

Short Communication

Expression of the Macrophage Scavenger Receptor, a Multifunctional Lipoprotein Receptor, in Microglia Associated with Senile Plaques in Alzheimer's Disease

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The macrophage scavenger receptor is a multifunctional receptor whose ligands include oxidized low density lipoprotein (LDL), as well as several other polyanionic macromolecules. Although the capacity of the receptor to bind modified LDL has implicated it in the process of atherosclerosis, its physiological role remains uncertain. We have examined human brain for expression of macrophage scavenger receptor as part of ongoing studies of lipoprotein receptors in the central nervous system. The receptor is expressed on microglia, but not on astrocytes, neurons, or vessel-associated structures. In Alzheimer disease, there is strong expression of the scavenger receptor in association with senile plaques. (Am J Pathol 1996, 148:399–403)

The observation that the $\epsilon 4$ allele of the apolipoprotein (Apo)E gene is associated with increased risk for and earlier onset of Alzheimer's disease (AD)^{1–7} has led us to study molecules related to lipoprotein metabolism in the brain. The best characterized lipoprotein receptor, the low density lipoprotein (LDL) receptor, provides receptor-mediated endocytosis for lipoproteins containing ApoB-100 and ApoE. Two other classes of molecules have been recently characterized as lipoprotein receptors. These are macrophage scavenger receptors⁸ and the LDL receptor-related protein (LRP).^{9,10} Both of these receptors

are characterized by high affinity and broad specificity, recognizing both lipoprotein and non-lipoprotein ligands. We have recently demonstrated that LRP, but not the LDL receptor, is associated with cortical senile plaques in AD.⁵ We have now examined the expression of the macrophage scavenger receptor in human brain.

The macrophage scavenger receptor was discovered after the observation that massive uptake of cholesterol into macrophages was possible even in individuals who had defective LDL receptors.^{11–13} The macrophage scavenger receptor mediates the endocytosis of chemically modified LDL rather than native LDL. Modifications that convert LDL into a scavenger receptor ligand include acetylation or oxidation.¹⁴ Uptake of ligands leads to internalization into lysosomal and endosomal compartments and lysosomal degradation.

The scavenger receptor also binds and internalizes multiple other molecules.¹⁵ Oxidized lipoproteins, polyribonucleotides, polysaccharides, such as dextran sulfate, ionic phospholipids, endotoxin, and other compounds including advanced glycation end products¹⁶ all appear to be specific ligands. These are all polyanionic molecules or macromolecules, although not all polyanions are macrophage scavenger receptor ligands. The putative ligand binding site of the scavenger receptor is a collagen-like domain (Figure 1).¹⁷ Truncation of this domain prevents li-

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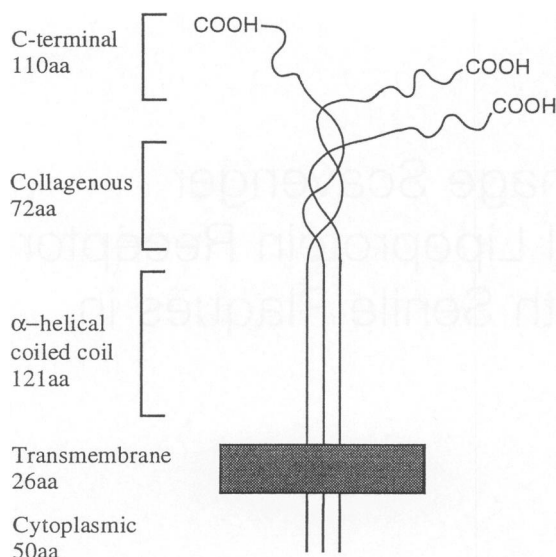


Figure 1. Predicted molecular structure of the type I scavenger receptor. The trimeric receptor consists of a short cytoplasmic tail, a transmembrane region, a spacer region (not shown), an α -helical coiled coil domain, a collagen-like ligand binding region, and a C-terminal cysteine-rich domain of unknown function (absent in the Type II receptor).

gand binding, and site-directed mutagenesis of lysines within this domain reduces binding and endocytosis.¹⁸

Materials and Methods

Brain tissue from control ($n = 4$, mean age 71 years) and AD patients ($n = 6$, mean age 75 years) was obtained from the Alzheimer Disease Research Center Brain Bank (Massachusetts General Hospital, Boston, MA). All AD cases met Khachaturian and CERAD diagnostic criteria for AD based on examination of Bielschowsky-stained paraffin sections of multiple cortical and subcortical areas^{26,27}. Tissue from six cases with neuropathological diagnoses other than AD (two Pick's disease, one Parkinson's disease, and three diffuse Lewy body disease cases) was also examined for scavenger receptor expression. Dissected regions containing the hippocampal formation and adjacent temporal neocortex were fixed in paraformaldehyde-lysine-metaperiodate for 24 to 36 hours, then transferred to cryoprotectant solution (15% glycerol in Tris-buffered saline, pH 7.4) at 4°C. Sections were prepared at 50 μm on a freezing sledge microtome and stored in a solution of 15% glycerol in Tris-buffered saline, pH 7.4 at -80°C , until use. Immunohistochemistry was performed on free-floating sections using the anti-scavenger receptor antibody hSRI-2.¹⁹ This is a rabbit polyclonal antibody raised against a synthetic

peptide corresponding to the collagenous (ligand-binding) domain of the human scavenger receptor. Double staining was carried out using antibody LN-3 (ICN Biochemicals, Irvine, CA) for human lymphocyte antigen-DR-positive microglia, antibody 10D5 for $\text{A}\beta$ (Athena Neuroscience, South San Francisco, CA; see ref. 20), or thioflavine S fluorescent histochemistry for senile plaques.

Immunohistochemistry was performed by overnight incubation at 4°C in primary antibody diluted 1:500 in 1% normal goat serum/Tris buffered saline, pH 7.4. Immunoreactivity was visualized using either a biotinylated secondary antibody with subsequent avidin-biotin amplification (Vector Elite Kit, Vector Laboratories, Burlingame, CA), and diaminobenzidine as chromagen, or, for double immunohistochemistry, via a gold-linked secondary antibody followed by silver enhancement (Amersham Life Science, Buckinghamshire, UK). Specific staining was absent if primary antisera were omitted, and when the hSRI-2 antiserum was precleared with excess rabbit scavenger receptor protein, with which the antibody cross-reacts.

The specificity of antibody hSRI-2 has been previously shown by specific staining of systemic macrophages.¹⁹ We have also demonstrated that this antibody specifically recognizes a rabbit scavenger receptor fusion protein,²¹ which shares 15 of 18 amino acids with the human scavenger receptor collagenous domain. Antibody specificity in human brain was established by Western blot analysis on a reducing, denaturing, 4 to 12% Tris-glycine gel. Brain tissue frozen immediately at autopsy and stored at -80°C was homogenized in a buffer containing 1 mmol/L EDTA, 30 mmol/L Tris, 1 mmol/L benzamidine, 1 mmol/L Na-o-vanadate, 10 $\mu\text{g}/\text{ml}$ aprotinin, 2% sodium dodecyl sulfate, and phenylmethylsulfonyl fluoride (1:200) added just before homogenization. 100 μg of total protein was added to each well, and the gel was transferred to Immobilon (Millipore, Bedford, MA) for subsequent immunological processing and visualization using enhanced chemiluminescence Western blotting detection reagent (Amersham Life Science).

Results

The macrophage scavenger receptor was present in microglia in cortical and subcortical areas in both control and Alzheimer disease brains (Figure 2, A and C). Counterstaining with monoclonal antibody LN3, specific for microglia, confirmed the cellular identity of the scavenger receptor positive cells (Fig-

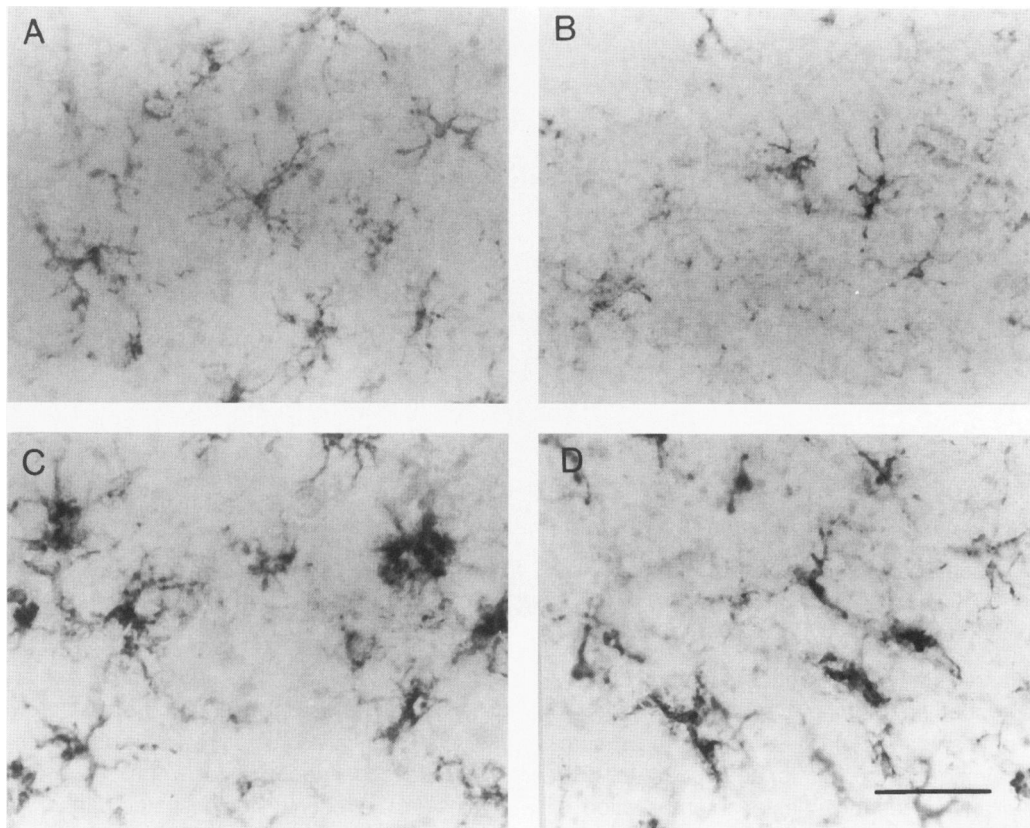


Figure 2. Resting microglia in a control brain (top) and activated microglia in an AD brain (bottom) are immunoreactive for both hSRI-2 (A, C) and LN-3 (B, D). Scale bar, 50 μ m.

ure 2, B and D). No astrocytic profiles were observed, nor was there expression of the scavenger receptor on neurons or vessel-associated cells. Both resting and activated microglial cells were immunoreactive, with increased immunoreactivity in activated microglia (Figure 2D).

In Alzheimer disease, the number of activated microglia was substantially larger than in control brains. Microglia were frequently associated with $A\beta$ deposits. These microglia strongly expressed the scavenger receptor. Figure 3 illustrates macrophage scavenger receptor immunoreactivity in a microglial cell associated with a thioflavine S-positive senile plaque. Scavenger receptor immunoreactivity was prominent in microglia associated with $A\beta$ deposits identified by thioflavine S or with a "compact" appearance using $A\beta$ immunoreactivity (antibody 10D5). The association of scavenger receptor with $A\beta$ was present but less prominent in "diffuse" $A\beta$ deposits such as those found in the presubiculum, in that such deposits contained fewer and less intensely stained microglia. Examination of other neuropathological cases revealed significant staining of activated and resting microglia in 2 of 2 cases with Pick's disease. Resting microglia were immunoreac-

tive in 1 (of 1) case with Parkinson's disease and in 1 of 3 cases with a diagnosis of diffuse Lewy body disease.

The specificity of hSRI-2 in human brain was assessed by immunoblot analysis (Figure 4). A band at ~75 kd in control human brain homogenates matched the size expected for the bovine scavenger receptor.¹⁴ Two much weaker bands, at ~55 kd and ~98 kd, were also present in brain homogenates. Staining of all three bands was abolished by preabsorption of the primary antibody with excess rabbit scavenger receptor protein.

Discussion

We report herein the localization of the macrophage scavenger receptor in microglia in the human brain. In AD, the scavenger receptor is prominent on activated microglia in the vicinity of senile plaques. Although the physiological function of the scavenger receptor remains unknown, it has been postulated to be involved in lipoprotein metabolism, atherosclerosis, and host defense mechanisms.²² The affinity of the macrophage scavenger receptor for a wide va-

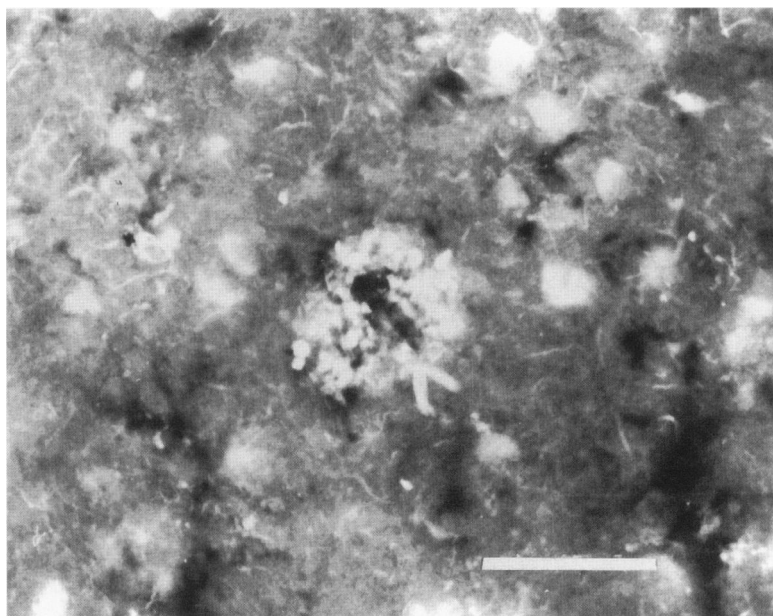


Figure 3. Senile plaque-associated microglia are strongly positive for the scavenger receptor. The A β deposit is visualized using a thioflavine S fluorescent stain, and the scavenger receptor is visualized using rabbit polyclonal antibody hSRI-2 with a peroxidase-linked secondary antibody and diaminobenzidine as chromagen. Scale bar, 50 μ m.

riety of ligands, via the collagenous domain, confers broad but specific binding capabilities similar to complement factor C_{1q}. In this regard, the macrophage scavenger receptor is analogous to LRP, which similarly is responsible for internalization of ApoE in addition to a variety of other ligands via multiple binding sites in the extracellular domain.

Our observations that both LRP⁵ and the macrophage scavenger receptor are expressed in the vicinity of senile plaques provide an initial step in understanding the molecular responses to the deposition of A β in the neuropil. Whether these multifunctional receptors interact directly with A β or with other plaque components is unknown. Increasing evidence suggests elevated free radical and oxidative damage in the AD

brain,²³ and recent data suggest that senile plaques contain advanced glycation end products, which are also a potential source of free radicals.²⁴ The avidity of the scavenger receptor for oxidatively modified proteins and its recently demonstrated capacity for mediating endocytosis of advanced glycation end products¹⁶ suggest a possible role for scavenger receptor-positive microglia in interacting with such proteins in the vicinity of A β deposits. In the case of LRP, we have recently demonstrated that all seven known naturally occurring ligands that are expressed in the brain are associated with senile plaques.²⁵ Thus, these multifunctional receptors may play a role to the pathophysiological processes that occur in the vicinity of senile plaques.

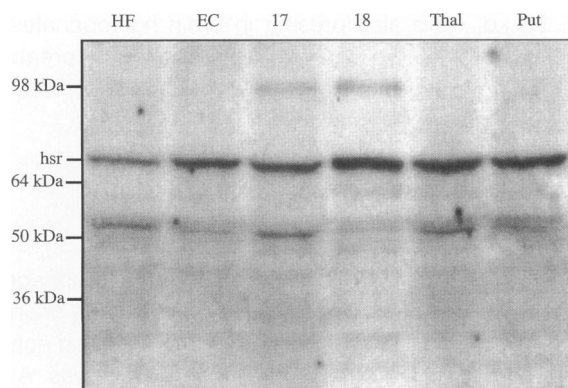


Figure 4. Antibody hSRI-2 recognizes a band at ~75 kd in homogenates made from control (non-AD) human cortex. Faint bands at ~55 kd and ~98 kd are also present. All three bands are absent upon preclearing antiserum with rabbit scavenger receptor protein. HF: hippocampus; EC: entorhinal cortex; 17, 18: Brodmann's areas 17 and 18; Thal: thalamus; Put: putamen.

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