Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9133–9137, December 1990
Medical Sciences

Human macrophage scavenger receptors: Primary structure, expression, and localization in atherosclerotic lesions

(modified low density lipoproteins/chromosome 8/collagen)

AKIYO MATSUMOTO*†, MAKOTO NAITO‡, HIROSHIGE ITAKURA*†, SHINJI IKEMOTO†, HITOSHI ASAOKA†, IKUHO HAYAKAWA†, HIROSHI KANAMORI*, HIROYUKI ABURATANI*§, FUMIMARO TAKAKU*, HIROSHI SUZUKI¶, YUKAGE KOBARI¶, TATSUYA MIYAI¶, KIYOSHI TAKAHASHI‡, EDWARD H. COHEN‖, ROBERT WYDRO‖, DAVID E. HOUSMAN§, AND TATSUHIKO KODAMA*†**

*The Third Department of Internal Medicine, University of Tokyo, Hongo, Tokyo 113, Japan; †Division of Clinical Nutrition, National Institute of Health and Nutrition, Tokyo, Japan; ‡The Second Department of Pathology, Kumamoto University, Kumamoto, Japan; ¶Laboratory Animal Center, Chugai Pharmaceutical, Tokyo, Japan; §Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139; and ‖Genzyme Corporation, Framingham, MA 01701

Communicated by Michael S. Brown, August 17, 1990

ABSTRACT **Two types of cDNAs for human macrophage scavenger receptors were cloned from a cDNA library derived from the phorbol ester-treated human monocytic cell line THP-1. The type I and type II human scavenger receptors encoded by these cDNAs are homologous (73% and 71% amino acid identity) to their previously characterized bovine counterparts and consist of six domains: cytoplasmic (I), membrane-spanning (II), spacer (III), α-helical coiled-coil (IV), collagen-like (V), and a type-specific C-terminal (VI). The receptor gene is located on human chromosome 8. The human receptors expressed in CHO-K1 cells mediated endocytosis of modified low density lipoproteins. Two mRNAs, 4.0 and 3.2 kilobases, have been detected in human liver, placenta, and brain. Immunohistochemical studies using an anti-peptide antibody which recognizes human scavenger receptors indicated the presence of the scavenger receptors in the macrophages of lipid-rich atherosclerotic lesions, suggesting the involvement of scavenger receptors in atherogenesis.**

The macrophage scavenger receptors are trimeric membrane glycoproteins implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis (1, 2). The molecular cloning of bovine scavenger receptor cDNAs has revealed several unexpected features (3, 4). Two types of receptor subunits exist, and both of these receptor proteins contain two extracellular domains that are predicted to form a long triple-stranded α-helical coiled coil and a collagen-like triple helix (3, 4). These receptors mediate the endocytosis of a diverse group of macromolecules, including modified low density lipoproteins (LDLs) (1–5). When cultured macrophages are exposed to appropriately modified LDLs, they are converted to cholesteryl ester-rich foam cells which are strikingly similar to the foam cells found in atherosclerotic plaques (1). Recent *in vivo* studies support the suggestion that modified LDLs, possibly internalized via the scavenger receptors, may play a critical role in the development of atherosclerosis (6–8).

To study the role of human scavenger receptors in atherogenesis, we have cloned human scavenger receptor cDNAs.†† From the deduced amino acid sequence, we generated rabbit anti-peptide antiserum that recognizes human macrophage receptors. Immunohistochemical studies using this antibody have revealed the presence of immunoreactive scavenger receptor protein in the macrophages of atherosclerotic lesions, suggesting the involvement of scavenger receptors in atherogenesis.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Molecular Cloning of Human Scavenger Receptor cDNAs. THP-1 cells were cultivated in the presence of 200 nM phorbol 12-myristate 13-acetate (PMA) for 4 days (2). Poly(A)$^+$ RNA was isolated from these cells and was used to construct a size-fractionated oligo(dT)-primed cDNA library in λ ZAP II (3). The *Xba* I–*Sph* I fragment of pBSR7 corresponding to the collagen-like domain of the bovine receptor (3) labeled by random priming was used as a hybridization probe to screen 6×10^5 plaques. Two positive clones were isolated, and the DNA was excised *in vivo* and sequenced. Both clones contained a long open reading frame homologous to the nucleotide sequence of the bovine scavenger receptor. The 3′ sequences of the open reading frames of these two clones were different. One, containing an extra cysteine-rich sequence of 110 amino acid residues at the C terminus, was designated type I, and the other, type II. Additional screening using the inserts of these clones as probes resulted in another 17 positive clones (14 type I clones and 3 type II clones) from 6×10^5 plaques. Sequence comparison was performed using GENETYX sequence analysis packages (SDC, Tokyo). For Northern blot hybridization analysis, poly(A)$^+$ RNA of human liver and cerebellum was prepared from noncancerous tissue resected with malignancy. The RNA samples were electrophoresed through a 1% agarose gel containing 7% formamide, transferred to a nylon filter, and subjected to hybridization.

Expression of Scavenger Receptors in CHO Cells. A type I scavenger receptor expression vector, pXhSR1, was prepared by excision of the insert of phSR1 by *Hin*dIII/*Xba* I in the vector sequence followed by ligation into RC/CMV (InVitrogen, San Diego, CA). A type II scavenger receptor expression vector, pXhSR2, was also prepared by using the *Hin*dIII/*Xba* I fragment of phSR2 and RC/CMV. These vector DNAs were transfected into CHO-K1 cells by the lipofection method (9). Two days after transfections, G418 was added to a final concentration of 480 μg/ml and resistant cells were selected over 2 weeks. Cells expressing high levels of scavenger receptor activity were detected by using acetyl-

Abbreviations: LDL, low density lipoprotein; PMA, phorbol 12-myristate 13-acetate; PCR, polymerase chain reaction; BSA, bovine serum albumin.

**To whom reprint requests should be addressed at: The Third Department of Internal Medicine, University of Tokyo, 7-3-1 Hongo, Tokyo 113, Japan.

††The sequences reported in this paper have been deposited in the GenBank data base (accession nos. D90187 for type I and D90188 for type II).

LDL fluorescently labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine as described elsewhere (2).

Chromosomal Mapping. Genomic DNA from 43 human/rodent somatic cell hybrid lines (10) and human, mouse, and hamster controls was digested with *Eco*RI or *Pst* I, electrophoresed, transferred, and hybridized overnight with ^{32}P-labeled human type I scavenger receptor cDNA. A specific DNA sequence for the human scavenger receptor gene was amplified from a panel of human/rodent hybrids by using the polymerase chain reaction (PCR) (11).

Immunohistochemical Studies. Synthetic peptide hSRI-2 and its bovine serum albumin (BSA) conjugate were purchased from Peptide Institute (Osaka, Japan). The amino acid sequence of hSRI-2 corresponds to the C terminus of the collagen-like domain (CRPGNSGPKGQKGEKGSGN in the one-letter code, residues 325–342 with an addition of a cysteine at the N terminus). Antiserum was generated by repeated injections of BSA-coupled peptide emulsified in Freund's complete adjuvant. The serum reacted with the hSRI-2 peptide in an enzyme immunoassay (12) at a dilution of greater than 1:10,000, which is at least 100-fold higher than that of nonimmune sera or sera from animals immunized against other peptides. IgG was purified from the serum by using staphylococcal protein A (13). Human aorta specimens were obtained from four postmortem examinations and from four surgical resections. Frozen sections of these tissues were incubated with antiserum in Dulbecco's phosphate-buffered saline (PBS). The binding of IgG was visualized by using peroxidase-conjugated goat anti-rabbit IgG.

RESULTS

Primary Structure of Human Scavenger Receptors. The nucleotide sequence of the type I human scavenger receptor cDNA (Fig. 1*A*) contains a 1350-base-pair (bp) open reading frame encoding a 451 amino acid protein with 78.8% identity to the bovine receptor type I cDNA at the nucleotide level and 73.5% identity at the amino acid level. The sequence of the type II cDNA (Fig. 1*B*) contains a 1074-bp open reading frame encoding a 358 amino acid protein with 81.0% identity to the bovine type II receptor at the nucleic acid level and 71.3% identity at the amino acid level. Nucleotides −46 to 1033 (amino acids 1–344) of the type II receptor are identical to those of the type I receptor cDNA. The predicted human receptor protein sequence contains six domains identical to those of the bovine receptor: I, N-terminal cytoplasmic (residues 1–50); II, transmembrane (51–76); III, spacer (77–109); IV, α-helical coiled-coil (110–272); V, collagenous (273–341); and VI, C-terminal type specific (342–451 and 342–358 for type I and II, respectively).

The structures of each domain are highly conserved (Fig. 2). The receptor contains a single hydrophobic stretch of 26 amino acids. The N-terminal cytoplasmic domain lacks a signal sequence or obvious functional domain (e.g., tyrosine kinase). There is a potential protein kinase substrate site (14), Arg-Xaa-Xaa-Ser/Thr (residues 26–29), which is conserved in the bovine receptor, whereas the other site found in the bovine receptor (residues 44–47) was not conserved. In the case of the human LDL receptor, aromatic residues (tyrosine, phenylalanine, or tryptophan) in the middle of the cytoplasmic domain are required for rapid endocytosis (15). Two phenylalanine residues (7 and 22) and a tryptophan (residue 4) in the cytoplasmic domain of the scavenger receptors are conserved. A tyrosine (residue 48) in the bovine receptor is another aromatic residue, phenylalanine, in the human receptor. In the extracellular domains, all seven potential N-glycosylation sites are conserved. There is an insertion of asparagine at residue 91 in the spacer domain, suggesting possible structural flexibility of this domain. The α-helical coiled-coil structure and two histidines interrupting

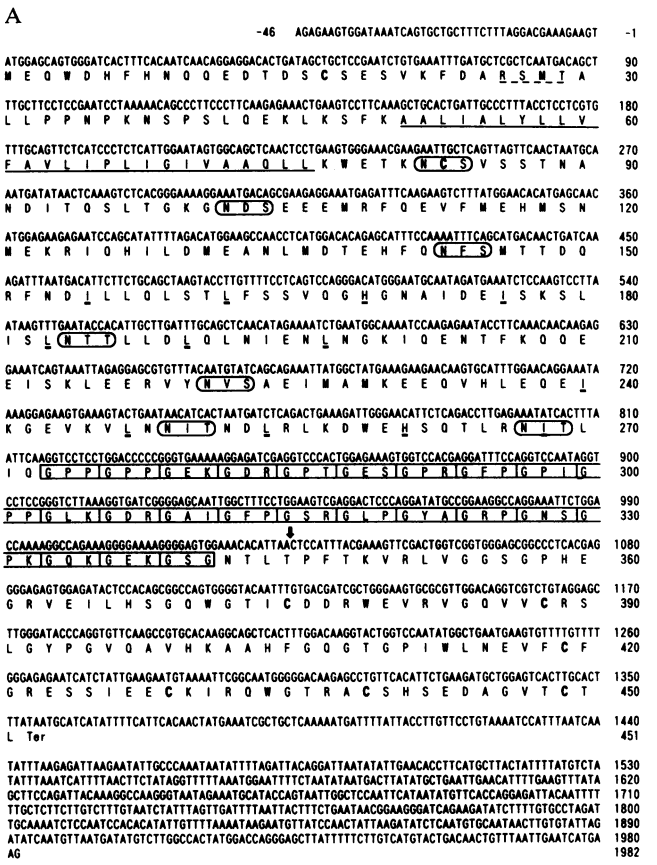

FIG. 1. Nucleotide sequence of the type I (*A*, phSR1) and type II (*B*, phSR2) human scavenger receptors, and the deduced amino acid sequence (single-letter code). The putative transmembrane domain is underlined; the potential N-linked glycosylation sites are enclosed by boxes with round corners; the heptad repeats of leucine and isoleucine residues and interrupting histidine residues are double underlined; and the collagen-like Gly-Xaa-Yaa repeats are indicated by boxes with square corners. The cysteine residues are in bold. Arrows indicate the position where the type-specific nucleotide sequence starts.

the heptad hydrophobic repeats (residues 169 and 261) are also conserved. The collagenous domain of the human scavenger receptor contains 23 Gly-Xaa-Yaa repeats, whereas the bovine receptor contains 24 repeats. Almost all of the Gly-Xaa-Yaa triplets are either uncharged or positively charged at neutral pH except for the sixth triplet, which is negatively charged. In the C-terminal domain of the type I receptor, all six cysteine residues and the length of the gaps between them are completely conserved between the bovine and human receptors. On the other hand, the C-terminal domain of the human type II receptor (17 residues) is longer than that of the bovine receptor (6 residues) and has little sequence identity to the bovine. We generated human scavenger receptor expression vectors (pXhSR1 and pXhSR2) and transfected them into CHO-K1 cells. Both types of transfected cells expressed receptor-mediated uptake of fluorescently labeled acetyl-LDL (data not shown).

Scavenger Receptor mRNA. Northern blot analysis showed expression of the cloned gene in human liver and placenta (Fig. 3*A*, approximately 4- and 3.2-kb bands). In the brain, low levels of these two mRNAs were also detected. The 3.2-kb message was detected by using the 0.7-kb *Xba* I/*Xba*

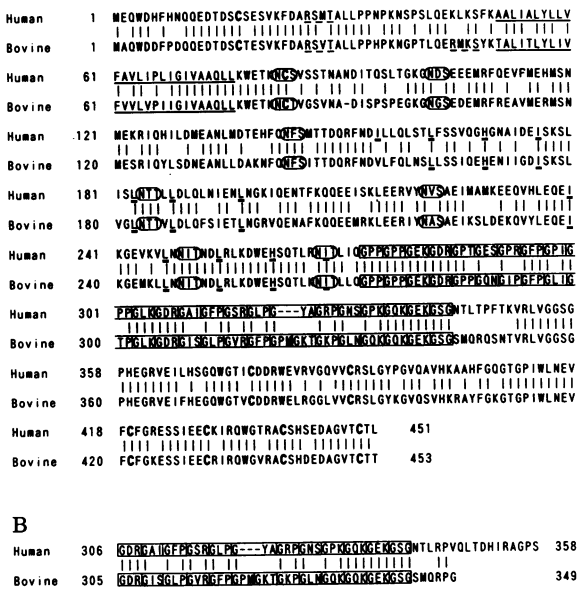

FIG. 2. Alignment of human and bovine type I (*A*) and type II (*B*) scavenger receptor amino acid sequences. Vertical lines indicate identical amino acids. The putative transmembrane domain is underlined; potential N-linked glycosylation sites are enclosed by boxes with round corners; the heptad repeats of leucine and isoleucine residues and interrupting histidine residues are double underlined; and collagen-like Gly-Xaa-Yaa repeats are indicated by boxes with square corners. Cysteine residues are in bold.

I fragment of phSR2, which is a type II specific sequence (Fig. 3*B*).

Chromosomal Localization. By means of a human scavenger receptor cDNA probe we detected human DNA fragments 11.5, 5.8, 4.3, and 2.9 kb long in *Eco*RI-digested DNA from a human/rodent somatic cell hybrid panel. A discordancy analysis of an entire panel of 43 hybrid cell lines indicated that the lowest discordancy values are obtained with respect to chromosome 8 (7.0%). All other chromosomes indicated significantly higher discordancies (higher than 16.3%). The only cell line which did not match this assignment was the HDM4 cell line. Previous characterization of this cell line had suggested that it contains only chromosomes 4 and 20 (10). To confirm these results, we used PCR to amplify a gene-specific DNA fragment from DNA of seven somatic hybrid cell lines containing human chromosome 4, 8, or 20 (Fig. 4). All cell lines except HDM4 that were positive for the scavenger receptor gene contained chromosome 8, and all cell lines that contained either chromosome 4 or 20 but did not contain chromosome 8, except HDM4, were negative from the scavenger receptor gene. These results indicate that the scavenger receptor gene localizes to chromosome 8 and that the cell line HDM4 contains a previously undetected fragment of chromosome 8.

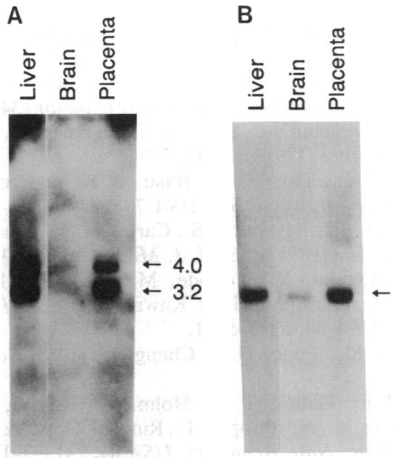

FIG. 3. Northern blot analysis of human scavenger receptor mRNA. Poly(A)$^+$ RNA obtained from human liver, brain, and placenta (each 5 μg) was electrophoresed through a formamide-containing gel, blotted onto a nylon filter, and probed with a random-prime ^{32}P-labeled type I receptor cDNA (*A*) or a type II cDNA specific probe, a 0.7-kb *Xba* I/*Xba* I fragment of phSR2 (*B*), labeled by random priming. The filter was washed at 50°C in 0.5× SSC/0.1% SDS (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7). RNA sizes are given in kilobases (kb).

Presence of Human Scavenger Receptor Protein in Atherosclerotic Lesions. Anti-hSR1-2 immunoreactive protein was detected in cells in the intima of atherosclerotic lesions (Fig. 5*D*), but nonimmune serum cannot recognize these cells (Fig. 5*A*). To identify the cell type in which the scavenger receptors were localized, these lesions were also immunostained with the monocyte/macrophage lineage-specific monoclonal antibody EBM11 (16, 17) (Fig. 5*C*), and these lesions were also stained for lipid contents (Fig. 5*B*). Immunoreactive cells possessing the characteristics of the macrophage were detected in various stages of atheromatous lesions in association with the accumulation of lipids (Fig. 5*B*). The presence of scavenger receptor positive cells was detected in atheromatous lesions of all eight specimens studied, and the immunoreactive cells also had EBM11 antigen. The immunoreactive cells were not detected in the intima without atherosclerotic lesion.

DISCUSSION

We have previously reported (2) that, in the human monocytic leukemia cell line THP-1, a 220-kDa scavenger receptor protein is induced after PMA treatment. Two cDNA clones homologous to the bovine type I and type II cDNAs were cloned from a cDNA library of THP-1 cells treated with PMA. Expression studies in CHO-K1 cells indicated that

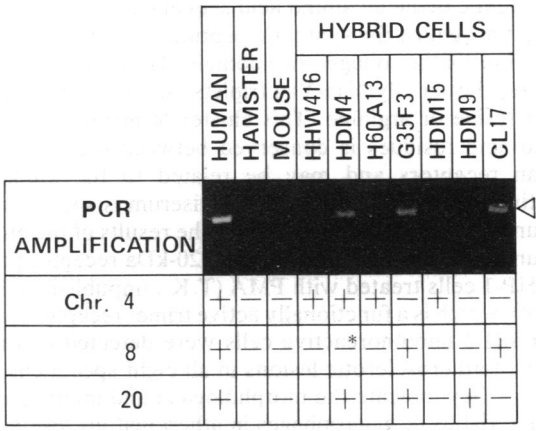

FIG. 4. Confirmation of the localization of the human scavenger receptor gene on chromosome 8 by PCR. Human scavenger receptor gene specific DNA was amplified from genomic DNA of human, hamster, mouse, and seven human/rodent somatic cell hybrid lines containing human chromosome 4, 8, or 20 (10). The sequences of synthetic oligonucleotide primers used were as follows: 5′-CAAAATTTCAGCATGACAACTG-3′ and 3′-CCTTACGTTATC-TACTT-5′. PCR was carried out with 0.2 μg of template DNA. An initial denaturation step, 1.5 min at 94°C, was followed by 35 cycles of 1 min at 94°C (denaturation), 2 min at 46°C (annealing), and 3 min at 72°C. A sample of the amplified DNA was electrophoresed on 1.5% agarose gel and stained with ethidium bromide. The triangle indicates the 102-bp amplified DNA.
*See text for discussion.

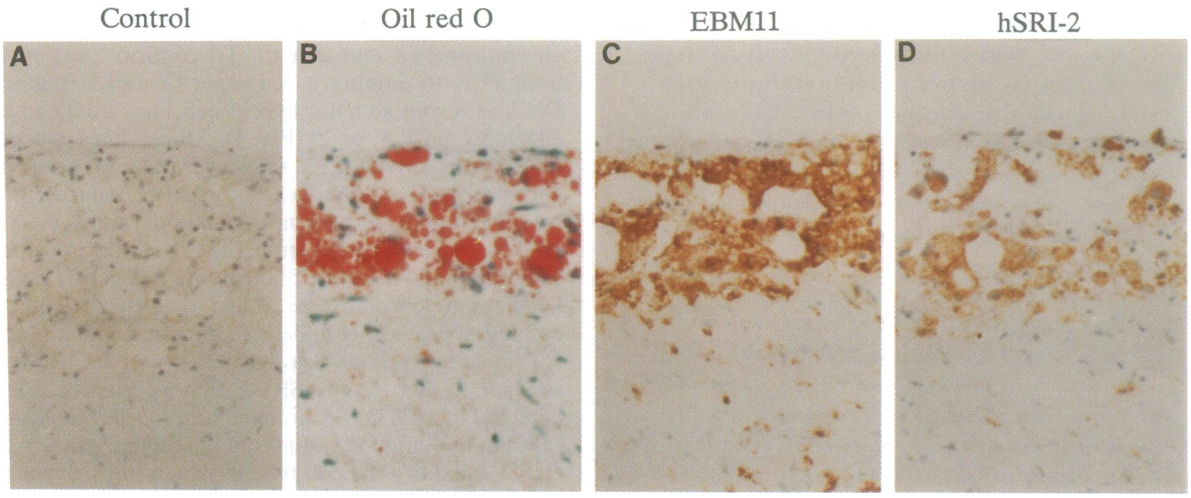

FIG. 5. Immunohistochemical detection of anti-hSRI-2 antigens in the atherosclerotic lesions of human aortic intima. Human aorta was fixed in periodate/lysine/paraformaldehyde fixative, embedded in OCT compound (Miles), and frozen in dry ice/acetone. Frozen sections (6 μm) were cut on a cryostat. The sections were incubated with control rabbit serum, EBM11 (anti-human macrophage monoclonal antibody, Dakopatts no. M718), or anti-hSRI-2. As secondary antibodies, horseradish peroxidase-linked F(ab') fragments of goat anti-mouse immunoglobulin or goat anti-rabbit immunoglobulin (Amersham) were used. After visualization with 3,3'-diaminobenzidine tetrahydrochloride, the nuclei were stained with hematoxylin. Histochemical demonstration of lipids was by oil-red O staining. (*A*) Immunostaining of the atherosclerotic lesion with nonimmune serum (control). (*B*) Lipid substances stained red in the atherosclerotic lesion. (*C*) Brown reaction products for EBM11 observed in lipid-laden macrophages in the atherosclerotic intima. (*D*) Reaction products for anti-hSRI-2 also localized in the lipid-laden macrophages.

both type I and type II human clones were functionally active for the uptake of modified lipoproteins. Comparison of the human and bovine sequences indicated that most of the structural characteristics of the bovine receptor are conserved in the human receptor, except for domain VI of the type II receptor. The coiled-coil fibrous domains (IV and V) which apparently mediate the ligand binding of the scavenger receptors (3, 4) are well conserved. The short C-terminal domain VI of the type II human receptor is quite different from that of the bovine receptor, but the difference did not prevent the receptor-mediated uptake of acetyl-LDL.

Using the deduced amino acid sequence, we generated an antiserum against the synthetic peptide corresponding to the C terminus of the collagen-like domain. The peptide sequence includes the six Gly-Xaa-Yaa triplets, and four of them have either lysine or arginine. This cluster of positively charged amino acid residues is conserved between the bovine and human receptors and may be related to the binding of negatively charged ligands. This antiserum is specific to the immunized peptide, as judged from the results of the enzyme immunoassay, and can bind to the 220-kDa receptor protein on THP-1 cells treated with PMA (T.K., unpublished observation), which is a functionally active trimer receptor (2). The anti-hSRI-2 immunoreactive cells were detected in various stages of atherosclerotic lesions in all eight specimens studied, and they indicate the morphological and immunological characteristics of macrophages in atheromatous lesions. We could not find significant immunoreactivity in cell types other than macrophages. The immunoreactive cells were detected in the lipid-rich lesions. These results strongly support the hypothesis of the involvement of the scavenger receptor in the formation of foam cells and in the development of atherosclerotic lesions in human subjects (1).

The scavenger receptor mRNA was detected in various human tissues, including the liver, lung, and placenta. In addition to these organs, previously known to possess scavenger receptors (2, 3, 18), the mRNA was also detected in the brain. Two mRNAs (4 and 3.2 kb) were detected in these organs, and 3.2-kb mRNA represents the type II mRNA. The scavenger receptor gene is located on chromosome 8. Independent chromosome mapping of the murine receptor confirms this assignment (19).

The molecular cloning of human scavenger receptor cDNAs enables deduction of the primary structure, generation of the specific antibody, and determination of the chromosomal localization. Further studies concerning their physiological role, pathological involvement, genetic polymorphism, and relationship to atherosclerotic disease are important for the understanding of the pathogenesis of atherosclerosis.

We thank Drs. M. Krieger, M. Freeman, L. Rohrer, and L. Hobbie for helpful advice, Drs. R. Sadahiro and K. Boru for reviewing the manuscript, Drs. Y. Uetsuka and S. Hosoda for the arterial wall specimens, and Dr. J. David Brook for hybrid cell DNAs.

1. Brown, M. S. & Goldstein, J. L. (1983) *Annu. Rev. Biochem.* **52,** 223–261.
2. Kodama, T., Reddy, P., Kishimoto, C. & Krieger, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9238–9242.
3. Kodama, T., Freeman, M., Rohrer, L., Zabrecky, J., Matsudaira, P. & Krieger, M. (1990) *Nature (London)* **343,** 531–535.
4. Rohrer, L., Freeman, M., Kodama, T., Penman, M. & Krieger, M. (1990) *Nature (London)* **343,** 570–572.
5. Goldstein, J. L., Ho, Y. K., Basu, S. K. & Brown, M. S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 333–337.
6. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witztum, J. L. (1989) *N. Engl. J. Med.* **320,** 915–924.
7. Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H. & Kawai, C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5928–5931.
8. Harberland, M., Fong, D. & Cheng, L. (1988) *Science* **241,** 215–218.
9. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. & Danielsen, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7413–7417.
10. Lugo, T. G., Handelin, B., Killary, A. M., Housman, D. E. & Fournier, R. E. (1987) *Mol. Cell. Biol.* **7,** 2814–2820.
11. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, M. A. (1988) *Science* **239,** 487–491.
12. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 564–565.
13. Johnstone, A. & Thorpe, R. (1982) in *Immunochemistry in Practice* (Blackwell, Oxford), pp. 202–232.

14. Edelman, A. M., Blumenthal, D. K. & Krebs, E. G. (1987) *Annu. Rev. Biochem.* **56,** 567–613.
15. Davis, C. G., van Driel, I. R., Russell, D. W., Brown, M. S. & Goldstein, J. L. (1987) *J. Biol. Chem.* **262,** 4075–4082.
16. Franklin, W. A., Mason, D. Y., Pulford, K., Falini, B., Bliss, E., Gatter, K. C., Stein, H., Clarke, L. C. & McGee, J. O. (1986) *Lab. Invest.* **54,** 322–335.
17. Bielefeldt-Ohmann, H., Sabara, M., Lawman, M. J. P., Griebel, P. & Babiuk, L. A. (1988) *J. Immunol.* **140,** 2201–2209.
18. Rebourcet, R., Alsat, E., Mondon, F., Berthelier, M., Pastier, D., Cedard, L., Goldstein, S. & Polonovski, J. (1986) *Biochimie* **68,** 1135–1140.
19. Freeman, M., Ashkemas, J., Rees, D. J. G., Kingsley, D. M., Copeland, N. G., Jenkins, N. A. & Krieger, M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8810–8814.