Dominant Negative Mutations of the Scavenger Receptor

Native Receptor Inactivation by Expression of Truncated Variants

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Abstract

The bovine scavenger receptor was truncated at amino acid 266 or 310 to delete either all or part, respectively, of the collagen-like domain. The truncated receptors were inactive in the binding and internalization of acetyl (Ac) low density lipoprotein (LDL). Coexpression of truncated receptor with the native receptor dramatically reduced the percentage of cells internalizing fluorescently labeled Ac LDL, compared with cells expressing the native receptor alone. The mutant truncated at amino acid 266 was most effective in receptor inactivation, resulting in a 42% or 80% decrease in the percentage of cells expressing active receptor when transfected in a 1:1 or 1:2 molar ratio (native:mutant), respectively, with native receptor. Degradation of ^{125}I -Ac LDL was reduced up to 90% when the native and truncated mutant receptors were coexpressed. Scavenger receptor inhibition was specific because the activity of the LDL receptor was not altered. Transient transfection of the mouse macrophage cell line P388D1 with truncated scavenger receptor resulted in a 65% decrease in the uptake and degradation of Ac LDL but did not decrease the degradation of β -migrating very low density lipoprotein, which is LDL receptor-mediated. These results demonstrate that expression of truncated bovine scavenger receptor inactivates both the native bovine and murine scavenger receptors, producing a dominant negative phenotype in vitro. (*J. Clin. Invest.* 1993. 92:894-902.) Key words: scavenger receptor • acetyl low density lipoprotein receptor • dominant negative mutations • acetylated low density lipoprotein • oxidized low density lipoprotein

Introduction

In 1979 Goldstein et al. (1) determined that acetylation of LDL inhibited its binding to the LDL receptor and stimulated its binding to an acetyl LDL receptor expressed by mouse and rat macrophages, Kupffer cells, and cultured human monocytes. The acetyl LDL receptor has subsequently been demon-

strated to bind various other forms of modified lipoproteins such as acetoacetylated (2, 3), malondialdehyde-modified (4), and oxidized LDL (5), as well as certain negatively charged high-molecular-weight molecules such as fucoidan, dextran sulfate, polyvinyl sulfate, and polyinosinic acid (6). Because of this broad ligand specificity, the acetyl LDL receptor is also referred to as the scavenger receptor.

The scavenger receptor has been purified from bovine liver membranes (7), from the murine macrophage cell line P388D1 (8), and from carrageenan-induced rabbit granulomas (9). The functional unit of the receptor appears to be a 220- to 260-kD trimer. In ligand blots of nonreduced sodium dodecyl sulfate-polyacrylamide gels acetyl (Ac)¹ LDL binds to a trimer, but not to the 160-kD dimer (7, 10, 11). Two types of scavenger receptor have been cloned from a bovine lung cDNA library and their amino acid (aa) sequences deduced (12, 13). The type I and type II scavenger receptors are identical, except that the former has an 110-aa cysteine-rich carboxy-terminal domain. In the type II receptor, this domain is replaced by a six-residue carboxy terminus. Both forms of the receptor have a cytoplasmic amino terminus, a transmembrane domain, a spacer region, a series of heptad repeats predicted to form an α -helical coiled coil, and a collagen-like domain. The deduced sequences of the receptors are consistent with the trimeric structure predicted from ligand-blotting studies (13). The scavenger receptor is a trimeric integral membrane glycoprotein, which spans the plasma membrane once and is oriented with the amino terminus in the cytoplasm. When type I and type II receptors are transiently expressed in COS cells, both forms bind Ac LDL and oxidized LDL, indicating that the cysteine-rich carboxy-terminal domain of the type I receptor is not required for ligand-receptor interaction (12, 13). Complementary DNAs for two forms of the human and mouse scavenger receptor have recently been obtained from cDNA libraries derived from a phorbol ester-treated human monocyte-derived cell line, THP1, and from the murine cell line p388D1, respectively (14, 15). Their domain structures are identical to those of the type I and type II forms of the bovine receptors (14, 15).

Although a number of ligands have been identified for the scavenger receptor, its physiologic function and significance remains obscure. It has been postulated that the receptor contributes to the formation of foam cells in atherosclerotic lesions (1, 5, 16, 17) and to the clearance and inactivation of endotoxin (18). One strategy to establish the role of the receptor in vivo would be to inactivate the receptor, and then determine the metabolic consequences of this inactivation. A method for disrupting scavenger receptor activity is suggested by the trimeric structure of the receptor. In theory, multimeric proteins

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1. Abbreviations used in this paper: aa, amino acid(s); Ac, acetylated; CHO, Chinese hamster ovary fibroblasts; DiI, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate.

can be inactivated by the interaction of variant forms of the protein with the normal subunits (19). Several examples of naturally occurring dominant negative mutations have been described; in particular, mutations of the collagen genes that lead to osteogenesis imperfecta in heterozygous individuals provide a useful analogy to the situation with the scavenger receptor (20, 21). In these cases, mutant forms of collagen that interact with normal collagen monomers lead to aberrant processing, premature degradation, and a dominant negative phenotype. In the studies reported herein, it was demonstrated that the expression of truncated variants of the bovine scavenger receptor in cells *in vitro* leads to the inactivation of both the bovine and murine scavenger receptors.

Methods

Materials. FBS, DME, Dulbecco's phosphate-buffered saline, penicillin, streptomycin, Na ¹²⁵I, and the fluorescent probe 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) were obtained as previously described (22). Ham's F12 medium, RPMI 1640, the neomycin analog G418, and lactalbumin hydrolysate were purchased from Gibco (Baltimore, MD). Chinese hamster ovary fibroblasts (CHO), the murine macrophage cell line P388D1, and COS-7 cells were purchased from American Type Culture Collection (Rockville, MD). Lipofectin and TransfectACE were purchased from BRL (Baltimore, MD), and dimethyl sulfoxide, CaCl₂, DEAE-dextran, and chloroquine from Sigma Chemical Co. (St. Louis, MO). The vector pcDNA1 was purchased from Invitrogen (San Diego, CA). An expression vector, pSV2-neo, conferring resistance to neomycin (23), was obtained from Dr. David Johnson (Gladstone Institute). The type I and type II bovine scavenger receptor expression vectors, pXSR7 and pXSR3, were a generous gift from Dr. Monty Krieger (MIT, Cambridge, MA). The cDNA for glycoprotein (gp)1b α in the vector pcDNA1 was obtained from Dr. José López (Gladstone Institute). A peptide corresponding to aa 1–15 of the bovine scavenger receptor (13) was synthesized at the Biomolecular Resource Center, University of California, San Francisco, CA. To facilitate coupling to keyhole limpet hemocyanine (24), before production of antibodies in rabbit, the peptide was synthesized with an amino terminal cysteine.

Lipoproteins. Lipoproteins were isolated from normal human plasma or from rabbits fed a hypercholesterolemic diet (25). Human LDL ($d = 1.02$ – 1.05 g/ml) and rabbit β -migrating very low density lipoproteins (β -VLDL) were obtained from plasma (1 mg/ml EDTA) by sequential density gradient ultracentrifugation (26) dialyzed against saline EDTA (0.15 M NaCl, 0.01% EDTA) following isolation, and sterilized by filtration (0.45 μ m). The lipoproteins were iodinated by the method of Bilheimer et al. (27) or labeled with the fluorescent probe DiI (28, 29). The ¹²⁵I-LDL and DiI-labeled LDL were then acetylated as described (30). Human lipoprotein-deficient serum was derived from plasma as previously described (22).

Transfection and site-directed mutagenesis. The cDNA encoding the bovine type I scavenger receptor was mutagenized using an oligonucleotide-directed *in vitro* mutagenesis system (version 2) supplied in kit form by Amersham Corp. (Arlington Heights, IL). The procedure is based on the methods of Eckstein and associates (31–33). The mutated plasmid was introduced into *Escherichia coli*, and plasmids prepared from clones were sequenced to confirm the mutation and to ensure that no other point mutations were inadvertently introduced. The bovine scavenger receptor was truncated at the codon for asparagine 266 (AAT $\rightarrow$ TAA) and in a separate construct at the codon for serine 310 (TCT $\rightarrow$ TAA) to delete all or part, respectively, of the collagen-like domain. These specific sites for mutation were chosen so that the stop codons were introduced using two base changes in the DNA sequence. This permitted the detection of mRNA for the native and truncated receptors in cotransfected cells by RNase protection assay (34). The mutated cDNAs are all in the vector pcDNA1. Plasmids

for transfection were purified twice by CsCl gradient centrifugation as described (35).

The type I bovine scavenger receptor expression vector pXSR7 and the vector containing cDNA for the receptor truncated at aa 310 were cotransfected separately in CHO cells with the neomycin resistance gene pSV2-neo (20:1 ratio), using a calcium phosphate DNA precipitation method (36). Control CHO cells (SR neg) were cotransfected with the vector (pcDNA1) and pSV2-neo in a 20:1 ratio. Stably transfected cells were selected by growth in medium containing the neomycin analog G418 (0.4 mg/ml). The COS cells were transiently transfected using a DEAE-dextran-chloroquine procedure as described (37). In cotransfection experiments the cells were transfected with the expression vector for the native and truncated scavenger receptors in either a 1:1 or 1:2 molar ratio. The P388D1 cells were transfected with cDNA for the truncated bovine scavenger receptor in the vector pcDNA1 by liposome-mediated transfection with Lipofectin or TransfectACE as described (36). Transiently transfected cells (72 h after transfection) were analyzed for receptor activity.

Cell culture experiments. The COS-7 cells were routinely grown in DME containing 10% heat-inactivated FBS, penicillin (100 U/ml), and streptomycin (100 μ g/ml). Wild-type CHO cells were maintained in 50% DME–50% Ham's F12 medium containing 10% heat-inactivated FBS, penicillin (100 U/ml), and streptomycin (100 μ g/ml). The murine macrophage cell line P388D1 was maintained in RPMI medium containing 10% FBS. Cells for analysis by FACS[®] were incubated for 16 h at 37°C with either DiI-labeled Ac LDL (5 μ g/ml) or DiI-labeled β -VLDL (5 μ g/ml) in DME. The cells were processed and analyzed as described previously (22).

Scavenger receptor activity was also assessed by measuring cellular degradation of ¹²⁵I-Ac LDL or ¹²⁵I- β -VLDL. For all lipoprotein degradation assays, the medium was removed from the cells and iodinated lipoprotein was added to the cells in serum-free DME. The cells were incubated at 37°C for 16 h, and the trichloroacetic acid-soluble lipoprotein degradation products in the medium were quantitated as described (38). All data points were the average of duplicate determinations. Nonspecific degradation (i.e., the amount of degradation obtained in the presence of a 100-fold excess of unlabeled Ac LDL) has been subtracted from all data. Cells were solubilized in 0.1 N NaOH, and the protein content was determined by the method of Lowry et al. (39) using BSA as a standard.

RNase protection assay. A specific cDNA template was produced by PCR amplification from pXSR7 using oligonucleotide primers corresponding to bases 760 to 780 (sense) and 1007 to 1039 (antisense) of the bovine scavenger receptor cDNA. A 29-base T7 promoter sequence was added at the 5' end of the antisense primer, and a random 21-base sequence was added to the 5' end of the sense primer. A ³²P-labeled antisense RNA probe was generated by reverse transcription under the control of the bacteriophage T7 promoter. The labeled probe of the expected size (329 bases) was gel purified. The RNase protection assay was performed on total RNA isolated from cells expressing the native and mutant receptors as described (34). Using this probe, native receptor mRNA would protect a 279-bp fragment, mRNA for the truncation at aa 310 would result in 170- and 109-bp fragments, and mRNA for the truncation at aa 266 would result in cutting of the probe into 240- and 39-bp fragments. The 39-bp fragment was not detected.

Western blot analysis. Membrane proteins for immunoblotting were obtained essentially as described by Via et al. (8). Cells were scraped from the tissue culture dishes in buffer containing protease inhibitors, sonicated, and octylglucoside-solubilized proteins from the 100,000-g pellet were obtained as described (8). The membrane proteins were applied to one-dimensional sodium dodecyl sulfate-polyacrylamide (5% acrylamide) slab gels, electrophoresed, and electrophoretically transferred to nitrocellulose (32, 40, 41). A rabbit antisera, R1B, raised to a synthetic peptide corresponding to aa 1 to 15 of the bovine scavenger receptor, was used for immunoblotting. The immunoblots were then incubated with protein A conjugated to horseradish peroxidase, with reagents for chemiluminescent detection (Amersham Corp.), and exposed to x-ray film.

Figure 1. Photomicrographs of transiently transfected COS cells following incubation with DiI-labeled acetyl LDL. The cells were transiently transfected to express the type I scavenger receptor (*A*), the receptor truncated at aa 310 (*B*), or the receptor truncated at aa 266 (*C*). The cells were incubated with DiI-labeled Ac LDL (5 μ g/ml) for 16 h, 48 h after transfection. The cells were examined by fluorescence microscopy.

Results

As a rapid means of assessing receptor activity in transfected cells, the internalization of DiI-labeled Ac LDL was examined by fluorescence microscopy. Cells transiently transfected with the native receptor bound and internalized fluorescently labeled Ac LDL as expected (Fig. 1 *A*). In contrast, in cells transiently transfected with the bovine scavenger receptors truncated at either aa 266 or 310 to delete all or part of the collagen-like domain, respectively, no uptake of DiI-labeled Ac LDL was observed (Fig. 1, *B* and *C*). These results were confirmed by FACS[®] analysis (data not shown).

Because the truncated receptors were not active, it was important to demonstrate that mutant receptor cDNA was in fact transcribed. For this purpose, RNase protection assays were performed on total RNA from control and transiently transfected COS cells. Scavenger receptor mRNA was not detected in nontransfected cells but was present in cells transfected to express the native receptor or the receptors truncated at either aa 310 or 266 (Fig. 2). In cotransfected cells, mRNA for both native and truncated receptor was detected (Fig. 2). The cells were cotransfected in a 1:2 ratio with the expression vectors for the native and truncated receptors, and the protected probe fragments were quantitated either by gamma scanning densitometry of the nylon filter or by densitometric scanning of the autoradiograms. In two experiments, the ratio of truncated receptor mRNA to native receptor mRNA was determined to be 1.8:1 and 2.0:1 for the receptor truncated at aa 310, and 1.6:1 and 3.5:1 for the receptor truncated at aa 266.

To demonstrate that truncated receptor mRNA was translated, membrane protein from CHO cells stably transfected with either the native or a truncated form of the receptor was examined by immunoblotting, using a polyclonal antiserum raised to a peptide corresponding to the amino terminus of the receptor. Receptor protein was not detected in immunoblots of cell membranes from the control cells, whereas in cells expressing the native receptor, proteins were detected that had an apparent molecular weight consistent with that expected for tri-

meric (~ 220 kD) and dimeric (~ 130 – 160 kD) forms of the scavenger receptor (Fig. 3). In this gel the dimeric forms of the receptor were more prominent than usual (12). In immunoblots of reduced gels, the trimeric and dimeric forms of the receptor were totally absent and only the monomer was detected (data not shown). The truncated receptor was also expressed and detected by the antibody, but at a lower apparent molecular mass as expected (Fig. 3). The trimeric form of

Figure 2. RNase protection assay of total RNA from control and transfected cells. RNase protection assay of total RNA (5 μ g) from control nontransfected COS cells and from COS cells transfected with the native Type I bovine scavenger receptor, the mutant receptors truncated at either aa 310 or 266, or cotransfected with the native and mutant receptors in a 1:2 ratio.

Figure 3. Immunoblots of octylglucoside-solubilized membrane proteins from control CHO cells and from cells transfected with the Type I bovine scavenger receptor or with the receptor truncated at aa 310. Cells were transfected and selected for stable transfectants in media containing G418. Octylglucoside-solubilized membrane proteins (160 μ g) were subjected to western blot analysis, and scavenger receptor was detected with an antipeptide antibody, R1B.

the truncated receptor had an apparent molecular mass of ~ 180 kD.

The mutant truncated receptors, which do not bind ligand, were tested for their ability to inactivate the native bovine scavenger receptor in transiently transfected COS cells. Native COS cells do not express the scavenger receptor and, as shown in Fig. 4, when the cells are transfected with the vector pcDNAI as a

control, only 1.7% of the cells were fluorescent to a significant extent. When the cells were cotransfected with the expression vector pXSR3, for the type II bovine scavenger receptor, in a 1:2 ratio with the expression vector for gp1b α , 27.4% of the cells internalized fluorescently labeled Ac LDL. This same level of uptake was observed when the scavenger receptor was coexpressed with the vector pcDNAI (data not shown). In contrast, when the scavenger receptor was coexpressed in a 1:2 molar ratio with the receptor truncated at aa 266 or 310, the percentage of cells internalizing DiI-labeled Ac LDL decreased to 7.7% and 4.7%, respectively (Fig. 4). This represents a 72 to 83% decrease in the percentage of cells expressing active receptor. Although in this single experiment the mutant truncated at aa 310 was the most effective in reducing native receptor activity, this case was not typical (discussed below).

A series of similar cotransfection experiments was performed to characterize further the dominant negative effect of the truncated receptor on type II scavenger receptor activity. As a control in each experiment, native receptors were coexpressed with either the vector pcDNAI or with glycoprotein Ib α , each in a 1:1 or 1:2 ratio. Coexpression of the vector or gp1b α with the native receptor had no consistent effect on the percentage of cells expressing active receptors (i.e., 92 to 103%). On the other hand, coexpression of the native receptor with the receptors truncated at aa 266 or 310 resulted in a dramatic decrease in the percentage of cells internalizing DiI-labeled Ac LDL (Fig. 5). This dominant effect was both dose- and mutant-dependent, with a greater inhibition of receptor activity with the higher amount of mutant receptor and with the receptor truncated at aa 266 (Fig. 5). Under these conditions, up to an 80% decrease in the percentage of cells expressing active receptor was observed. The truncated receptors also inhibited the activity of the type I bovine scavenger receptor (data not shown). Similar cotransfection experiments were performed and LDL receptor activity was assessed to demonstrate that the suppression of receptor activity was specific. Transient expression of either the native scavenger receptor alone or coexpression with the truncated receptors in COS cells

Figure 4. Fluorescence-activated cell sorter analysis showing the effect of coexpression of native scavenger receptor with truncated scavenger receptor on the uptake of DiI-labeled Ac LDL. The COS cells were transiently transfected with the vector pcDNAI (5 μ g), or with the expression vector pXSR3 (5 μ g), for the native type II bovine scavenger receptor, in a 1:2 ratio with the expression vectors for gp1b α or with the receptor truncated at aa 266 or 310. The uptake of DiI-labeled Ac LDL was assessed by flow cytometry 72 h later. Cotransfection with the receptors truncated at aa 266 or 310 resulted in a 72% and 83% reduction, respectively, in the percentage of cells expressing active receptor.

Figure 5. Summary of transient transfection experiments showing the effect of coexpression of native and truncated scavenger receptor on receptor activity. Cells were prepared and analyzed by flow cytometry as described in Fig. 4. The data for eight separate experiments are compiled. In each experiment, cells were transfected with the native receptor alone (type II scavenger receptor, pXSR3) or cotransfected with the vector pcDNA1, or with the truncated receptors in the ratios shown. The percentage of cells internalizing DiI-labeled Ac LDL was determined by FACS[®] analysis as shown in Fig. 4. The average percentage of cells expressing native receptor was $33 \pm 5.6\%$. There was no effect of expression of the vector. Data are reported as the mean $\pm$ range ($n = 2$) or the mean $\pm$ standard deviation ($n = 4$).

did not decrease the uptake of DiI-labeled LDL, as determined by FACS[®] analysis (data not shown).

The degradation of ^{125}I -Ac LDL and ^{125}I -LDL by COS cells transiently transfected with the native scavenger receptor alone or in a 1:1 or 1:2 ratio with each of the mutant receptors was also studied. In agreement with the FACS data, coexpression of mutant receptor truncated at aa 266 resulted in a 60% (1:1 ratio) to 90% reduction (1:2 ratio) in the specific degradation of Ac LDL (Fig. 6). The decrease of scavenger receptor activity was clearly specific, as the degradation of native LDL was not diminished in the cotransfected cells when they were grown in medium containing either fetal bovine serum or lipoprotein-deficient serum to upregulate LDL receptor activity (Fig. 6). Essentially identical results were obtained with the 310 mutant (data not shown).

In an attempt to determine the mechanism of receptor inactivation, immunoblotting experiments were performed with membrane proteins from transiently transfected COS cells (Fig. 7). When the type I scavenger receptor (Fig. 7, *top*) or the receptor truncated at aa 266 (Fig. 7, *bottom*) was expressed individually, each displayed a complex pattern of bands representing trimeric and dimeric forms of the receptor. When the native and truncated receptors were coexpressed (Fig. 7, *middle*), the amount of trimeric full-length native receptor expressed was reduced. Due to the complexity of the gels, it is impossible to determine if the reduction in native trimers resulted from the formation of heterotrimers of native and mutant receptor monomers, or from decreased synthesis or premature degradation of the native receptor.

Transient transfection experiments were performed in the mouse macrophage cell line P388D1 to determine if the truncated bovine scavenger receptor would inactivate the mouse scavenger receptor. After transient transfection of the cells with the receptor truncated at aa 266 or 310, the uptake of DiI-la-

Figure 6. Degradation of Ac LDL and LDL by COS cells transfected with the native scavenger receptor alone or in a 1:1 or 1:2 ratio with the receptor truncated at aa 266. The cells were split into smaller tissue culture dishes 24 h after transfection and incubated an additional 24 h in medium containing either 10% FBS or 10% lipoprotein-deficient serum to upregulate LDL receptor activity before the degradation experiments. The cells were then incubated for 16 h in DME containing either ^{125}I -Ac LDL or ^{125}I -LDL, and the lipoprotein degradation products in the medium were measured as described in Methods. Each data-point is the average of duplicate determinations. Nonspecific degradation, i.e., that which occurred in the presence of a 100-fold excess of nonlabeled ligand, has been subtracted from all data.

beled Ac LDL was examined by fluorescence microscopy. In cells transfected with the vector alone, all cells internalized the DiI-labeled Ac LDL to a similar extent (Fig. 8A). This pattern of uptake looked no different than the uptake by control nontransfected cells (data not shown). In contrast, in the cells transfected with the receptor truncated at aa 310, two populations of cells were observed: cells that internalized DiI-labeled

Figure 7. Densitometric scans of immunoblots of membrane proteins from cells transiently expressing the type I scavenger receptor, a truncated scavenger receptor, or the type I and truncated receptors together. The COS cells were transiently transfected either with the expression vector pXSR7 to express the type I bovine scavenger receptor (*top*), with the expression vector for the receptor truncated at aa 266 (*bottom*), or with the two expression vectors together (*middle*). Octyl glucoside-solubilized membrane proteins, isolated 72 h following transfection, were subjected to western blot analysis, and scavenger receptor was detected with an antipeptide antibody, R1B. The fluorograms were scanned by densitometry. The crosshatched areas of the scans indicate the trimeric forms of the native and truncated receptors.

Figure 8. Photomicrographs showing the effect of transient transfection of the mouse macrophage cell line P388D1, with truncated bovine scavenger receptor, on the internalization of DiI-labeled Ac LDL. Mouse macrophage cells (P388D1) were transiently transfected with either the vector pcDNAI (*A* and *B*) or the mutant receptor truncated at aa 310 (*C* and *D*). The day after TransfectACE-mediated transfection, the cells were split into six-well dishes and maintained for 32 h in their regular medium before the 16 h incubation in DME-containing DiI-labeled Ac LDL (5 μ g/ml). The cells were then examined by fluorescence microscopy. Both fluorescent (*A* and *C*) and phase contrast photomicrographs (*B* and *D*) are shown.

Ac LDL as efficiently as control cells, and cells that internalized less ligand, presumably due to inactivation of the mouse scavenger receptor (Fig. 8 *C*). Similar results were obtained with the receptor truncated at aa 266 (data not shown). These results are consistent with the inhibition of receptor activity in those cells that incorporated and expressed the transfected cDNA. In Fig. 8 *C*, some of the cells appear to be brighter than controls. This is an artifact of the photography, since FACS[®] analysis did not demonstrate enhanced uptake (see data below).

The effect of transfection with mutant receptor on the uptake of DiI-labeled Ac LDL in P388D1 cells was also examined by flow cytometry (Fig. 9). When the control cells were transfected with the vector pcDNAI, there was no effect on receptor activity (data not shown). However, when the cells were transfected with the bovine scavenger receptor that was truncated at aa 266, a significant decrease in receptor activity was observed (Fig. 9, *left*). Based on the mean fluorescence intensity of the cells, transfection with the mutant receptor resulted in a 65% decrease in the uptake of DiI-labeled Ac LDL. There was, however, no effect on LDL receptor activity because the internalization of the β -VLDL, which is LDL receptor-mediated (42), was not affected (Fig. 9, *right*). In similar experiments, examination of the degradation of ¹²⁵I-Ac LDL and ¹²⁵I- β -VLDL, yielded essentially identical results: the degradation of the ¹²⁵I-Ac LDL was dramatically reduced (50%) while the degradation of ¹²⁵I- β -VLDL was unaffected (Fig. 10).

Figure 9. Fluorescence-activated cell sorter analysis showing the effect of transfection with truncated bovine scavenger receptor on the uptake of DiI-labeled Ac LDL and β -VLDL by mouse macrophages. The cells were transiently transfected (Lipofectin-mediated) with the receptor truncated at aa 266. They were then processed as described in the legend to Fig. 8. The uptake of DiI-labeled Ac LDL and DiI-labeled β -VLDL was assessed by flow cytometry. Results from the control cells transfected with the vector alone are shown by the dashed line and those from the cells expressing the truncated mutant are shown by the solid line.

Discussion

This study reports a strategy to inactivate the scavenger receptor by expression of dominant negative mutations. Truncation mutations were made in the collagen-like domain of the scavenger receptor. The truncated mutants of the bovine scavenger receptor, lacking half (310 mutant) or all (266 mutant) of the collagen-like domain, were expressed as determined by RNase protection assay and immunoblotting of membrane protein, but were incapable of internalizing DiI-labeled Ac LDL. These mutant receptors were then examined for their ability to inactivate the native receptor. Using transient cotransfection with the native receptor in COS cells, it was demonstrated that the truncated bovine scavenger receptors inactivate the native bovine receptor. These results are in agreement with a recent report by Acton et al. (43). This inhibition was specific, since expression of an unrelated protein (glycoprotein 1b α) had no effect on scavenger receptor activity and since there was no effect of mutant receptor expression on LDL receptor activity.

Figure 10. Effect of expression of truncated bovine scavenger receptor on the degradation of Ac LDL and β -VLDL by mouse macrophages. The mouse macrophage cell line (P388D1) was transiently transfected with either the vector pcDNAI (control cells)

or the receptor truncated at aa 266, as described in Fig. 9. The cells were split into smaller tissue culture dishes 24 h after transfection and incubated in their regular medium for 48 h before the degradation experiment. The cells were incubated for 16 h in DME-containing either ¹²⁵I-Ac LDL or ¹²⁵I- β -VLDL, and the lipoprotein degradation products in the medium were measured as described in Methods. Each data-point is the average of duplicate determinations. Nonspecific degradation, i.e., that which occurred in the presence of a 100-fold excess of nonlabeled ligand, has been subtracted from all data.

Furthermore, expression of the truncated bovine scavenger receptor inactivated the mouse scavenger receptor in the macrophage cell line P388D1. The decrease in scavenger receptor activity is clearly not due to a nonspecific effect on receptor-mediated endocytosis or activity, because the LDL receptor is not affected. Although we did not demonstrate the mechanism for this inhibition, we have demonstrated that the loss of activity is associated with a decrease in the expression of native scavenger receptor trimers in the cotransfected cells.

The data suggest that the collagen-like domain of the scavenger receptor is the receptor-binding domain. This contention is supported by the observation that binding activity was eliminated by truncation of the receptor at either aa 266 or 310 to delete all or part of the collagen-like domain. Because the type II scavenger receptor is 349 aa long (12), the data suggest that the receptor-binding domain falls between aa 310 and 349. This conclusion is in agreement with a report by Kodama et al. (44) and is supported by the observation that the collagenous domain of the complement component C1_q also binds several polyanions that are ligands for the scavenger receptor (43).

Probable mechanisms for the dominant negative effect of the truncated receptors are suggested by the multimeric structure of the scavenger receptor. Dominant negative suppression of function results when mutant polypeptides disrupt the activity of the wild-type protein. When the protein is multimeric, a defective subunit capable of interacting with the wild-type monomer can be inhibitory (19). Some examples of dominant negative mutations of receptors are dysfunctional heterodimers of mutant and normal forms of the insulin receptor, found in certain cases of insulin resistance (45, 46), a mutation of the *c-kit* proto-oncogene in mice (47), and the production of dominant negative forms of the platelet-derived growth factor (48) and the EGF receptors (49). The most numerous cases of naturally occurring dominant negative mutations, however, and also the most relevant to scavenger receptor inactivation, are mutations in collagen genes that lead to lethal forms of osteogenesis imperfecta in heterozygous individuals (20, 21). A number of dominant negative mutations in pro- α 1 and pro- α 2 collagen interfere with formation of the normal type I collagen triple helix (20). Dominant negative mutations of collagen result from both deletion mutations and from single base changes most often in the codon for glycine (20). The mutant protein is dominant because it interacts with the products of the normal gene, leading to aberrant processing or to premature degradation of the native protein, or both (20, 21, 50).

Because the scavenger receptor contains a collagen-like domain and is known to form trimers and dimers, the truncated receptors are probably inhibiting the activity of the native receptor through the formation of dysfunctional heteromers with the native monomer. The fact that the bovine receptor monomer can disrupt the function of the mouse receptor suggests that sequences involved in association of receptor monomers are conserved between species. In fact, there is close aa identity between the mouse and bovine scavenger receptors (51). The hypothesis that heterotrimer formation is responsible for receptor inactivation is supported by the dose-dependent effect of the mutant. When more mutant monomer is expressed, there is a lower probability of forming native homotrimers. The precise mechanism by which this inhibition of receptor function occurs remains unknown. Penman et al. (10) recently showed that scavenger receptor trimerization is required for

efficient transport from the endoplasmic reticulum to the Golgi and that only mature receptor trimers are then transported to the cell surface, where they can participate in the receptor-mediated endocytosis of ligands. In addition, α , α' -dipyridyl alters the processing of the scavenger receptor, suggesting that the trimerized receptor undergoes posttranslational processing similar to collagen (10). Formation of heterotrimers of the truncated and native receptor monomers could disrupt any of these intracellular steps. The mutant could, therefore, exert its inhibitory effect by forming heteromers with the native receptor, leading to aberrant processing, to premature degradation of the native receptor, or to inefficient transport to the cell surface. Alternatively, it is possible that heterotrimers may be expressed on the cell surface but are not competent to bind ligand. Finally, increased expression of the mutant receptor may lead to reduced synthesis of the native receptor. The 266 mutation is more effective than the 310 mutation in receptor inactivation. This could be because aa 267 to 310 are important in the stabilization of the collagen-like domain. Deletion of the residues could therefore lead to more defective processing and premature degradation.

A recent report by Via et al. (52) indicates that the murine scavenger receptor trimer, as well as dimers and monomers generated under mild reducing conditions *in vitro*, bind Ac LDL on ligand blots. Radiation inactivation experiments, which were used to determine the functional molecular weight of the receptor *in situ*, also suggest that ligand can be bound by each receptor monomer present within cell surface trimers (52). On the other hand, we have not observed ligand binding to dimeric scavenger receptors (11) that are often present (Fig. 3) when the bovine scavenger receptor is isolated from transfected cells. The reason for this difference is unknown. If ligand can in fact bind to each individual monomer present in trimers and dimers, as the data of Via et al. (52) suggest, the dominant effect we observed would probably result from intracellular heteromer formation leading to premature degradation of the native monomer, defective processing, and/or reduced transport to the cell surface, rather than from the expression of heterotrimers on the cell surface.

Although the molecular mechanism responsible for receptor inactivation has not been determined, we have clearly demonstrated a dominant negative effect of mutant receptor on normal receptor function. These *in vitro* observations suggest that dominant negative mutations can be used *in vivo* for receptor inactivation in transgenic mice. Transgenic animals with a scavenger receptor-negative phenotype would provide an exciting opportunity to determine the physiologic role of this receptor. In addition, these data strongly suggest that naturally occurring dominant negative mutations of the scavenger receptor will eventually be identified in humans. Whether or not these lead to severe metabolic defects remains to be determined.

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