 37), it is widely assumed that a decrease in lipid accumulation in macrophages in the arterial wall is responsible for the reduced atherosclerosis. However, this has not been proved. Other aspects of SR-A biology have been suggested to contribute to the reduced atherosclerosis (11, 15–19). Our results support a potential alternative mechanism for the decrease in atherosclerosis in SR-A−/− mice. These data suggest that the adhesion and retention of SR-A−/− macrophages in the extracellular matrix may be lower than the retention of SR-A+/+ macrophages, thereby resulting in reduced development of atherosclerosis. Biglycan, versican, and decorin, normally present in arteries, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesions, are substantially elevated in atherosclerotic lesio