Expression of α_2-Macroglobulin Receptor/Low Density Lipoprotein Receptor-related Protein and Scavenger Receptor in Human Atherosclerotic Lesions

Jukka Luoma, Timo Hiltunen, Terttu Särkioja,* Søren K. Moestrup,‡ Jørgen Gliemann,‡ Tatsuhiko Kodama,§ Tapio Nikkari, and Seppo Ylä-Herttuala

*Department of Biomedical Sciences, University of Tampere, FIN-33101 Tampere, Finland; *Department of Forensic Sciences, University of Oulu, FIN-90220 Oulu, Finland; ‡Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus, Denmark; and §The Third Department of Internal Medicine, University of Tokyo, Tokyo 113, Japan*

Abstract

Macrophage- and smooth muscle cell (SMC)-derived foam cells are typical constituents of human atherosclerotic lesions. At least three receptor systems have been characterized that could be involved in the development of foam cells: α_2-macroglobulin receptor/LDL receptor-related protein (α_2MR/LRP), scavenger receptor, and LDL receptor.

We studied the expression of these receptors in human atherosclerotic lesions with in situ hybridization and immunocytochemistry. An abundant expression of α_2MR/LRP mRNA and protein was found in SMC and macrophages in both early and advanced lesions in human aortas. α_2MR/LRP was also present in SMC in normal aortas. Scavenger receptor mRNA and protein were expressed in lesion macrophages but no expression was found in lesion SMC. LDL receptor was absent from the lesion area but was expressed in some aortas in medial SMC located near the adventitial border. The results demonstrate that (*a*) α_2MR/LRP is, so far, the only lipoprotein receptor expressed in lesion SMC in vivo; (*b*) scavenger receptors are expressed only in lesion macrophages; and (*c*) both receptors may play important roles in the development of human atherosclerotic lesions. (*J. Clin. Invest.* 1994. 93:2014–2021.) Key words: apolipoprotein E · low density lipoprotein receptor · macrophages · smooth muscle cells · foam cells

Introduction

Human atherosclerotic lesions are characterized by the presence of lipid-loaded foam cells which are derived from monocyte/macrophages and smooth muscle cells (SMC)[1] (1). Most of the lipid that accumulates in the foam cells is derived from plasma lipoproteins (1). Since native LDL does not cause foam cell formation in vitro, it has been proposed that plasma lipoproteins must first undergo some kind of modification before they can be metabolized in the arterial wall cells (2,3). One of the modifications that has been implicated in the foam cell formation is oxidation of LDL (3).

Oxidized LDL could lead to the formation of macrophage-derived foam cells since it can be metabolized through macrophage scavenger receptors (3,4). Scavenger receptors are trimeric membrane proteins that exist in two closely related forms (types I and II receptors) which arise from alternative splicing of a single gene (4). Scavenger receptors bind several ligands (4) and are expressed in human lesion macrophages (5–7). Human lesion SMC do not express detectable amounts of scavenger receptors (6,7) or LDL receptors (6). Thus, the mechanism(s) by which lesion SMC take up lipoproteins is less well characterized than that of macrophages and may involve receptor-mediated endocytosis and other processes such as phagocytosis.

α_2-Macroglobulin receptor/LDL receptor-related protein (α_2MR/LRP) is a 600-kD multifunctional endocytotic receptor which belongs to the LDL receptor gene family (8–10). After synthesis, α_2MR/LRP is cleaved into 515-(α chain) and 85-kD (β chain) subunits (11). It is expressed by a number of different human cell types including macrophages and SMC of various tissues (12). α_2MR/LRP is essential for normal embryogenesis since the disruption of the α_2MR/LRP gene in mice blocks the development of embryos (13).

The α chain contains several repeated sequences, e.g., cysteine-rich complement-type repeats homologous with the ligand binding repeats of the LDL receptor (8), and binds several classes of ligands to separate or overlapping sites. The ligands include α_2-macroglobulin (9,10,14) and the homologous pregnancy zone proteins (15), which achieve receptor-active conformations when complexed with target proteases, plasminogen activator inhibitor type 1 complexed with urokinase-type (16) or tissue-type plasminogen activators (17), and *Pseudomonas* exotoxin A (18). α_2-macroglobulin–protease and plasminogen activator inhibitor–plasminogen activator complexes bind to separate sites in the region of α_2MR/LRP α chain containing eight complement-type repeats (19). Cytokines such as TGF-β bind to the receptor-active form of α_2-macroglobulin, and the α_2-macroglobulin–TGF-β complex is still capable of binding to α_2MR/LRP (20). Recent results also demonstrate that receptor-active α_2-macroglobulin markedly enhances the TGF-β–induced growth response of cultured rat aortic SMC and that this effect is dependent upon the binding of the complex to α_2MR/LRP (21).

α_2MR/LRP can also bind apo E–enriched chylomicron and VLDL remnants (22,23), lipoprotein lipase (LPL) (24), and LPL–triglyceride-rich lipoprotein complexes (25,26). Thus, it is possible that α_2MR/LRP may play a role in the uptake of lipoproteins in the arterial wall cells. However, there is no data about the expression of α_2MR/LRP in human atherosclerotic lesions. In this paper we have studied the expression of

Address correspondence to Seppo Ylä-Herttuala, M.D., Department of Biomedical Sciences, University of Tampere, P.O. Box 607, FIN-33101 Tampere, Finland.

Received for publication 12 October 1993 and in revised form 24 December 1993.

1. *Abbreviations used in this paper:* LPL, lipoprotein lipase; α_2MR/LRP, α_2-macroglobulin receptor/LDL receptor-related protein; SMC, smooth muscle cells.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/94/05/2014/08 $2.00
Volume 93, May 1994, 2014–2021

α_2MR/LRP, scavenger receptors, and LDL receptor in human arteries. It was found that α_2MR/LRP was actively expressed in lesion SMC and macrophages. Scavenger receptors were expressed in lesion macrophages, but no expression of LDL receptor was found in the lesions. The results suggest that α_2MR/LRP and scavenger receptors may play important roles in the development of atherosclerotic lesions.

Methods

Tissue samples. Human aortic samples were obtained from nine medicolegal autopsies (six males, three females, age 29–79 yr) 4–13 h postmortem. Adrenal cortex was obtained from an organ donor for a renal transplant immediately after the surgery. Tissue samples were embedded immediately in OCT compound (Miles Laboratories, Inc., Elkhart, IN), frozen in liquid nitrogen, and kept at -70°C until used for in situ hybridization and immunocytochemistry. We cannot exclude the possibility of postmortem changes in the tissue samples. However, in previous studies no major differences have been found in tissue samples obtained at similar postmortem intervals as compared with results obtained from organ donors or from perfusion-fixed animals (6,27,28). All procedures were approved by the Ethical Committee of the Tampere University Hospital.

In situ hybridization. In situ hybridizations were done using either oligonucleotide probes or riboprobes as described (6,27,28). Oligonucleotide probes Ol47 (47 mer) and Ol49 (49 mer) were designed to recognize two unrelated sequences of human α_2MR/LRP mRNA (nucleotides 2705–2751 and 1184–1232, respectively). Control oligonucleotides were of the same size and contained the same sequence but in an opposite direction. Oligonucleotides were synthesized by Biotechnical Institute (Helsinki, Finland). The homologies of Ol47 and Ol49 sequences with human LDL receptor (29), GP 330 (30), and VLDL receptor (31) were only 43–51%, which makes the detection of mRNA of the other members of the LDL receptor gene family unlikely. Oligonucleotides were end-labeled with ^{35}S-ATP (1,000–1,500 Ci/mmol)(New England Nuclear, Boston, MA) using terminal transferase (Amersham Intl., Buckinghamshire, UK) (32).

For riboprobe synthesis, human LDL-receptor cDNA (29) and bovine type I scavenger receptor cDNA (4) were subcloned in plasmid vectors using standard techniques (32). Human type I scavenger receptor (5) shows a 79% similarity with bovine type I scavenger receptor, which has been used previously for in situ hybridization studies with human tissue (6). Antisense and sense riboprobes were synthesized using Sp6, T7, or T3 RNA polymerases with ^{35}S-UTP (1,200 Ci/mmol)(New England Nuclear) as described (6). All reagents used for riboprobe synthesis were from Promega Biotec (Madison, WI).

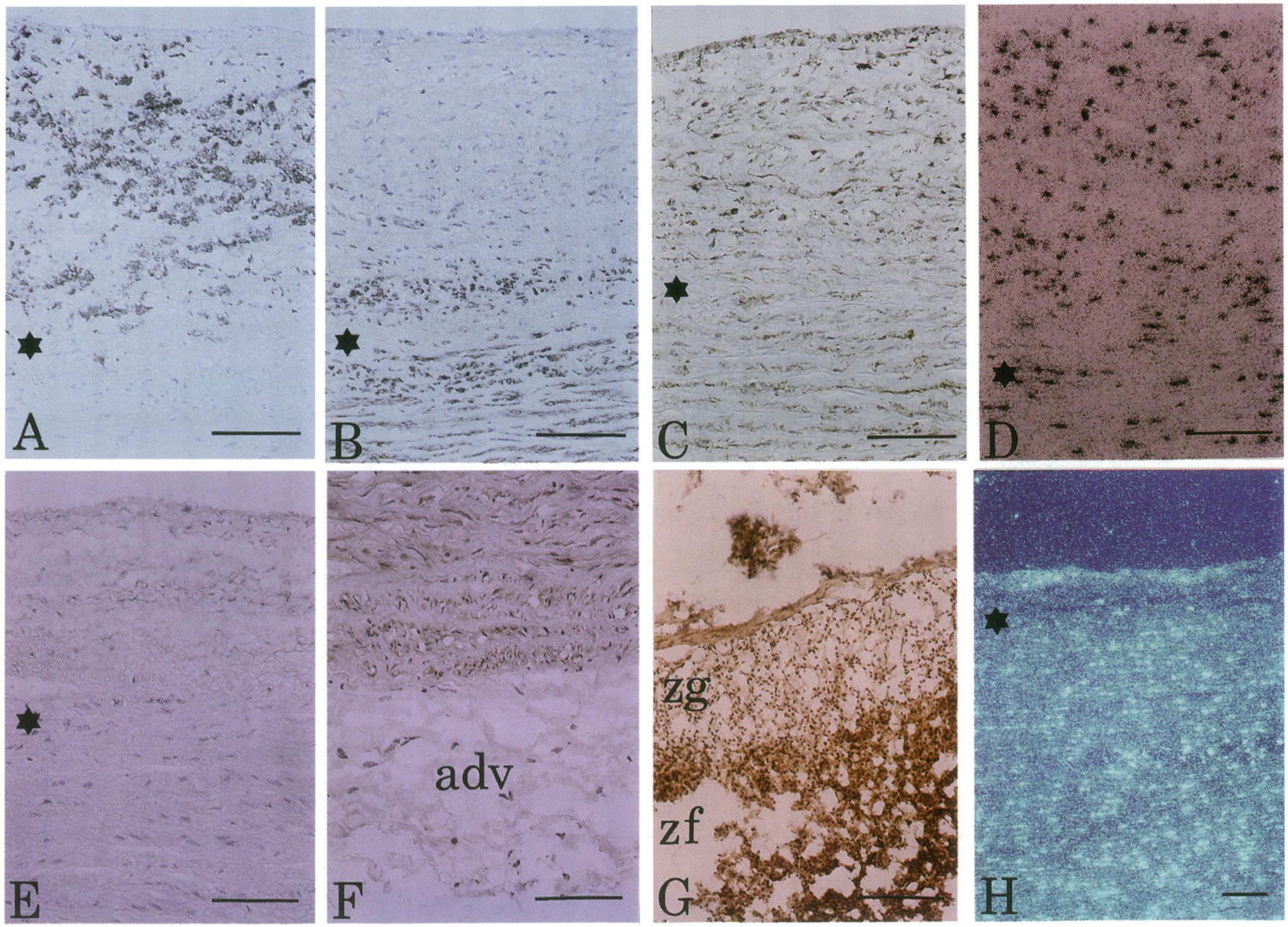
In situ hybridization studies were done on a set of serial sections (5–10 μm) (6,27,28). Briefly, cryostat sections were fixed with formaldehyde/sucrose for 10 min (4% paraformaldehyde, 5% sucrose, 1 mM EDTA, 50 μM butylated hydroxytoluene, pH 7.4), dehydrated, and dried in vacuo. Sections were hybridized for 14 h with 6×10^6 cpm/ml of each probe in a hybridization solution containing 50% formamide (Fluka Chemie AG, Buchs, Switzerland), 2×SSC, 20 mM Tris (pH 7.4) 1×Denhardt's solution, 1 mM EDTA, 10% dextran sulfate (Pharmacia LKB Biotechnology, Sweden), 1 mM DTT, and 0.5 mg/ml yeast transfer RNA (Boehringer Mannheim GmbH, Mannheim, Germany). Hybridization temperatures were 42 and 53°C for oligonucleotide probes and riboprobes, respectively. The final wash after the hybridizations was at 52°C in 1×SSC for oligonucleotide probes and at 55°C in 0.1×SSC for riboprobes. Slides were then dehydrated, dried, dipped in a photographic emulsion (NTB-2; Eastman-Kodak Co., Rochester, NY), and developed after a 2–10-wk exposure time. After development, the slides were counterstained with hematoxylin-eosin. Controls for the in situ hybridizations included incubations with control oligonucleotides or sense riboprobes. In addition, RNAseA-pretreated sections were used as controls during the optimization of the in situ hybridization protocols.

Immunocytochemistry. Serial cryostat sections (5–10 μm) were used for immunostainings. Immunocytochemistry studies were done using two mAbs ($A_2MR\alpha$-1 and $A_2MR\alpha$-5) against human α_2-macroglobulin receptor α chain and one mAb ($A_2MR\beta$-1) against β chain (33). These three antibodies react with distinct epitopes of α_2-macroglobulin receptor α and β chains (19). $A_2MR\alpha$-1, $A_2MR\alpha$-5, and $A_2MR\beta$-1 antibodies do not cross-react with LDL receptor or GP 330 (12,14,33). Rabbit antiserum (hSRI-2) against synthetic human scavenger receptor peptides was used for the scavenger receptor immunostaining (7). The antiserum recognizes both types I and II receptors (7). We also used mouse mAbs specific for bovine and human LDL receptor (clone C7/RPN 537; Amersham Intl), human macrophages (HAM-56) (34), SMC (HHF-35) (35), apo B–100 (MB-47) (36), and apo E (clone 2E1; Boehringer Mannheim GmbH). Avidin–biotin–horseradish peroxidase system (Vector Laboratories, Burlingame, CA) was used for the immunostainings (37). After the immunostainings the slides were counterstained with hematoxylin. Controls for the immunostainings included sections incubated with irrelevant class- and species-matched immunoglobulins and incubations where the first antibody was omitted.

Results

Human fatty streaks contain both macrophages and SMC. A typical human fatty streak is shown in Fig. 1. Macrophages occupied the upper part of the lesion (Fig. 1 *A*), whereas SMC were located mostly in the deeper part of the lesion (Fig. 1 *B*). Immunostaining with a monoclonal $A_2MR\alpha$-1 antibody revealed an abundant presence of α_2MR/LRP protein in both macrophage- and SMC-containing areas (Fig. 1 *C*). In situ hybridization with Ol49 oligonucleotide probe showed a similar expression pattern for α_2MR/LRP mRNA (Fig. 1 *D*). Immunostainings with $A_2MR\alpha$-5 and $A_2MR\beta$-1 mAbs and in situ hybridization with Ol47 oligonucleotide probe for α_2MR/LRP gave similar results, whereas control immunostainings with irrelevant class- and species-matched immunoglobulins and in situ hybridizations with control oligonucleotide probes were negative (data not shown). No immunostaining was found for LDL receptor protein in the lesion area (Fig. 1 *E*). However, medial SMC (identified by a positive HHF-35 immunostaining) located close to the adventitial border were positive for LDL receptor protein (Fig. 1 *F*). Positive immunostaining for LDL receptor protein in this location was found in two out of nine aortas studied. Human adrenal cortex was used as a positive control for LDL receptor immunostaining. LDL receptors have been shown to be densely expressed in zona fasciculata and to be absent or expressed at much lower level in zona glomerulosa (6, 38, 39). As expected, zona fasciculata stained intensely positive for LDL receptor protein, whereas zona glomerulosa showed no or very little specific immunostaining (Fig. 1 *G*). α_2MR/LRP mRNA and protein were also expressed by SMC in macroscopically normal aortas, as shown in Fig. 1 *H*. Similar results were obtained with immunostaining (data not shown).

A representative micrograph of an advanced human atherosclerotic lesion is shown in Fig. 2. The lesion contains both SMC (Fig. 2 A) and macrophages (Fig. 2 *B*). The lesion stained positive with all three antibodies against α_2MR/LRP. Fig. 2, *C* and *D* show immunostainings for α_2MR/LRP protein with antibodies $A_2MR\alpha$-1 and $A_2MR\beta$-1, respectively. In situ hybridizations for α_2MR/LRP mRNA with end-labeled oligonucleotide probes gave similar results (data not shown). An abundant presence of apo B–100 (LDL) was detected in the same area that contained α_2MR/LRP (Fig. 2 *E*). Apo E was also present,


A
B
C
D
E
F
adv
zg
zf
G
H

but it showed a less intense staining than apo B–100, and was located mostly in macrophage-rich areas (Fig. 2 *F*). Control immunostainings with irrelevant class- and species-matched immunoglobulins and in situ hybridizations with control oligonucleotide probes were negative (data not shown).

Scavenger receptor mRNA and protein were detected by immunostaining and in situ hybridization in lesion macrophages in the same areas that contained α_2MR/LRP. An example of macrophages expressing scavenger receptors is shown in Fig. 3. Macrophages (Fig. 3 *A*), scavenger receptor protein (Fig. 3 *B*), and scavenger receptor mRNA (Fig. 3 *C*) colocalize in the same area that also stains positive for α_2MR/LRP protein (Fig. 3 *D*) and negative for LDL receptor protein (Fig. 3 *E*). Controls for immunostainings (Fig. 3 *F*) and in situ hybridizations with sense riboprobes (data not shown) were negative. No expression of the scavenger receptor mRNA or protein was seen in arterial SMC in this lesion or in any other lesion studied.

Discussion

The first visible atherosclerotic lesions are fatty streaks, which consist of lipid-loaded macrophages and SMC (1). At least three receptor systems have been characterized that could mediate the uptake of lipoproteins by macrophages and SMC: α_2MR/LRP (8–10), scavenger receptors (2,4), and LDL receptor (2,29). In addition, other receptors (40) and processes, such as phagocytosis, may be involved in the uptake of lipids by arterial wall cells (1–3).

For several reasons, α_2MR/LRP could contribute to the formation of foam cells: (*a*) the present results show that α_2MR/LRP is expressed in lesion macrophages and SMC; (*b*) α_2MR/LRP can bind and internalize apo E–enriched remnant lipoproteins (22,23) and LPL–triglyceride-rich lipoprotein complexes (25,26); (*c*) α_2MR/LRP is not downregulated by an increased cellular cholesterol content (23), which could allow the formation of foam cells; (*d*) human aortic intima contains as much as 5–15% of the total lipoprotein particles as the VLDL and intermediate density lipoproteins (41). These remnantlike particles also contain significant quantities of apo E (41) and could be potential ligands for α_2MR/LRP; and (*e*) apo E and LPL can mediate the binding to α_2MR/LRP of β-VLDL and triglyceride-rich lipoproteins. Both apo E and LPL are present in lesions (28,42–44) and can affect cellular lipoprotein metabolism (45). As expected, apo B–100 (LDL) and apo E were present in the lesions, but apo E tended to have a more localized staining pattern than apo B–100 (43,44,46).

Scavenger receptors are likely to be involved in the uptake of modified forms of LDL by macrophages (2–4). These receptors are not downregulated by increased cellular cholesterol content and can lead to foam cell formation in vitro (47). The present results confirm earlier reports that scavenger receptors are expressed in human lesion macrophages (5–7) and extend the findings by showing that macrophages in the same areas express α_2MR/LRP as well. No expression of the scavenger receptors was detected in lesion SMC.

LDL receptor activity in the liver plays an important role in the regulation of plasma LDL levels (2). In vitro, LDL receptor is downregulated effectively by an increased cellular cholesterol content and does not lead to foam cell formation (2). No expression of LDL receptor protein was found in the lesion area, which confirms previous observations (6). However, in two out of nine aortas, LDL receptor expression was seen in medial SMC located near the adventitial border, but this expression was unrelated to lesion development.

As was mentioned above, α_2MR/LRP and scavenger receptors are not downregulated by an increased cellular cholesterol content. Since both receptors bind multiple ligands, including native and/or modified lipoproteins, they are attractive candidates for the uptake mechanisms responsible for foam cell formation. The regulation of α_2MR/LRP and scavenger receptor expression is not fully understood, but both α_2MR/LRP and scavenger receptors are downregulated by lipopolysaccharide and interferon-γ (48) and upregulated during monocyte differentiation into macrophages (12,49). Colony-stimulating factors, which are expressed in lesions (50,51), also increase the expression of both receptors (52,53).

α_2MR/LRP mRNA and protein were expressed in every lesion studied. Whether the level of the α_2MR/LRP expression was increased or decreased in lesion cells as compared with normal artery cannot be answered reliably by the current studies. However, in some macrophage-rich areas of the lesions, the level of α_2MR/LRP immunostaining seemed to be somewhat decreased, whereas SMC expression appeared to be more constant. Interferon-γ is secreted by lesion T cells (54) and could account for the variability of the α_2MR/LRP immunostaining in lesion macrophages. The expression of scavenger receptors in only a subpopulation of lesion macrophages (6) also points to the importance of local regulation in the lesion microenvironment by cytokines and other similar substances (55). Binding and internalization of various ligands by α_2MR/LRP, including apo E/β-VLDL and LPL/β-VLDL, can also be regulated by a 39–40-kD receptor-associated protein (14,26,56), but there is no data about the expression of this protein in human atherosclerotic lesions.

Our results show that α_2MR/LRP is expressed by SMC and macrophages in human atherosclerotic lesions, whereas scaven-

Figure 1. Expression of α_2MR/LRP protein and mRNA in macrophages and SMC in a human fatty streak (thoracic aorta of a 41-yr-old male, postmortem time 4 h). Immunostaining and in situ hybridization autoradiography of serial sections (*A–E*): (*A*) Antibody specific for human macrophages (HAM-56, dilution 1:500); (*B*) Antibody specific for SMC (HHF-35, dilution 1:500); (*C*) Antibody specific for the α chain of α_2MR/LRP (A_2MRα-1, dilution 1:50); (*D*) In situ hybridization with a ^{35}S-ATP–labeled oligonucleotide probe Ol49 for α_2MR/LRP; (*E*) Antibody specific for human LDL receptor (clone C7, dilution 1:100). (*F–G*) Immunostaining with the same antibody as in *E*. (*F*) Adventitia (*adv*) and one-fourth of the media at the adventitial side of the same tissue sample as shown in *E*; (*G*) Adrenal cortex showing an intense positive immunostaining for LDL receptor protein in zona fasciculata (*zf*) whereas zona glomerulosa (*zg*) contained no or very little specific staining; (*H*) In situ hybridization with a ^{35}S-ATP–labeled oligonucleotide probe Ol47 for α_2MR/LRP in a macroscopically normal aorta containing diffuse intimal thickening (thoracic aorta of a 44-yr-old male, postmortem time 11 h). Controls for immunostainings and in situ hybridizations were negative (data not shown). Hematoxylin counter stain, except in *D* and *H*, which were counterstained with hematoxylin and eosin. A star marks the boundary between intima and media. *H* was photographed using polarized light epiluminescence. ×200 (A–G), ×100 (*H*). Bars = 100 μm.

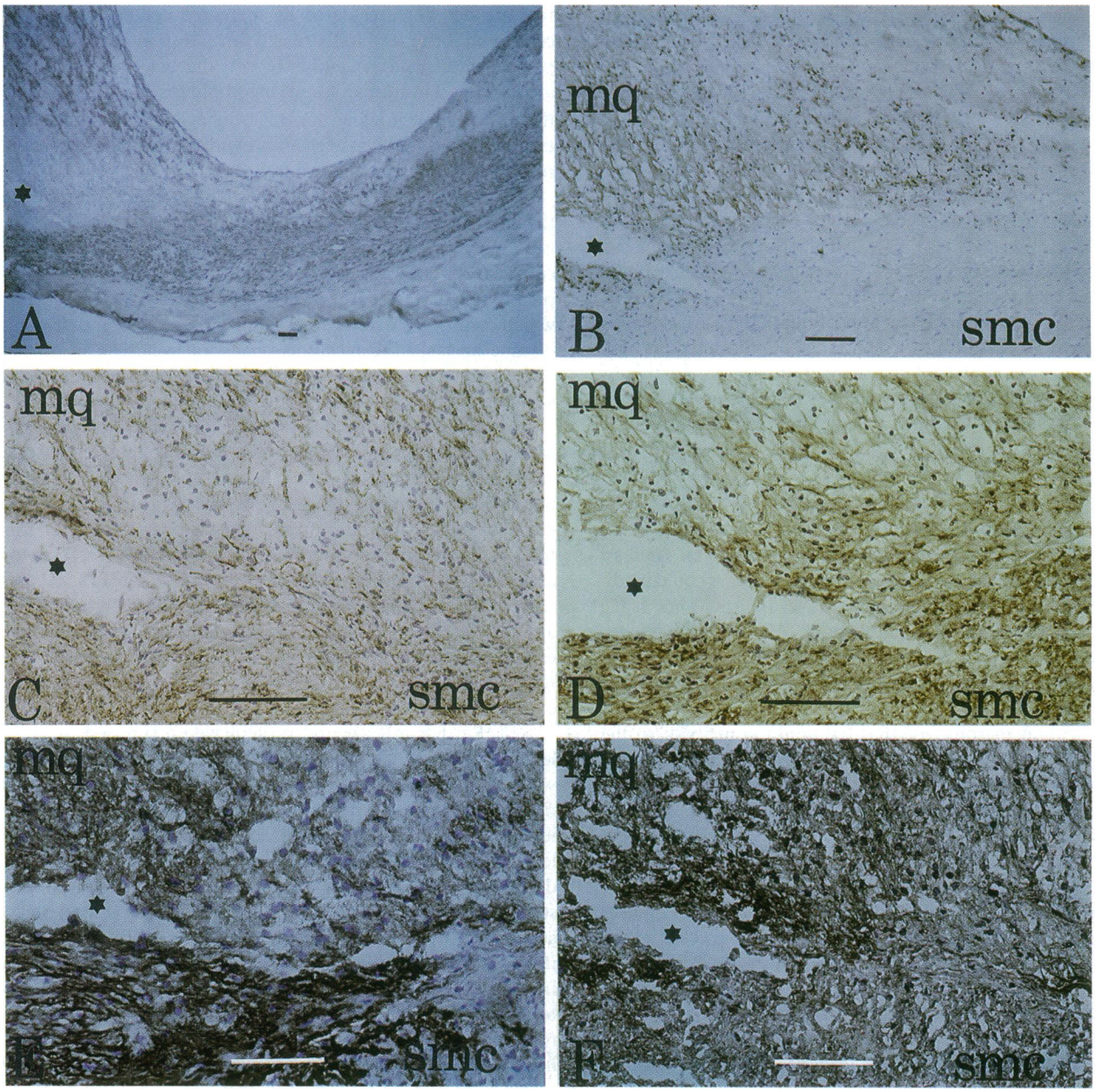


Figure 2. Expression of α_2MR/LRP protein in macrophages (mq) and SMC (smc) in an advanced human atherosclerotic lesion (abdominal aorta of a 44-yr-old male, postmortem time 11 h). Immunostaining of serial sections (A–F): (*A*) Antibody specific for SMC (HHF-35, dilution 1:500); (*B*) Antibody specific for human macrophages (HAM-56, dilution 1:500). (C–F) Higher magnification of the immunostainings of serial sections. (*C*) Antibody specific for the α chain of α_2MR/LRP (A_2MRα-1, dilution 1:50); (*D*) Antibody specific for the β chain of α_2MR/LRP (A_2MRβ-1, dilution 1:50); (*E*) Antibody specific for apo B–100 (MB-47, dilution 1:500); (*F*) Antibody specific for apo E (clone 2E1; dilution 1:100). A star identifies the same location in A–F. Controls for immunostainings were negative (data not shown). Hematoxylin counterstain. ×40 (*A*), ×100 (*B*), ×200 (*C–F*). Bars = 100 μm.

ger receptors are only expressed in lesion macrophages. Both receptors could contribute to the formation of macrophage-derived foam cells. α_2MR/LRP is, so far, the only receptor expressed in arterial SMC in vivo that is capable of mediating the uptake of lipoproteins. Thus, it may play an important role in the formation of SMC-derived foam cells. Further studies are needed to establish the role of α_2MR/LRP as a functional lipoprotein receptor in arterial cells and to clarify the regulation of the expression of α_2MR/LRP and scavenger receptors in lesion cells. Better understanding of the functions of these receptors could give further insights into the development of foam cells and could lead to new therapeutic approaches for the prevention of atherosclerotic lesion formation in human arteries.

Acknowledgments

The authors thank Dr. Joseph L. Witztum (University of California, San Diego) for MB-47 antibodies; Dr. Allen Gown (University of Wash-

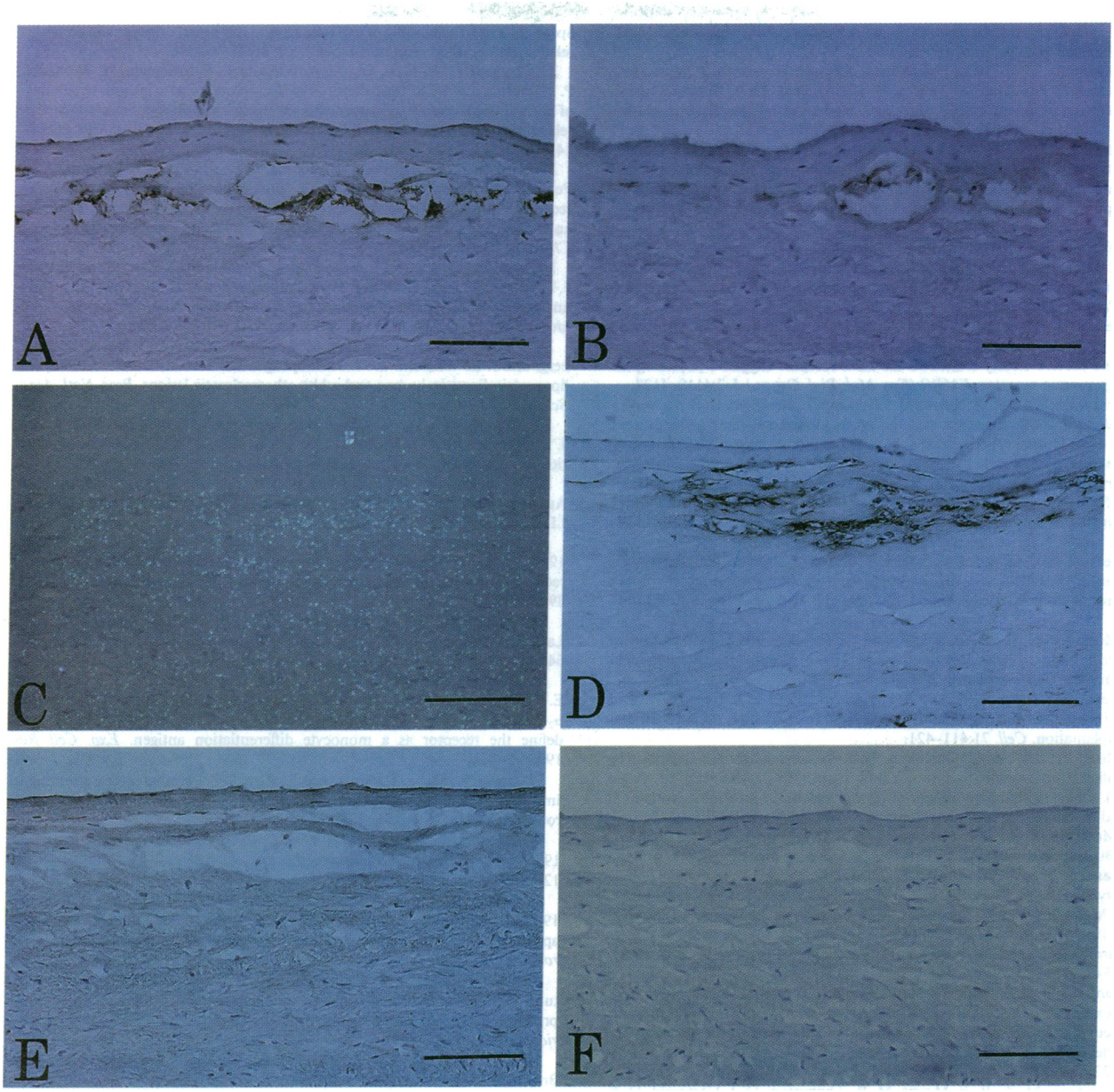


Figure 3. Expression of scavenger receptors and α_2MR/LRP in macrophages in an extracellular matrix–rich area of a fibrous lesion (abdominal aorta of a 51-yr-old female, postmortem time 13 h). Immunostaining and in situ hybridization autoradiography of serial sections (*A–F*): (*A*) Antibody specific for human macrophages (HAM-56, dilution 1:500); (*B*) Antibody specific for human scavenger receptor types I and II (hSRI-2, dilution 1:500); (*C*) In situ hybridization with a ^{35}S-UTP–labeled antisense riboprobe for types I and II scavenger receptors; (*D*) Antibody specific for the β chain of α_2MR/LRP (A_2MRβ-1, dilution 1:50); (*E*) Antibody specific for LDL receptor shows no positive reaction (clone C7, dilution 1:100). (*F*) Control for immunostaining (primary antibody replaced with an irrelevant mouse IgG). Control in situ hybridizations with sense riboprobes were negative (data not shown). Hematoxylin counterstain. *C* was photographed using polarized light epiluminescence. ×200 (*A–F*). Bars = 100 μm.

ington, Seattle) for HAM-56 and HHF-35 antibodies; Dr. Hiroshi Suzuki (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) for help in producing hSRI-2 antibodies; Dr. Markku Pelto-Huikko for help in labeling oligonucleotides; Ms. Marja Jousimies for skillful technical assistance, and Ms. Eija Kyrölä for preparing the manuscript.

This study was supported by grants from the Academy of Finland, The Elli and Elvi Oksanen Fund of the Pirkanmaa Regional Fund under the auspices of the Finnish Cultural Foundation, the Sigrid Jusélius Foundation, the Finnish Heart Foundation, and the Danish Medical Research Council.

References

1. Ross, R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* (Lond.). 362:801–809.

2. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* 52:223–261.

3. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 320:915–924.

4. Kodama, T., M. Freeman, L. Rohrer, J. Zabrecky, P. Matsudaira, and M.

Krieger. 1990. Type I macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils. *Nature (Lond.).* 343:531–535.

5. Matsumoto, A., M. Naito, H. Itakura, S. Ikemoto, H. Asaoka, I. Hayakawa, H. Kanamori, H. Aburatani, F. Tataku, H. Suzuki, et al. 1990. Human macrophage scavenger receptors: primary structure, expression, and localization in atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* 87:9133–9137.

6. Ylä-Herttuala, S., M. E. Rosenfeld, S. Parthasarathy, E. Sigal, T. Särkioja, J. L. Witztum, and D. Steinberg. 1991. Gene expression in macrophage-rich human atherosclerotic lesions. 15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J. Clin. Invest.* 87:1146–1152.

7. Naito, M., H. Suzuki, T. Mori, A. Matsumoto, T. Kodama, and K. Takhashi. 1992. Coexpression of type I and type II human macrophage scavenger receptors in macrophages of various organs and foam cells in atherosclerotic lesions. *Am. J. Pathol.* 141:591–599.

8. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, and K. K. Stanley. 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:4119–4127.

9. Kristensen, T., S. K. Moestrup, J. Gliemann, L. Bendtsen, O. Sand, and L. Sottrup-Jensen. 1990. Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the α_2-macroglobulin receptor. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 276:151–155.

10. Strickland, D. K., J. D. Ashcom, S. Williams, W. H. Burgess, M. Migliorini, and W. S. Argraves. 1990. Sequence identity between the α_2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J. Biol. Chem.* 265:17401–17404.

11. Herz, J., R. C. Kowal, J. L. Goldstein, and M. S. Brown. 1990. Proteolytic processing of the 600 kd low density lipoprotein receptor-related protein (LRP) occurs in a *trans*-Golgi compartment. *EMBO (Eur. Mol. Biol. Organ.) J* 9:1769–1776.

12. Moestrup, S. K., J. Gliemann, and G. Pallesen. 1992. Distribution of α_2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res.* 269:375–382.

13. Herz, J., D. E. Clouthier, and R. E. Hammer. 1992. LDL Receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell.* 71:411–421.

14. Moestrup, S. K., and J. Gliemann. 1991. Analysis of ligands recognition by the purified α_2-macroglobulin receptor (low density lipoprotein receptor-related protein). Evidence that high affinity of α_2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. *J. Biol. Chem.* 266:14011–14017.

15. Moestrup, S. K., E. I. Christensen, L. Sottrup-Jensen, and J. Gliemann. 1987. Binding and receptor-mediated endocytosis of pregnancy zone protein-proteinase complex in rat macrophages. *Biochim. Biophys. Acta.* 930:297–303.

16. Nykjaer, A., C. M. Petersen, B. Moller, P. H. Jensen, S. K. Moestrup, T. L. Holtet, M. Etzerodt, H. C. Thøgersen, M. Munch, P. A. Andreasen, and J. Gliemann. 1992. Purified α_2-macroglobulin receptor/LDL receptor-related protein binds urokinase-plasminogen activator inhibitor type-1 complex. *J. Biol. Chem.* 267:14543–14546.

17. Orth, K., E. L. Madison, M. J. Gething, J. F. Sambrook, and J. Herz. 1992. Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen activator inhibitor type 1 are internalized by means of the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. *J. Biol. Chem.* 267:7422–7426.

18. Kounnas, M. Z., R. E. Morris, M. R. Thompson, D. J. Fitzgerald, D. K. Strickland, and C. B. Saelinger. 1992. The α_2-macroglobulin receptor/low density lipoprotein receptor-related protein binds and internalizes *Pseudomonas* exotoxin A. *J. Biol. Chem.* 267:12420–12423.

19. Moestrup, S. K., T. L. Holtet, M. Etzerodt, H. C. Thøgersen, A. Nykjaer, P. A. Andreasen, H. H. Rasmussen, L. Sottrup-Jensen, and J. Gliemann. 1993. α_2-macroglobulin-proteinase complexes, plasminogen activator inhibitor type-1-plasminogen activator complexes, and receptor-associated protein bind to a region of the α_2-macroglobulin receptor containing a cluster of eight complement-type repeats. *J. Biol. Chem.* 268:13691–13696.

20. LaMarre, J., M. A. Hayes, G. K. Wollenberg, I. Hussaini, S. W. Hall, and S. L. Gonias. 1991. An α_2-macroglobulin receptor-dependent mechanism for the plasma clearance of transforming growth factor-β1 in mice. *J. Clin. Invest.* 87:39–44.

21. Stouffer, G. A., J. LaMarre, S. L. Gonias, and G. K. Owens. 1993. Activated α_2-macroglobulin and transforming growth factor-β1 induced a synergistic smooth muscle cell proliferative response. *J. Biol. Chem.* 268:18340–18344.

22. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature (Lond.).* 341:162–164.

23. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* 86:5810–5814.

24. Beisiegel, U., W. Weger, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* 88:8342–8346.

25. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, M. W. Pladet, P. H. Iverius, and D. K. Strickland. 1993. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor in vitro. *J. Biol. Chem.* 268:14168–14175.

26. Nykjaer, A., G. Bengtsson-Olivecrona, A. Lookene, S. K. Moestrup, C. M. Petersen, W. Weber, U. Beisiegel, and J. Gliemann. 1993. The α_2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and β-migrating very low density lipoprotein associated with the lipase. *J. Biol. Chem.* 268:15048–15055.

27. Ylä-Herttuala, S., M. E. Rosenfeld, S. Parthasarathy, C. K. Glass, E. Sigal, J. L. Witztum, and D. Steinberg. 1990. Colocalization of 15-lipoxygenase mRNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* 87:6959–6963.

28. Ylä-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. L. Witztum. 1991. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* 88:10143–10147.

29. Yamamoto, T., C. G. Davis, M. S. Brown, W. J. Schneider, M. L. Casey, J. L. Goldstein, and D. W. Russell. 1984. The human LDL receptor: a cysteine-rich protein with multiple alu sequences in its mRNA. *Cell.* 39:27–38.

30. Raychowdhury, R., J. L. Niles, R. T. McCluskey, and J. A. Smith. 1989. Autoimmune target in Heymann nephritis is a glycoprotein with homology to the LDL receptor. *Science (Wash. DC).* 244:1163–1165.

31. Takahashi, S., Y. Kawarabayasi, T. Nakai, J. Sakai, and T. Yamamoto. 1992. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. USA.* 89:9252–9256.

32. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.

33. Moestrup, S. K., K. Kaltoft, C. M. Petersen, S. Pedersen, J. Gliemann, and E. I. Christensen. 1990. Immunocytochemical identification of the human α_2-macroglobulin receptor in monocytes and fibroblasts: monoclonal antibodies define the receptor as a monocyte differentiation antigen. *Exp. Cell Res.* 190:195–204.

34. Gown, A. M., T. Tsukuda, and R. Ross. 1986. Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. *Am. J. Pathol.* 125:191–207.

35. Tsukada, T., D. Tippens, H. Mar, D. Gordon, R. Ross, and A. M. Gown. 1986. HHF-35: a muscle-actin-specific monoclonal antibody. *Am. J. Pathol.* 126:51–60.

36. Young, S. G., J. L. Witztum, D. C. Casal, L. K. Curtiss, and S. Bernstein. 1986. Conservation of the low density lipoprotein receptor-binding domain of apoprotein B: Demonstration by a new monoclonal antibody, MB47. *Arteriosclerosis.* 6:178–188.

37. Rosenfeld, M. E., W. Palinski, S. Ylä-Herttuala, S. Butler, and J. L. Witztum. 1990. Distribution of oxidation specific lipid-protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis.* 10:336–349.

38. Swanson, L. W., D. M. Simmons, S. L. Hofmann, J. L. Goldstein, and M. S. Brown. 1988. Localization of mRNA for low density lipoprotein receptor and a cholesterol synthetic enzyme in rabbit nervous system by *in situ* hybridization. *Proc. Natl. Acad. Sci. USA.* 85:9821–9825.

39. Fong, L. G., E. Bonney, J. C. Kosek, and A. D. Cooper. 1989. Immunohistochemical localization of low density lipoprotein receptors in adrenal gland, liver, and intestine. *J. Clin. Invest.* 84:847–856.

40. Sparrow, C. P., S. Parthasarathy, and D. Steinberg. 1989. A macrophage receptor that recognizes low density lipoprotein but not acetylated low density lipoprotein. *J. Biol. Chem.* 264:2599–2604.

41. Ylä-Herttuala, S., O. Jaakkola, C. Ehnholm, M. J. Tikkanen, T. Solakivi, T. Särkioja, and T. Nikkari. 1988. Characterization of two lipoproteins containing apolipoproteins B and E from lesion-free human aortic intima. *J. Lipid Res.* 29:563–572.

42. O'Brien, K. D., D. Gordon, S. Deeb, M. Ferguson, and A. Chait. 1992. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J. Clin. Invest.* 89:1544–1550.

43. Babaev, V. R., A. D. Dergunov, A. A. Chenchik, E. M. Tararak, E. V. Yanushevskaya, I. N. Trakht, C. Sorg, and V. N. Smirnov. 1990. Localization of apolipoprotein E in normal and atherosclerotic human aorta. *Atherosclerosis.* 85:239–247.

44. Rosenfeld, M. E., S. Butler, V. A. Ord, B. A. Lipton, C. A. Dyer, L. K. Curtiss, W. Palinski, and J. L. Witztum. 1993. Abundant expression of apoprotein E by macrophages in human and rabbit atherosclerotic lesions. *Arterioscler. Thromb.* 13:1382–1389.

45. Stein, O., M. Ben-Naim, Y. Dabach, G. Hollander, G. Halperin, and Y.

Stein. 1993. Can lipoprotein lipase be the culprit in cholesteryl ester accretion in smooth muscle cells in atheroma? *Atherosclerosis.* 99:15–22.

46. Hoff, H. F., R. L. Jackson, J. T. Mao, and A. M. Gotto. 1974. Localization of low density lipoproteins in arterial lesions from normolipidemics employing a purified fluorescent-labeled antibody. *Biochim. Biophys. Acta.* 351:407–418.

47. Freeman, M., Y. Ekkel, L. Rohrer, M. Penman, N. J. Freedman, G. M. Chisolm, and M. Krieger. 1991. Expression of type I and type II bovine scavenger receptors in Chinese hamster ovary cells: lipid droplet accumulation and nonreciprocal cross competition by acetylated and oxidized low density lipoprotein. *Proc. Natl. Acad. Sci. USA.* 88:4931–4935.

48. LaMarre, J., B. B. Wolf, E. L. W. Kittler, P. J. Quesenberry, and S. L. Gonias. 1993. Regulation of macrophage α_2-macroglobulin receptor/low density lipoprotein receptor-related protein by lipopolysaccharide and interferon-γ. *J. Clin. Invest.* 91:1219–1224.

49. Fogelman, A. M., M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards. 1981. Factors regulating the activities of the low density lipoprotein receptor and the scavenger receptor on human monocyte-macrophages. *J. Lipid Res.* 22:1131–1141.

50. Rosenfeld, M. E., S. Ylä-Herttuala, B. A. Lipton, V. A. Ord, J. L. Witztum, and D. Steinberg. 1992. Macrophage colony-stimulating factor mRNA and protein in atherosclerotic lesions of rabbits and humans. *Am. J. Path.* 140:291–300.

51. Clinton, S. K., R. Underwood, L. Hayes, M. L. Sherman, D. W. Kufe, and P. Libby. 1992. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. *Am. J. Pathol.* 140:301–316.

52. Hussaini, I. M., K. Srikumar, P. J. Quesenberry, and S. L. Gonias. 1990. Colony-stimulating factor-1 modulates α_2-macroglobulin receptor expression in murine bone marrow macrophages. *J. Biol. Chem.* 32:19441–19446.

53. Ishibashi, S., T. Inaba, H. Shimano, K. Harada, I. Inoue, H. Mokuno, N. Mori, T. Gotoda, F. Takaku, and N. Yamada. 1990. Monocyte colony-stimulating factor enhances uptake and degradation of acetylated low density lipoproteins and cholesterol esterification in human monocyte-derived macrophages. *J. Biol. Chem.* 265:14109–14117.

54. Hansson, G. K., J. Holm, and L. Jonasson. 1989. Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am. J. Pathol.* 135:169–175.

55. Ylä-Herttuala, S. 1992. Gene expression in atherosclerotic lesions. *Herz.* 17:270–276.

56. Herz, J., J. L. Goldstein, D. K. Strickland, Y. K. Ho, and M. S. Brown. 1991. 39-kDa protein modulates binding of ligands to the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. *J. Biol. Chem.* 266:21232–21238.