Characterization of inherited scavenger receptor overexpression and abnormal macrophage phenotype in a normolipidemic subject with planar xanthomas

Claude Giry, Louise-Marie Giroux, Madeleine Roy, Jean Davignon, and Anne Minnich[1]

Clinical Research Institute of Montreal, 110 Pine Avenue, West, Montreal, P.Q., Canada H2W 1R7

Abstract It is proposed that, in hyperlipidemia, foam cells develop in cutaneous xanthomas from the uptake by the macrophage scavenger receptor (SR) of low density lipoproteins (LDL) that are modified due to increased residence time in plasma. We have observed extensive xanthelasmas and planar xanthomas in the absence of hyperlipidemia in two siblings. In blood monocytes from one sibling, ^{125}I-labeled acetylated LDL (Ac-LDL) degradation and SR mRNA were 4 and 7 times higher, respectively, than in four control subjects. Among monocytes from these five individuals, variation in Ac-LDL degradation was completely accounted for by SR mRNA levels ($R^2 = 0.98$, $P < 0.001$). Monocyte SR mRNA was induced upon maturation into macrophages during 7 days in culture. Mean monocyte and macrophage SR mRNA values from one sibling and six additional family members were elevated 5- and 4-fold compared to that of 16 control subjects, and elevated monocyte SR mRNA was associated with abnormally high cell-surface expression of SR epitopes. Monocytes from eight of nine family members examined displayed an unusual phenotype characterized by increased adhesion and rapid maturation into large macrophages which overaccumulated lipids. Monocyte-macrophage SR overexpression relative to control persisted even in the absence of autologous serum, consistent with a cellular abnormality. This is the first demonstration of an inherited abnormality in scavenger receptor expression and its occurrence in association with planar xanthomas.—**Giry, C., L-M. Giroux, M. Roy, J. Davignon, and A. Minnich.** Characterization of inherited scavenger receptor overexpression and abnormal macrophage phenotype in a normolipidemic subject with planar xanthomas. *J. Lipid Res.* 1996. **37:** 1422–1435.

Supplementary key words monocytes • lipoprotein receptors • gene expression • human • foam cells • atherosclerosis • CD36

Monocyte-derived macrophages are implicated in the formation of foam cells in atherosclerotic lesions (1). These foam cells are characterized by a high content of cellular cholesteryl esters. Most cell types do not accumulate cholesteryl esters because of the potent down-regulation of the LDL-receptor in the presence of excess intracellular sterols (2). In contrast, macrophages, when exposed to modified LDL, can be induced to accumulate large amounts of intracellular cholesterol due to the activity of an additional lipoprotein receptor whose expression is not regulated by cellular sterols (3). One receptor believed to be responsible for this activity is the macrophage scavenger receptor (SR) (3, 4). The bovine SR cDNA has recently been cloned and shown to induce uptake of modified lipoproteins when transfected into Chinese hamster ovary (CHO) cells (5). The human cDNA has also been cloned (6).

Both the human and bovine cloned scavenger receptors consist of an N-terminal cytoplasmic domain (exon 2), a transmembrane domain (exon 3), a spacer domain, α-helical coiled-coil domain that mediates trimerization (exons 4 and 5), a collagen-like domain (exons 6–8) essential for ligand binding, and a C-terminal type specific domain of unknown function (7). The ligand specificity of this receptor is broad (8) and includes negatively charged lipoproteins: oxidized LDL, a physiological ligand, and acetylated LDL, used often in vitro to assay SR activity. It is known that two forms of the SR are generated from alternative splicing of the same transcript (7). These forms, designated as type I and type II, differ in the carboxyl-terminal region in that the type I receptor contains a 110 amino acid cysteine-rich carboxyl terminal domain of unknown function while the 17-amino acid carboxyl terminus of the type II receptor contains no cysteines. As both forms take up acetylated

Abbreviations: SR, scavenger receptor; LDL, low density lipoprotein; Ac-LDL, acetylated LDL; CHO, Chinese hamster ovary; VLDL, very low density lipoprotein; HDL, high density lipoprotein; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; apo, apolipoprotein; M-CSF, macrophage-colony stimulating factor; PCR, polymerase chain reaction; RT, reverse transcription; HPLC, high performance liquid chromatography; Ox-LDL, oxidized LDL.

[1]To whom correspondence should be addressed.

Downloaded from www.jlr.org by guest, on June 18, 2012

and oxidized LDL equally well in transfected cells, the relative physiological significance of the two forms is unknown (5).

Some evidence suggests the existence of more than one receptor for modified lipoproteins. Such evidence consists of experiments in which oxidized and acetylated LDL exhibit nonreciprocal cross competition for binding and/or degradation by macrophages (5, 9–12). In addition, some additional cloned receptors have been reported to recognize modified lipoproteins as ligands (12–17). However, the macrophage SR is widely suspected to be responsible for the uptake of modified lipoproteins leading to foam cell formation (18).

In addition to arterial foam cell formation, it is also believed that foam cells may develop in cutaneous xanthomas, as occurs in familial hypercholesterolemia, from the uptake by monocyte-macrophage SR of plasma LDL which may be modified due to a prolonged residence time in circulation or to greater oxidizability (19). We have observed in two siblings, the proband (FC) and her brother (LC), attending our lipid clinic extensive planar xanthomas in the absence of hyperlipidemia or any other obvious dyslipoproteinemia or apolipoprotein defect. Isolated blood monocytes from FC displayed a markedly abnormal phenotype characterized by an apparent early maturation into foam cell-like macrophages and a 60-fold higher rate of cholesterol esterification induced by oxidized LDL compared to normal cells. The present report provides evidence to support the hypothesis that this phenotype is related to SR overexpression.

METHODS

Characterization of plasma lipoproteins

Plasma from venous blood of fasting subjects was prepared by low speed centrifugation at 4°C in the presence of 1.5 mg/ml EDTA. Lipoproteins were isolated by ultracentrifugation at d 1.006 g/ml to obtain very low density lipoprotein (VLDL) and precipitation of apoB in the d > 1.006 g/ml fraction to separate LDL from high density lipoprotein (HDL) (20). Plasma and lipoprotein cholesterol and triglyceride concentrations were determined enzymatically on an automated analyzer (Abbott Biochromatic Analyzer model 100, Abbott Laboratories, Pasadena, CA). Plasma lipoproteins were subjected to agarose gel electrophoresis (21) with a Beckman Paragon LipoGel system and visualized by Sudan Black staining or by autoradiography for ^{125}I-labeled LDL.

Characterization of the C kindred

The proband FC (II-6, Fig. 3), a 65-year-old Caucasian female, was referred to the lipid clinic at the Clinical Research Institute of Montreal by a dermatologist at age 48 because of recurrence of xanthelasmas after surgical removal. She had been adhering to a low fat diet since age 36. The only abnormal finding on physical examination at that time was the presence of extensive bilateral xanthelasmas of the upper and lower eyelids. Her body mass index and her plasma lipid and lipoprotein levels were consistently normal. Her apoE phenotype was E3/3. Cardiac function, including a 24-h Holter test, was consistently normal. During follow-up, orange elevated plaques, at times extensive, were noted at age 54 in the neck and upper thoracic areas. She developed thick yellowish lesions on her nose which were removed but also recurred. Microscopic examination of these lesions and of the neck lesions showed large numbers of foam cells and Touton-type cells, and the lesions were diagnosed as xanthomas. Potential causes of these xanthomas were investigated. A test for hyperbeta-sitosterolemia was negative. Post-heparin plasma lipase activity, apoA-I and apoC isoforms, and zonal ultracentrifugation of plasma lipoproteins gave a normal profile. Other routine biochemical tests and immunoelectrophoresis were consistently normal. Cryoglobulins were absent. FC died at age 65 of pancreatic cancer which had become symptomatic five months previously.

Her brother (LC, II-2, Fig. 3) was first examined at age 60, having no previous medical history. Examination revealed an aortic systolic murmur and bilateral arcus corneae. He had extensive orange slightly elevated plaques on the abdomen, inguinal areas, popliteal and cubital folds, and around the neck. Two scars on the limbs also had an orange discoloration and two subcutaneous lipomas were detected on the lower back and the para-umbilical area. He consumed a typical North American diet and was approximately 20 lbs. overweight. His apoE phenotype was E3/3. Immunoelectrophoresis of proteins revealed a monoclonal IgG-λ gammopathy. His resting EKG was normal and the echocardiogram showed slight to moderate sclerosis of the aortic valve. The flat plate of the abdomen revealed discrete calcifications of the abdominal aorta and iliac arteries. This research was approved by the institutional ethics committee. Informed consent was obtained from all C family members who participated in this study.

Cell culture

Monocytes were isolated from whole blood with Ficoll Paque (Pharmacia, Baie d'Urfe, Quebec) gradient centrifugation (22) and resuspended in DMEM (Gibco, Burlington, Ontario) containing 100 U/ml penicillin and 100 μg/ml streptomycin. The cell suspension was plated at a density of 2×10^6 monocytes per 35×10 mm well (Falcon) or 50,000 monocytes per chamber in Lab-tek chamber slides (Nunc, Naperville, IL) and incubated

Downloaded from www.jlr.org by guest, on June 18, 2012

for 1 h at 37°C in a humidified atmosphere containing 5% CO_2. Adherent cells were fed with DMEM supplemented with 30% autologous serum and antibiotics. Medium was changed 1 day after cell isolation and every 3 days. For some experiments, cells were grown in RPMI 1640 containing 1% Nutridoma® (Boehringer Mannheim). Recombinant macrophage-colony stimulating factor (M-CSF) (R&D Systems, Minneapolis, MN) was dissolved in PBS to 5 μg/ml and added to cells in one experiment. The human monocytic cell line THP-1 was obtained from American Type Culture Collection (Rockville, MD). THP-1 cells were cultured in RPMI with 10% FCS, and were incubated with 0.1 μg/ml phorbol myristate acetate (PMA, Gibco) to promote monocyte differentiation into macrophages.

Cell lipoprotein receptor activity assays

LDL were iodinated with carrier-free $Na^{125}I$ (Amersham, Oakville, Ontario) with Iodogen (Pierce, Rockford, IL) (23), and acetylated (24). ^{125}I-labeled acetyl (Ac)-LDL were dialyzed at 4°C overnight, sterilized by filtration through a 0.22 μM filter, and checked by gel electrophoresis with the Paragon system (Beckman). ^{125}I-labeled Ac-LDL degradation assays were performed with monocytes at varying stages of differentiation (days 1, 3, and 7 after isolation). In one experiment, the rate of incorporation of [^{14}C]oleate (Amersham) into cellular cholesteryl esters was performed as described (25) after incubation of macrophages with oxidized LDL (26). Cellular protein was measured by the method of Lowry et al. (27). In other experiments, binding and internalization of Ac-LDL were assayed qualitatively with DiI-acetyl LDL (Biomedical Technologies Inc., Stoughton, MA). After incubation with the ligand, cells were visualized by fluorescence microscopy with a rhodamine filter.

Cell-surface binding of Ac-LDL and antibodies

Binding of polyclonal anti-human SR antibodies hSRI-II (6) and SRII-I (28) (generous gifts from Dr. T. Kodama) and of FITC-conjugated monoclonal anti-human CD36 (Sigma Immunochemicals) to monocyte-macrophages was analyzed by flow cytometry. Approximately 10^6 cells were incubated with anti-SR antibodies (1:200) for 1 h at 4°C, washed 3 times, incubated 30 min with FITC-labeled swine anti-rabbit IgG (Dako, Carpinteria, CA), washed 3 times, and fixed in 2% paraformaldehyde/PBS. Cells incubated with FITC anti-rabbit IgG only were used as negative controls for these experiments. Alternatively, cells were incubated with FITC-conjugated anti-CD36. Cells were analyzed on an FACScan flow cytometer (Becton Dickinson) equipped with an argon laser (488 nm) and fluorescence was collected in FL1 (525 nm). Data were analyzed with CellQuest software.

mRNA quantification

Oligonucleotides were synthesized by the solid phase triester method on a Pharmacia LKB Gene Assembler Plus DNA synthesizer. Polyacrylamide gel-purified oligonucleotides labeled with JOE® fluorescent dye (Applied Biosystems, Foster City, CA) were obtained from Regional DNA synthesis Laboratories (Calgary, Alberta).

Total RNA was isolated from 6–8 × 10^6 cultured cells with RNAzol® (29) and quantified by UV absorbance at 260 nm. Four hundred ng RNA was reverse transcribed with the reverse transcription system of Promega (Madison, WI) according to the recommendations of the manufacturer and with random hexanucleotide primers (Boehringer Mannheim). SR and β-actin cDNA were PCR amplified (95°, 30 sec; 54°, 45 sec; 72°, 90 sec) simultaneously with primers indicated in **Table 1.** Alternatively, SR cDNA was co-amplified with glyceraldehyde-3-phosphate dehydrogenase (GAPDH A, Table 1). Apolipoprotein E cDNA was co-amplified with GAPDH A (Table 1). FcγRIII (CD16) cDNA was co-amplified with GAPDH B, in the presence of 250 nM fluorescein-12-labeled dUTP (Boehringer Mannheim). The relative quantity of amplified DNA fragments was measured by fluo-

TABLE 1. Oligonucleotides used for mRNA quantification

cDNA	5′-Oligo	3′-Oligo	Fragment Length	Sequence Location	Ref.
			bp		
β-Actin	atggtgcatctctgccttac[a]	catctcttgctcgaagtcca	340	1833–2170	56
GAPDH (A)	ggaaggtgaaggtcggagtc	cagaggggcagagatgag[a]	376	-2–372	64
GAPDH (B)	cgagccacatcgctcagaca	gcatcgccccacttgattttg	295	-30–263	64
SR	tcgctcaatgacagctttgc[a]	ccatgttgctcatgtgttcc	290	75–364	6
ApoE	cccggctgggcgcggacatggaggacgttt[a]	tcgcgggccccggcctggtaca	203	3711–3914	65
CD16	cacctgaggtgtcacagct	ggtcatttgtcttgagggtc	395	406–800	66

All oligonucleotide sequences are written in the 5′ → 3′ direction. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SR, scavenger receptor; apoE, apolipoprotein E.

[a]Oligonucleotides labeled with JOE® or TAMRA® fluorescent dye.

Downloaded from www.jlr.org by guest, on June 18, 2012

rescence detection after polyacrylamide gel electrophoresis on a model 373A Automated DNA Sequencer® (Applied Biosystems) (30). Amplification of RNA before reverse-transcription yielded no signal corresponding to the above fragments. In measurements of cycle number versus fluorescence signal, 25 cycles was shown to be within the log-linear range (data not shown) for all fragments and subsequent assays were performed with this number of cycles. All PCR reactions were performed in triplicate. Among monocytes or macrophages from 10 subjects, SR mRNA expressed as a ratio to β-actin and to GAPDH as internal standard were strongly correlated ($r = 0.97$, $P < 0.001$) when assayed in the same amplification. Among 38 samples assayed on different days, the correlation coefficient was $r = 0.82$ ($P < 0.001$).

Cellular histochemistry and electron microscopy

Chamber slide-plated cells were washed twice in PBS and fixed in 10% formaldehyde in PBS for 1 h at room temperature. Cells were then washed in propyleneglycol for 2 min, incubated 30 min in 0.5% Oil Red O in propyleneglycol, washed 3 times in 70% propyleneglycol and 15 min in water, and stained with Harris' hematoxylin for 5 min. For electron microscopy, cells were fixed in 3.5% glutaraldehyde in PBS, washed in 0.1 M cacodylate buffer with 2% sucrose, postfixed with 2% OsO_4 for 1 h and incubated with 1% *p*-phenylenediamine in 75% ethanol for 25 min. Cells were then dehydrated in ethanol, embedded in LX 112 (Ladd, Burlington, Ontario). Ultrathin sections were cut with an ultramicrotome (Reichert) and visualized with a Jeol 1200 EX electron microscope.

Measurement of anti-LDL reactive autoantibodies

IgG was purified from the plasma of subject LC (see Results) and from two normal subjects by affinity chromatography on Protein G Sepharose, and IgG concentrations were monitored by UV absorbance at 280 nm. One hundred µl of normal LDL was diluted to 30 µg/ml protein in 5 mM glycine, pH 9.2, and adsorbed overnight onto polystyrene microtitre wells. The wells were washed with 0.15 M NaCl, 0.025% Tween 20 and incubated 30 min with 1% bovine serum albumin (BSA) in PBS. One hundred µl aliquots of IgG diluted in 1% BSA were added to the wells and incubated for 2 h at room temperature. Wells were washed as above and incubated with 100 µl alkaline phosphatase-conjugated rabbit anti-human IgG. After the addition of 100 µl *p*-nitrophenyl phosphate, absorbance at 415 nm was quantitated when sufficient color was visible in positive controls (human IgG directly bound to plastic).

Measurement of plasma antioxidant levels

Plasma carotenoids (zeaxanthine, cryptoxanhine, lycopene, α-carotene, and β-carotene) and α-tocopherol were quantitated by HPLC essentially as described (31). The Beckman Gold system with a C18 column was used. Detector wavelengths were 450 and 284 nm for carotenoids and α-tocopherol, respectively. Echinenone and tocopheryl acetate were used as internal standards for carotenoids and α-tocopherol, respectively. Concentrations were calculated with absorption coefficients as described (32).

Statistical analyses

Unless otherwise indicated, data are reported as means ± standard error. Means were compared with a Student's *t*-test.

RESULTS

Monocyte-macrophage scavenger receptor activity in the proband and her brother

The presence of xanthomas in the absence of hyperlipidemia in the proband FC (**Fig. 1**) was probably not due to modification of plasma LDL from FC, as this LDL displayed normal agarose gel electrophoretic mobility (data not shown). In addition, no difference in macrophage degradation of ^{125}I-labeled native or oxidized LDL from FC and control was detected (**Table 2,** upper portion). These circumstances, therefore, suggested a possible abnormality of monocyte-macrophage function in FC. Blood monocytes (cultured in vitro in autologous serum) from FC were markedly abnormal in that they appeared to mature rapidly into macrophages and eventually acquired a foam cell-like appearance, unlike blood monocytes from a control subject. In addition, oxidized LDL induced 60 times as much cholesterol esterification

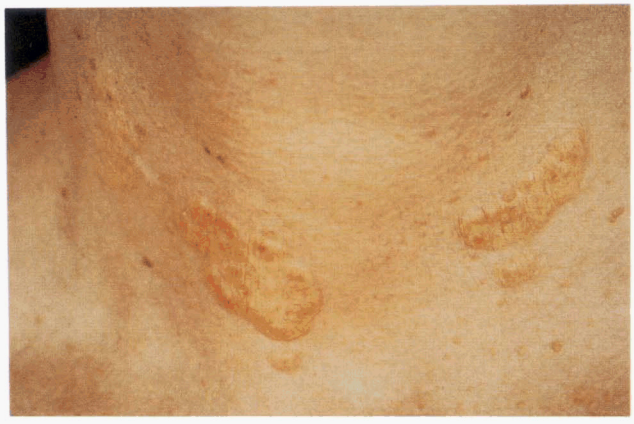

Fig. 1. Planar xanthomas in the proband FC. Shown is a frontal view of the neck.

Downloaded from www.jlr.org by guest, on June 18, 2012

in macrophages from FC relative to control macrophages (Table 2, lower portion), suggesting abnormally high activity of a receptor for modified lipoproteins, possibly the macrophage SR.

Due to the death of FC from pancreatic cancer, subsequent studies of monocyte-macrophage phenotype were performed in cells from her brother (LC), in whom similar xanthomas were observed. Compared to four control subjects, 1-day-old monocytes from LC degraded more than 3 times as much ^{125}I-labeled Ac-LDL as did those from controls [**Fig. 2,** 1523 ng/mg cell protein per 5 h (open triangle) vs. 464 ± 97 (open circles)]. The rate of ligand degradation increased on average approximately 3-fold in cells from control subjects, but not in those from LC, upon differentiation into macrophages after 7 days in culture. In 7-day macrophages, the ^{125}I-labeled Ac-LDL degradation rate was similar for LC (filled triangle) and controls (filled circles).

Scavenger receptor mRNA levels in monocytes and macrophages from affected and unaffected subjects

To address the possible identity of the receptor responsible for the abnormally high ^{125}I-labeled Ac-LDL degradation in LC monocytes, mRNA for the cloned human macrophage SR (6) was quantitated in monocytes and macrophages. In monocytes from normal subjects, the ratio of SR to β-actin mRNA peak areas increased with time in culture (Fig. 2 open vs. closed circles) several hundred-fold after 7 days, but this induction was much less marked in monocytes from LC (3.5-fold). Monocyte, but not macrophage, SR mRNA was elevated 3- to 20-fold in LC compared to controls (Fig. 2 open triangle vs. open circles), strongly implicating precocious SR expression in the higher rate of ^{125}I-labeled Ac-LDL degradation observed in monocytes from LC. Inter-individual variability in monocyte SR mRNA completely accounted for differences in ^{125}I-labeled Ac-LDL degradation rates: $Y = 2083X + 248$, $R^2 = 0.98$ (Fig. 2, open symbols). No such correlation was detectable among macrophages, suggesting the contribution of either posttranscriptional regulatory influences or the activity of other Ac-LDL receptors to inter-individual variability in SR activity among macrophages.

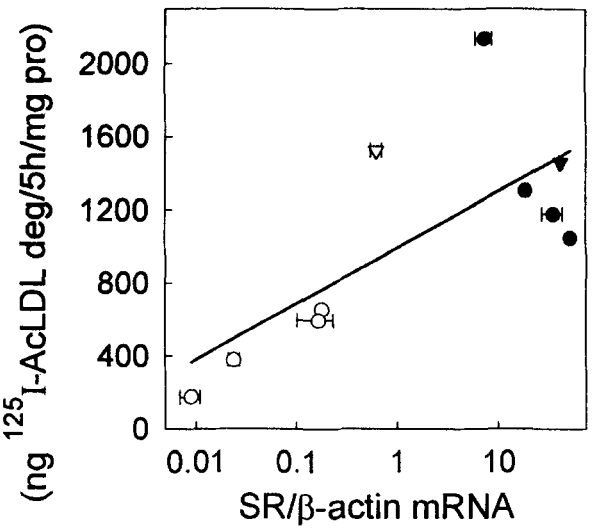

Fig. 2. Scavenger receptor activity versus mRNA levels in monocytes and macrophages from LC and controls. Each point represents the mean of duplicate determinations for SR activity obtained with 10 μg/ml ^{125}I-labeled Ac-LDL and the mean ± SEM for triplicate amplifications for SR mRNA determinations for one individual. Relative cellular SR mRNA levels were measured by RT-PCR and fluorescence detection as described in Methods. Shown on the X-axis is the ratio of peak areas corresponding to fluorescent DNA fragments amplified from SR and β-actin cDNA sequences. Empty and filled symbols represent monocytes and macrophages, respectively. Circles and triangles represent controls and LC, respectively. Controls were laboratory or Institute personnel, 3 women aged 30, 33, and 40 years and a 33-year-old male.

TABLE 2. Reactivity of native and oxidized LDL with monocyte-macrophages from patient (FC) or control

	^{125}I-Labeled Degradation	
	Control LDL	Patient LDL
	ng/mg cell protein	
Native LDL	171 ± 17	175 ± 13
Ox-LDL	1039 ± 44	603 ± 2
	Incorporation of [^{14}C]oleate into Cellular Cholesteryl Esters	
	Control Macrophages	Patient Macrophages
	nmol/mg/h	
Native LDL	0.27 ± 0.03	0.28 (n = 2)
Ox-LDL	1.7 (n = 2)	101 ± 3

Seven-day monocyte-macrophages were incubated 4 h at 37°C with 10 μg/ml ^{125}I-labeled or unlabeled oxidized (see Methods) or native LDL. Values are mean ± SD of three dishes except where indicated. Upper portion: ^{125}I-labeled oxidized or native LDL from patient and control were incubated with macrophages from the patient (FC). Lower portion: unlabeled oxidized or native LDL from control was incubated with macrophages from FC and control.

Other potential sources of macrophage cholesterol over-accumulation

Another instance of xanthomas in a normolipidemic subject with paraproteinemia has been attributed to the presence of an LDL-monoclonal IgG complex in plasma that is recognized by the SR, thereby promoting macrophage cholesteryl ester accumulation (33). The absence of an anti-LDL antibody in the plasma of LC ruled out this phenomenon as responsible for the presence of xanthomas in LC (data not shown, see Methods). In addition, LDL from LC displayed electrophoretic mobility indistinguishable from that of a control, both before and after acetylation (data not shown). Finally, plasma from LC contained normal levels of the lipid-sol-

Downloaded from www.jlr.org by guest, on June 18, 2012

uble antioxidants β-carotene and α-tocopherol (data not shown).

Family study of C kindred

Monocyte-macrophages from several members of the C family were examined (**Fig. 3**). Although subjects II-7, II-9, and III-1-4 (Fig. 3) did not have detectable cutaneous xanthomas, their monocyte-macrophages displayed marked differences in cellular phenotype compared to those of normal subjects (see below), as had been noted for FC. Of the subjects examined in the C-kindred, only II-8 displayed a relatively normal monocyte-macrophage phenotype. The mean peak area ratio corresponding to SR/GAPDH mRNA in 1-day-old monocytes from members of the C-kindred with the abnormal cellular phenotype (affected members) was 2.2 ± 0.6 (n = 7). The mean ratio among 13 male controls ranging in age from 32 to 69 years (mean 47 ± 3.4 years) was 0.44 ± 0.09 ($P < 0.002$ vs. affected members of the C-kindred), and in three females was 0.25 ± 0.1. Among control subjects, no effect of subject age or sex on monocyte SR expression was noted. In 7-day macrophages, these ratios rose to 10.3 ± 3.4 (n = 7) and 2.9 ± 0.4 (n = 9) for affected members of the C-kindred and controls, respectively ($P < 0.03$). Thus, in the C-kindred SR overexpression appears to be vertically transmitted in a dominant or codominant fashion.

The 2-fold greater expression of SR mRNA and the abnormal cellular phenotype in monocytes from II-9 compared to those from II-8 was associated with markedly greater SR activity in 2-day-old monocytes as assessed by uptake of DiI-labeled Ac-LDL (**Fig. 4**). The difference in SR activity between these two subjects was also apparent in 7-day-old macrophages (Fig. 4).

Cell-surface expression of SR proteins

Flow cytometric analysis revealed higher expression of SR protein epitopes, detected with anti-SR antibody hSRI-2, against the collagenous domain common to both isoforms (6), and with anti hSRII-1, against the type II-specific domain (28), on the cell surface of 1-day-old monocytes from subject II-2 and his son III-1, compared to controls (**Fig. 5B and C**). In fact, in monocytes from affected subjects, SR expression was commensurate with macrophage-like levels (Fig. 5A and B), as was the case for SR mRNA levels. Mean expression of CD36 in monocytes from affected subjects was elevated compared to controls (Fig. 5C, bottom panel). However, in

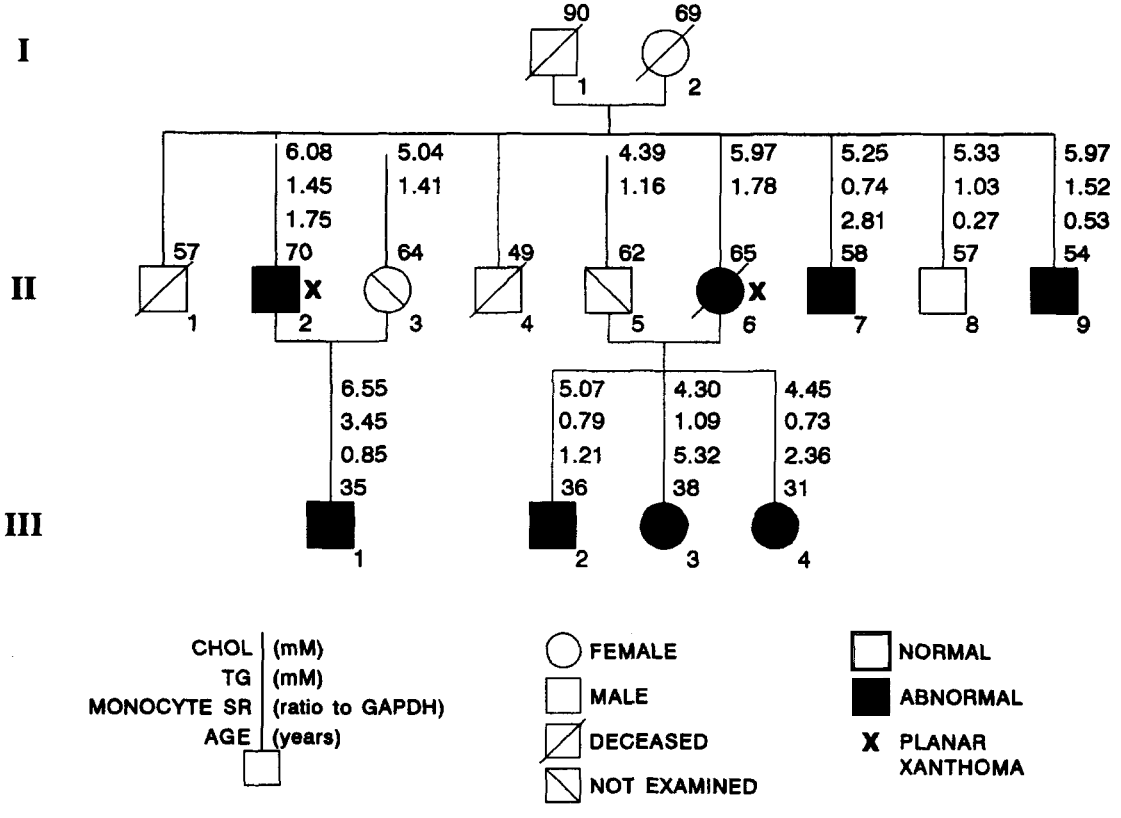

Fig. 3. Pedigree of C-kindred. II-2 and II-6 represent probands LC and FC, respectively. Subjects III-1–4 showed no signs of cutaneous lipid deposits. I-1 had a history of myocardial infarction and hypercholesterolemia diagnosed at age 72 and died at 90 of unknown causes. I-2 died of cancer at age 69, II-1 of a non-cardiovascular-related illness, and II-4 of lung cancer. Among all relatives studied, there was no history of premature cardiovascular disease. Normal and abnormal refer to monocyte-macrophage phenotype in vitro (see text).

Downloaded from www.jlr.org by guest, on June 18, 2012

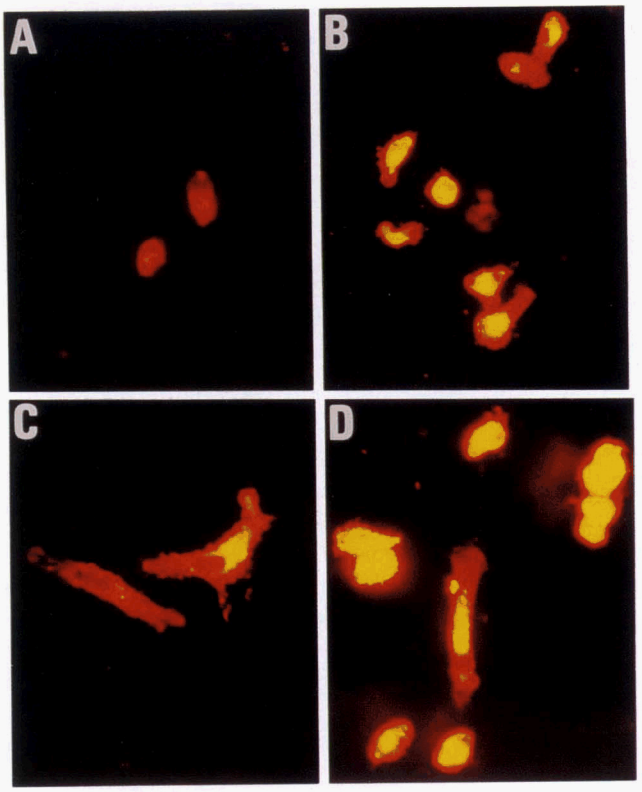

Fig. 4. SR activity in monocyte-macrophages from an affected and an unaffected member of the C-kindred. Monocytes plated in LabTek slides were incubated 4 h with 2.5 μg/ml DiI-AcLDL and the uptake of ligand was visualized with fluorescence microscopy as in Methods. A and B: 2-day-old monocytes from II-8 and II-9, respectively. C and D: 7-day-old macrophages from II-8 and II-9, respectively (SR/GAPDH mRNA peak area 1.8 and 11.0, respectively).

contrast to control subjects, the fluorescence peak for CD36 in affected subjects was broad and diffuse, indicating a wider range of CD36 expression within the monocyte population (Fig. 5B, bottom panel).

Cellular phenotype in affected and normal subjects

Monocytes from affected subjects consistently exhibited a morphology typical of macrophages as evidenced by larger cell size, flattening or spreading out, cytoplasmic extensions, more rounded or irregularly shaped nuclei, a greater cytoplasm to nucleus ratio, and the presence of vacuoles (34–36). Whereas monocytes from normal subjects developed these features after approximately 7 days in culture, the macrophage-like phenotype was apparent as early as day 2 in cultured monocytes from affected subjects (**Fig. 6**). Accumulation of cellular neutral lipid was assessed qualitatively by Oil Red O staining or quantitatively by gas–liquid chromatography, depending on the availability of material. **Figure 7** shows substantially greater cellular neutral lipid accumulation in macrophages from members of the C-kindred, in contrast to those from a control subject with a monocyte SR level of 0.39. Cells from affected subjects appeared to accumulate esterified cholesterol as early as days 1 and 4 as evidenced by a low ratio of free to total cellular cholesterol (0.76, 0.89 for II-2 and III-1, respectively at day 1 and 0.86, 0.86 at day 4) unlike cells from four control subjects (1.0 ± 0.03 and 0.96 ± 0.04 for day 1 and day 4, respectively). The accumulation of cellular neutral lipid in large foam cell-like macrophages of affected subjects (Fig. 7B) was already apparent at day 4 (Fig. 7A). These foam cells were clearly larger for affected members of the C family than for controls (Fig. 7 A–D). In addition, monocyte-macrophages from C family members were more numerous at all stages, with 50–100% more cells than for controls remaining after plating (Fig. 7, legend), despite identical numbers of blood mononuclear cells plated. This difference was not due to higher blood monocyte counts in the affected subjects, which were approximately 25% of white blood cells in both normal and affected subjects. In addition, cells from affected subjects relatively devoid of lipid tended to aggregate around the particularly large foam cells, forming clusters visible at days 4 and 7 (see arrows in Fig. 7), despite their uniform distribution at day 1 (not shown). No differences in cell morphology between controls and affected subjects were apparent in 1-day-old monocytes. Thus, apparent SR overexpression in monocytes from C-kindred members is associated with an abnormal cellular phenotype characterized by rapid accumulation of cellular lipid in a subpopulation of cultured macrophages and possibly with greater cellular adhesion.

Expression of other genes associated with monocyte maturation

For 1-day-old monocytes, the peak area corresponding to apoE relative to GAPDH mRNA was 0.03 ± 0.02 ($n = 10$) in controls and 0.2 ± 0.1 ($n = 7$) in affected members of the C family ($P < 0.06$ vs. controls). Monocyte apoE mRNA levels were induced > 50-fold and peaked after 4 days in culture. The peak area ratio of FcγIII (CD16)/GAPDH was 4.0 ± 0.9 ($n = 9$) in controls and 1.9 ± 0.6 ($n = 3$) in affected subjects (NS vs. controls). The mean induction of CD16 mRNA relative to GAPDH during monocyte maturation into 7-day macrophages was 3.4-fold, excluding one control and two affected subjects in whom there was no CD16 induction after 7 days in culture. Thus, no statistically significant differences in monocyte expression of the apoE or CD16 genes between controls and affected members of the C-kindred were observed.

SR gene expression in the absence of autologous serum

As human blood monocytes were cultured in the presence of autologous serum, SR overexpression in

Downloaded from www.jlr.org by guest, on June 18, 2012

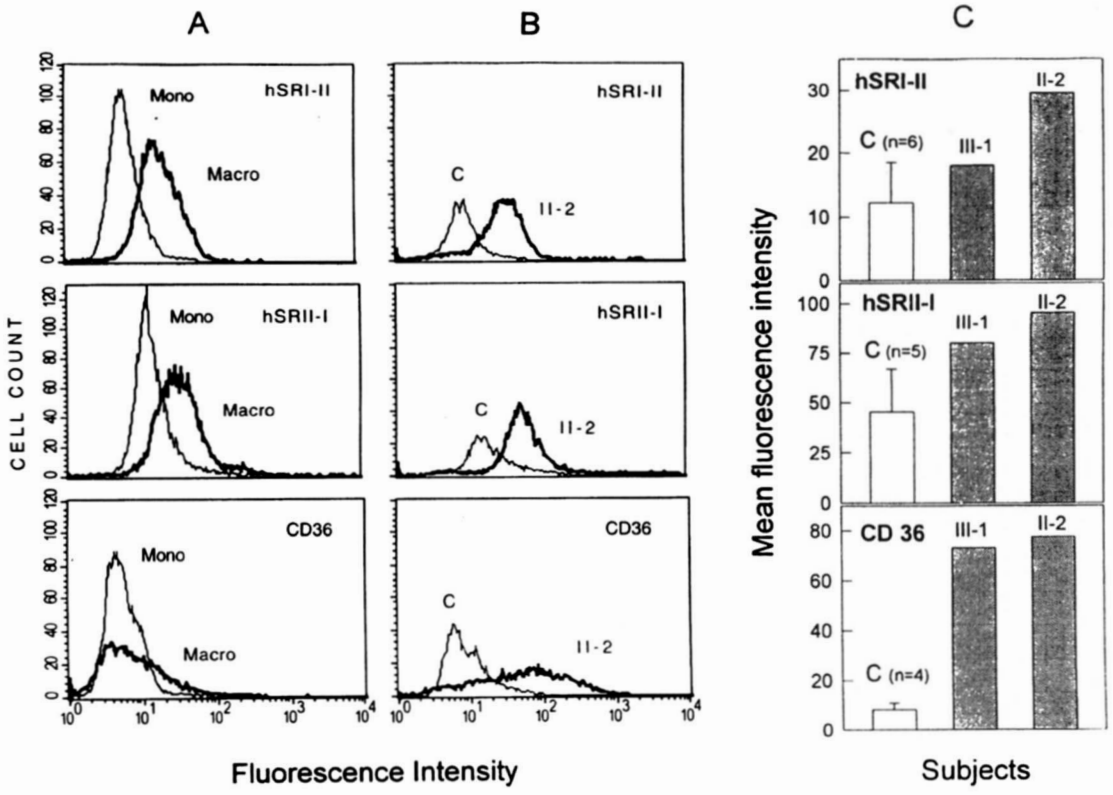

Fig. 5. Expression of SR protein on the cell surface of monocytes from affected and nonaffected subjects. Monocytes were subjected to flow cytometry after incubation with appropriate antibodies as in Methods. A: THP-1 cells, incubated overnight in the presence of 10% human serum, were analyzed with (macro) or without (mono) a 48-h induction with PMA. B: One-day-old monocytes from a control subject (C) or from subject II-2 (Fig. 3). C: Relative cell-surface expression of SR type I (hSRI-II), type II (hSRII-I), and CD36 epitopes in controls (C, empty bars) and affected subjects II-2 and III-1 (Fig. 3) (shaded bars). Specific fluorescence intensity was obtained after subtraction of cell autofluorescence or of the signal corresponding to cells incubated with FITC-anti-rabbit IgG only.

monocytes from LC and his family members might be related to some component in their serum. To investigate this possibility, SR gene expression in monocytes and macrophages, cultured in the presence and absence of autologous serum, from LC, his son (III-1 from Fig. 3), and a control subject was compared. For this purpose, a media supplement known to support growth and maturation of human monocytes (37) was used. In monocytes from a control subject, LC, and his son, SR mRNA levels were similar whether cells were incubated in the presence or absence (Nutridoma®) of autologous serum, and were 10- and 4-fold higher in LC and his son, respectively, than in monocytes from a normal control (**Table 3**). In 7-day macrophages from all three subjects, SR expression was increased to a similar degree (10- to 15-fold) in the presence or absence of autologous serum (Table 3), consistent with the previous observation that Nutridoma® supports monocyte differentiation even in the absence of autologous serum (37). Thus, monocyte SR overexpression in the C-kindred does not appear to depend on a serum component.

Effect of M-CSF on SR gene expression in LC and controls

Consistent with the apparent independence of SR overexpression from serum components, plasma M-CSF concentrations were normal in LC, his son, and his siblings (data not shown). The possibility that monocytes from affected members of the C-kindred respond differently to a serum component with known potent influences on monocyte-macrophage behavior was therefore tested. Incubation of monocytes from a normal subject with M-CSF resulted in no induction of SR mRNA, while monocytes from LC and his son responded to M-CSF with a 3.5- and 8-fold induction, respectively (**Fig. 8**). In contrast, incubation of macrophages from controls with M-CSF induced SR gene expression, as expected, while macrophages from LC and his son appeared refractory to further mRNA induction by M-CSF (Fig. 8).

DISCUSSION

The present study was prompted by the unusual observation of planar xanthomas on the neck in a nor-

Downloaded from www.jlr.org by guest, on June 18, 2012

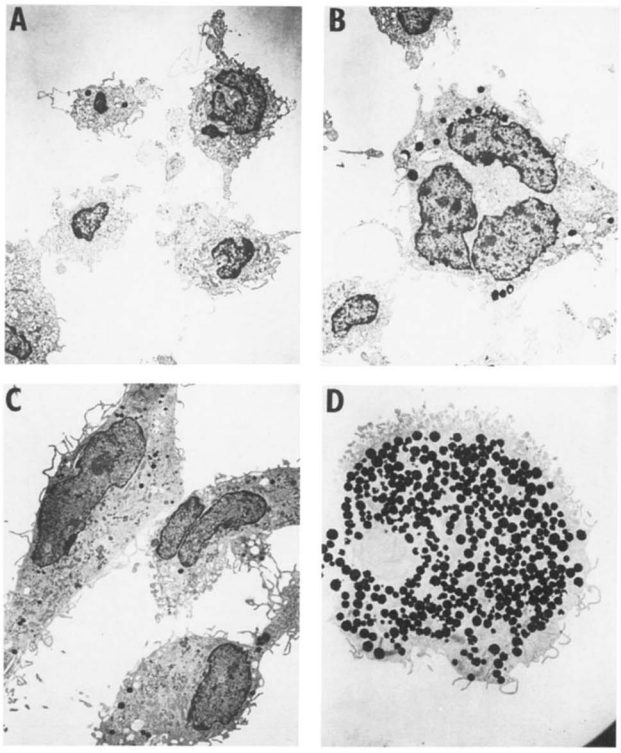

Fig. 6. Cellular phenotype of monocytes and macrophages from LC siblings with normal and high SR mRNA expression. Cells were stained with *p*-phenylenediamine and uranyl-acetate, fixed, sectioned, and visualized by electron microscopy as in Methods. A–D are as in Fig. 4.

mocholesterolemic subject (FC), previously observed only in the context of genetic apoA-I deficiency (38). Blood monocytes from FC matured abnormally rapidly into macrophages in culture and these macrophages appeared foam cell-like, unlike those of a normal subject. This cellular phenotype did not appear to be due to the presence of modified lipoproteins in plasma of FC, whose LDL displayed normal electrophoretic mobility. These observations suggested that the xanthoma phenotype in FC might be due to an abnormal property of her blood monocytes. This was confirmed by the observation that FC monocyte-macrophage uptake of modified LDL was substantially greater than normal. In addition, the xanthoma phenotype appeared to be hereditary, occurring also in the brother of FC (LC), in whom subsequent studies were performed. Monocytes from LC exhibited an approximately 3-fold greater rate of ^{125}I-labeled Ac-LDL degradation than did those from several control subjects. The level of SR activity in monocytes from LC was similar to that of macrophages from control subjects. RT-PCR assay of SR mRNA with primers specific for the cloned human SR cDNA (6) showed that in cells from normal subjects, this mRNA was induced over the 7-day period of monocyte maturation into macrophages, and that its level correlated strongly with Ac-LDL uptake among monocytes from different subjects. Furthermore, the steady-state SR mRNA levels and cell-surface expression of SR protein in monocytes from LC were several-fold higher than that of normal subjects.

Examination of monocytes from several members of two generations of the C-kindred consistently revealed an abnormal cellular phenotype. This phenotype was characterized by rapid acquisition of a macrophage-like morphology (34–36), increased adhesion, as evidenced by greater numbers of cells adhering to the cell culture matrix and cell clustering, and a subpopulation of cells that rapidly accumulated a large deposition of lipid as assessed by Oil Red O staining. Although only two of the affected members presented with xanthomas, all but one of the seven additional family members tested manifested the abnormal monocyte phenotype. Monocyte SR mRNA in these subjects was significantly elevated 5-fold compared to the average among 16 control subjects. These data suggest an association between precocious expression of the SR gene and observed greater rates of modified lipoprotein degradation and abnormal cellular phenotype observed in monocytes from members of the C family compared to normal subjects. These characteristics are vertically transmitted. Whether members of the C-kindred in whom these unusual monocytes are observed will eventually develop xanthomas, or whether an additional genetic or environmental factor such as age is important for expression of this clinical manifestation, remains to be determined.

Various reports have demonstrated that SR expression accompanies monocyte maturation. In THP-1 cells, a human monocytic cell line frequently used to study monocyte maturation into macrophages, SR activity (39–41), mRNA (41, 42), and protein (present study) are induced after stimulation of cell differentiation with phorbol ester. Most recently, induction of SR gene expression has been demonstrated upon maturation of human monocytes (43). It is not clear whether SR overexpression in affected members of the C-kindred is a cause or a consequence of the apparently precocious monocyte maturation. To determine whether the SR was overexpressed uniquely or in parallel with other genes in monocytes from affected subjects, we measured the expression of other genes suspected to be induced during monocyte maturation. The induction of apoE mRNA has been demonstrated upon phorbol ester-stimulated THP-1 cell differentiation into macrophages (44, 45). Although monocyte apoE mRNA levels tended to be higher in affected subjects than in controls, they were not commensurate with those observed in normal macrophages as were SR mRNA levels. FcγRIII (CD16) mRNA has been shown to be expressed on a subpopulation of human monocytes (46) and (immunologically)

Downloaded from www.jlr.org by guest, on June 18, 2012

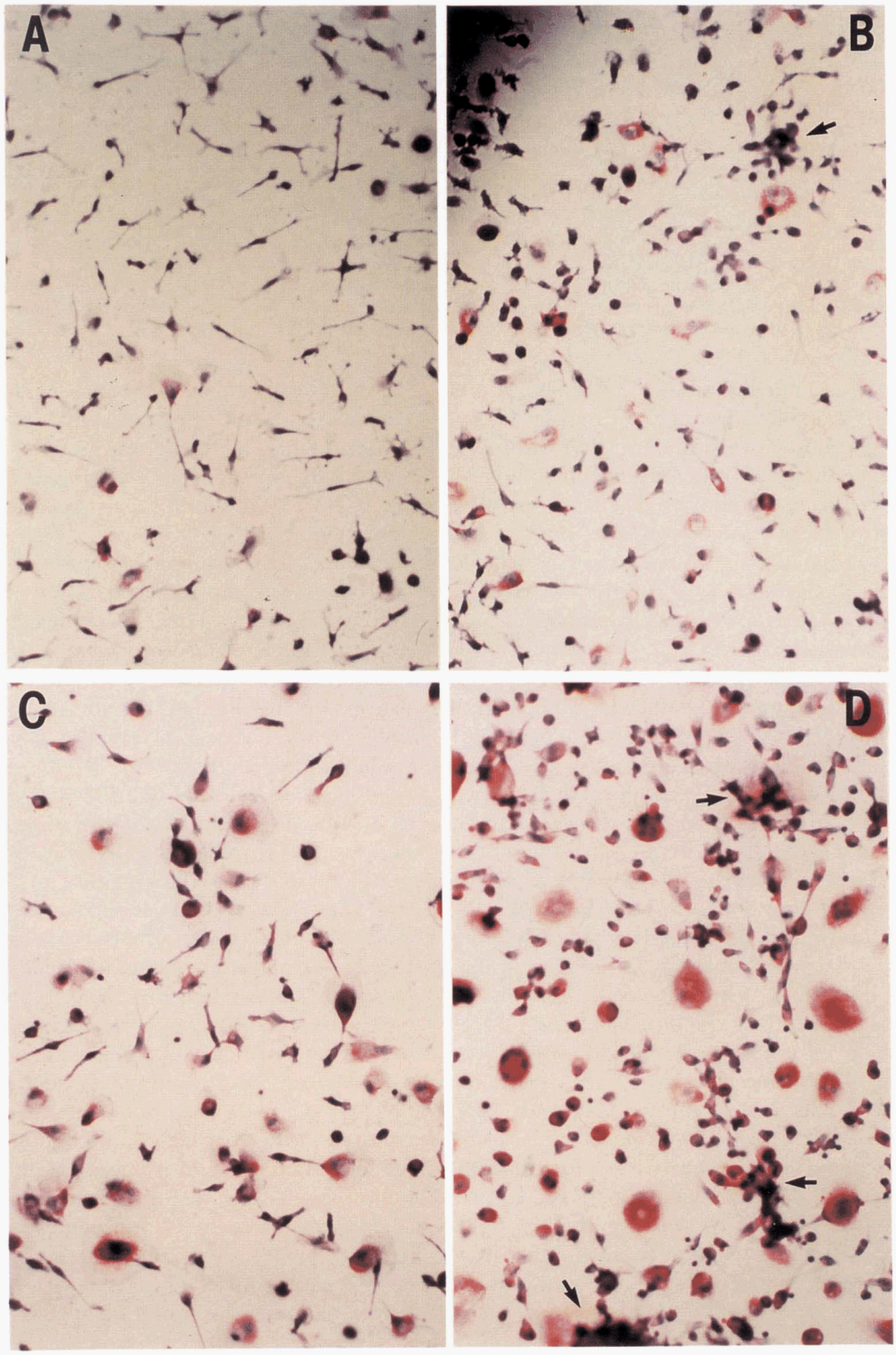

Fig. 7. Oil Red O staining of monocyte-macrophages from C family members and control. Cells were cultured for 4 or 7 days, fixed, stained as in Methods, and visualized by phase contrast microscopy (approximately 220 × magnification). A and C: 4- and 7-day macrophages from a normal 37-year-old male with SR/GAPDH mRNA of 0.39. B and D; 4- and 7-day monocyte-macrophages, respectively, from III-2 and III-3 (Fig. 3). The average number of cells/field as counted in duplicate fields of cells from subjects III-2–4 by three blinded observers were 262, 203, and 182, respectively, for day 4, and 145, 144, and 142 for day 7, versus 98 ± 1 and 92 ± 6 for two and three control subjects at days 4 and 7, respectively.

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 3. Monocyte/macrophage SR gene expression in the presence and absence of autologous serum

Subjec	Time in Culture	Autologous Serum	Nutridoma®
Control	1 day (monocytes)	0.03 ± 0.01	0.06 ± 0.04
II-5	1 day (monocytes)	0.14 ± 0.06	0.23 ± 0.08
LC (I-5)	1 day (monocytes)	0.31 ± 0.03	0.26 ± 0.02
Control	7 days (macrophages)	0.91 ± 0.04	0.93 ± 0.01
II-5	7 days (macrophages)	2.40 ± 0.13	2.93 (n = 2)
LC	7 days (macrophages)	3.45 ± 0.32	2.92 (n = 1)

Blood monocytes from indicated subjects were cultured in RPMI-1640 containing 30% autologous serum or 1% Nutridoma®. Scavenger receptor mRNA (mean ± SD of three dishes unless otherwise indicated) is expressed as the ratio of fluorescence peaks corresponding to SR/GAPDH.

to be induced in human monocytes upon maturation (35), although it is apparently not expressed by THP-1 cells (47). The level of expression of FcγRIII was indistinguishable in monocytes from affected members of the C-kindred and normal subjects. The lack of parallel overexpression of these two genes whose induction is associated with monocyte maturation suggests that SR overexpression is at least somewhat specific and perhaps not a mere consequence of precocious monocyte maturation in affected subjects.

The physiological function of the SR has been difficult to assess due to the unusually large range of ligands which it binds in vitro (8). Interestingly, recent data have supported the role of the SR as a cellular adhesion molecule (48), consistent with a possible role in monocyte maturation. In the present study, SR overexpression was associated with large multinucleated foam cells surrounded by clusters of cells. The generation of these structures, typical of granulomatous reactions, is inhibited by antibodies against cell adhesion molecules and is induced in vitro by cytokines, most notably γ-interferon (γ-IFN) (49). The reported effects of γ-IFN on SR expression are varied (50, 51), and may depend on the maturation state of monocyte-macrophages. In any case, the association of SR overexpression with apparently increased cellular adhesion in the present study supports a possible role for the SR in monocyte adhesion.

While the association between SR overexpression and foam cell formation in the present study is consistent with a role for the SR in macrophage neutral lipid accumulation, it does not exclude the possibility that induction of another putative receptor for modified lipoproteins results from monocyte adhesion and subsequent maturation. Recently, other receptors have been implicated in the cellular uptake of oxidized lipoproteins (12, 14–17). CD36 is a potential candidate for a physiological Ox-LDL receptor, because it is expressed on platelets and macrophages, mediates Ox-LDL binding in transfected cells (52), and because macrophages from CD36-deficient subjects take up 40% less Ox-LDL and accumulate 40% less CE than those from normal subjects (53). However, the ligand specificity of CD36 is distinct from that of the SR in that CD36 does not bind Ac-LDL (13), with which SR activity was assessed in the present study. Nevertheless, monocytes from affected subjects expressed abnormally high amounts of cell-surface CD36, although this expression was unusually heterogeneous.

The initial experiments described in this report were conducted with monocytes cultured in the presence of autologous serum, a general requirement for culturing human monocyte-macrophages. It has been demonstrated that products of T-lymphocytes stimulate SR expression in human monocyte-macrophages (54). Other reports have shown that cytokines such as γ-IFN inhibit (50) or increase (51), TNF-α inhibits (55) or has no effect (56), and M-CSF, GM-CSF, and IL-1 increase (57) SR expression in human monocyte-macrophages. However, as lymphocytes were not present in our experiments and these cytokines are unstable, it is unlikely that the SR overexpression observed in the present study was due to abnormal serum concentrations of these cytokines. As in normal subjects, serum M-CSF was below the limit of detection by ELISA (data not shown). Neither is the apparent overexpression likely to depend on the presence of lipoproteins, as cholesterol loading has not been observed to increase SR activity in macrophages (43, 58). In fact, monocytes from LC and his son cultured in the absence of autologous serum exhibited SR gene expression several-fold greater than control, as in autologous serum. Beyond the observation that SR overexpression in the C-kindred appears to be intrinsic to the cell, the present report does not address molecular mechanisms underlying this phenomenon. For example, although the condition appears to be hereditary it is not clear whether SR overexpression is related to the SR gene itself, to some *trans*-acting factor affecting its expression, or to a more general stimulus of monocyte maturation. Studies addressing these questions will undoubtedly elucidate some aspects of the regulation of SR gene expression and of monocyte maturation.

Although serum did not appear necessary for SR overexpression, the induction of SR mRNA by M-CSF was markedly different in LC and his son relative to

Downloaded from www.jlr.org by guest, on June 18, 2012

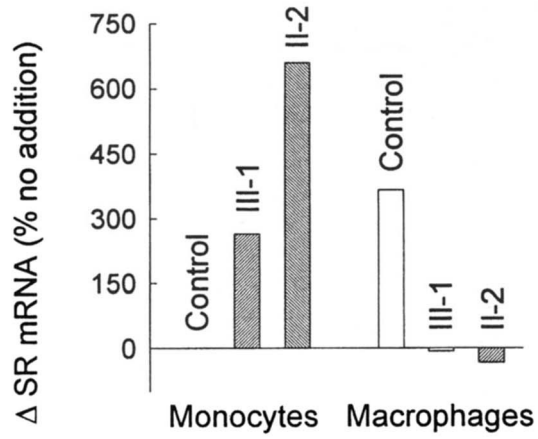

Fig. 8. Effect of M-CSF on SR gene expression in monocytes-macrophages from LC and controls. Empty and filled bars represent controls and C-family members, respectively (see Fig. 3). The control value for macrophages treated with M-CSF represents the mean of two controls (35 and 700%). Fifty ng/ml M-CSF was added 1 h and 96 h after isolation. SR mRNA was quantitated as a peak area ratio to GAPDH or to β-actin as in Fig. 2.

controls. M-CSF stimulates differentiation of hematopoietic stem cells into monocytes, and more recently has been shown to increase SR activity (57) and mRNA in human (59) and mouse peritoneal (60) macrophages. M-CSF does not up-regulate SR activity or mRNA in normal human blood monocytes, presumably due to the absence of the protooncogene-encoded receptor, *c-fms*. The abnormal response of monocytes from LC and his son to M-CSF is probably a consequence of premature maturation, as M-CSF is absent from the media shown to be sufficient for SR overexpression (Table 3). Given the role of monocyte maturation and adhesion in atherogenesis, it is unexpected that C-kindred presents no evidence for premature coronary artery disease. Though macrophage foam cells are implicated in atherosclerosis, it has recently been demonstrated that administration of recombinant M-CSF protects rabbits from granuloma foam cell formation, a model for atherosclerotic lesions (61). This effect may be attributable to increased apoE expression (45), which may promote cholesterol efflux (62) or provide apoE as ligand for increased rate of removal of plasma lipoproteins (63). Whatever the mechanism for the protective effect of M-CSF on atherosclerosis, hyper-response to M-CSF in monocytes from members of the C-kindred may confer protection from atherosclerosis and may be related to the absence of premature cardiovascular disease in this family.

This is the first report of inherited SR overexpression associated with planar xanthomas in normolipidemic subjects. Our study raises the possibility that a similar phenomenon could be involved in xanthomatosis where there is a large discrepancy between the dyslipoproteinemia and the extent of the skin lesions. These observations should prompt a systematic study of such cases to further assess the role of the SR in xanthoma and atheroma formation.

We are grateful to Dr. Ross Milne for measurement of anti-LDL antibody and to Dr. R. Schaub for measurement of plasma M-CSF. We thank Thanh-Dung Nguyen for technical assistance and Nathalie Tessier for assistance with flow cytometry. We are grateful to Dr. T. Kodama for providing anti-human SR antibodies, to Dr. C. Banka for quantitation of cellular cholesterol, and to Dr. M. Naruzcevicz for helpful discussions during the project. We are deeply indebted to FC, LC and their family for their cooperation in these studies. C.G. was supported by a stipend from the University of Montreal. This work was funded by the Medical Research Council of Canada (A.M.), the MRC/Ciba-Geigy/IRCM University Industry program (UI-11407, J.D.), and the Heart and Stroke Foundation of Quebec (A.M.).

Manuscript received 5 June 1995, in revised form 6 March 1996, and in re-revised form 15 April 1996.

REFERENCES

1. Gerrity, R. G. 1981. The role of monocytes in atherogenesis. *Am. J. Pathol.* **103:** 181–200.
2. Goldstein, J. L., and M. S. Brown. 1977. The low density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46:** 897–930.
3. Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA.* **76:** 333–337.
4. Rohrer, L., M. Freeman, T. Kodama, M. Penman, and M. Krieger. 1990. Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II. *Nature.* **343:** 570–572.
5. 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.
6. Matsumoto, A., M. Naito, H. Itakura, S. Ikemoto, A. Hitoshi, I. Hayakawa, H. Kanamori, H. Abutatani, F. Takaku, R. Wydro, D. E. Housman, and T. Kodama. 1990. Human macrophage scavenger receptors: primary structure, expression, and localization in atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA.* **87:** 9133–9137.
7. Emi, M., H. Asaoka, A. Matsumoto, H. Itakura, Y. Kurihara, Y. Wada, H. Kanamori, Y. Yazaki, E. Takahashi, M. Lepert, J-M. Lalouel, T. Kodama, and T. Mukai. 1993. Structure, organization, and chromosomal mapping of the human macrophage scavenger receptor gene. *J. Biol. Chem.* **268:** 2120–2125.
8. 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.

Downloaded from www.jlr.org by guest, on June 18, 2012

9. Arai, H., T. Kita, M. Yokode, S. Narumiya, and C. Kawai. 1989. Multiple receptors for modified low density lipoproteins in mouse peritoneal macrophages: different uptake mechanisms for acetylated and oxidized low density lipoproteins. *Biochem. Biophys. Res. Commun.* **159:** 1375–1382.
10. Kamps, J. A. A. M., J. K. Kruijt, J. Kuiper, and T. J. C. Van Berkel. 1992. Characterization of the interaction of acetylated LDL and oxidatively modified LDL with human liver parenchymal and Kupffer cells in culture. *Arterioscler. Thromb.* **12:** 1079–1087.
11. Sparrow, C. P., S. Parthasarathy, and D. Steinberg. 1989. A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. *J. Biol. Chem.* **264:** 2599–2604.
12. Stanton, L. W., R. T. White, C. M. Bryant, A. A. Protter, and G. Endemann. 1992. A macrophage Fc receptor for IgG is also a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* **267:** 22446–22451.
13. Endemann, G., L. W. Stanton, K. S. Madden, C. M. Bryant, R. T. White, and A. A. Protter. 1993. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* **268:** 11811–11816.
14. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
15. De Rijke, Y. B., and T. J. C. Van Berkel. 1994. Rat liver Kupffer and endothelial cells express different binding proteins for modified low density lipoproteins. Kupffer cells express a 95-kDa membrane protein as a specific binding site for oxidized low density lipoproteins. *J. Biol. Chem.* **269:** 824–827.
16. Pearson, A., A. Lux, and M. Krieger. 1995. Expression cloning of dSR-CI, a class C macrophage-specific scavenger receptor from *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA.* **92:** 4056–4060.
17. Elomaa, O., M. Kangas, C. Sahlberg, J. Tuukkanen, R. Sormunen, A. Liakka, I. Thesleff, G. Kraal, and K. Tryggvason. 1995. Cloning of a novel bacteria-binding receptor structurally related to scavenger receptors and expressed in a subset of macrophages. *Cell.* **80:** 603–609.
18. Kurihara, Y., A. Matsumoto, H. Itakura, and T. Kodama. 1991. Macrophage scavenger receptors. *Curr. Opin. Lipidol.* **2:** 295–300.
19. Raal, F. J., A. J. Areias, R. Waisberg, and M. Von Arb. 1995. Susceptibility of low density lipoprotein to oxidation in familial hypercholesterolaemia. *Atherosclerosis.* **115:** 9–15.
20. Lipid Research Clinics Program. 1974. Manual of Laboratory Operations, Volume 1. US Government Printing Office, Washington, DC.
21. Noble, R. P. 1968. Electrophoretic separation of plasma lipoprotein species by paper electrophoresis in albumin-containing buffer. *J. Lipid Res.* **9:** 693–700.
22. Fogelman, A. M., F. Elahi, K. Sykes, B. J. Van Lenten, M. C. Territo, and J. A. Berliner. 1988. Modification of the Recalde method for the isolation of human monocytes. *J. Lipid Res.* **29:** 1243–1247.
23. Salacinski, P. R. P., C. McLean, J. E. C. Sykes, V. V. Clement-Jones, and P. J. Lowry. 1981. Iodination of proteins, glycoproteins, and peptides using a solid phase oxidizing agent, 1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril (Iodogen). *Anal. Biochem.* **117:** 136–146.
24. Horiuchi, S., K. Takata, H. Maeda, and Y. Morino. 1985. Scavenger function of sinusoidal liver cells. *J. Biol. Chem.* **259:** 53–56.
25. Dallongeville, J., J. Davignon, and S. Lussier-Cacan. 1990. Relationship between plasma cholesterol levels and cholesterol esterification in isolated human mononuclear cells. *Life Sci.* **47:** 2351–2357.
26. Kuzuya, M., K. Yamada, T. Hayashi, C. Funaki, M. Naito, K. Asai, and F. Kuzuya. 1991. Oxidation of low-density lipoprotein by copper and iron in phosphate buffer. *Biochim. Biophys. Acta.* **1084:** 198–201.
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
28. Naito, M., H. Suzuki, T. Mori, A. Matsumoto, T. Kodama, and K. Takahashi. 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.
29. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
30. Pieretti, M., F. Zhang, Y. Fu, S. T. Warren, B. A. Oostra, C. T. Caskey, and D. L. Nelson. 1991. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell.* **66:** 817–822.
31. Bieri, J. G., E. D. Brown, and J. C. Smith. 1985. Determination of individual carotenoids in human plasma by high performance liquid chromatography. *J. Liq. Chromatogr.* **8:** 473–484.
32. Craft, N., H. Brown, and C. Smith. 1988. Effects of storage and handling conditions on concentrations of individual carotenoids, retinol, and tocopherol in plasma. *Clin. Chem.* **34:** 44–48.
33. Feingold, K. R., G. R. Castro, Y. Ishikawa, P. E. Fielding, and C. J. Fielding. 1989. Cutaneous xanthoma in association with paraproteinemia in the absence of hyperlipidemia. *J. Clin. Invest.* **83:** 796–802.
34. Nguyen, H. Q., B. Hoffman-Liebermann, and D. A. Liebermann. 1993. The zinc finger transcription factor Egr-1 is essential for and restricts differentiation along the macrophage lineage. *Cell.* **72:** 197–209.
35. Clarkson, S. B., and P. A. Ory. 1988. CD16. Developmentally regulated IgG Fc receptors on cultured human monocytes. *J. Exp. Med.* **167:** 408–420.
36. Turpin, J. A., and G. Lopez-Berestein. 1993. Differentiation, maturation, and activation of monocytes and macrophages: functional activity is controlled by a continuum of maturation. *In* Mononuclear Phagocytes in Cell Biology. G. Lopez-Berestein and J. Klostergaard, editors. CRC Press, Boca Raton. 71–99.
37. Banka, C. L., A. S. Black, C. A. Dyer, and L. K. Curtiss. 1991. THP-1 cells form foam cells in response to coculture with lipoproteins but not platelets. *J. Lipid Res.* **32:** 35–43.
38. Norum, R. A., J. B. Lakier, S. Goldstein, A. Angel, R. B. Goldberg, W. D. Block, D. K. Noffze, P. J. Dolphin, J. Edelgalss, D. D. Bogorad, and P. Alaupovic. 1982. Familial deficiency of apolipoproteins A-I and C-III and precocious coronary artery disease. *N. Engl. J. Med.* **306:** 1513–1519.
39. Hara, H., H. Tanishita, S. Yokoyama, S. Tajima, and A. Yamamoto. 1987. Induction of acetylated low density lipoprotein receptor and suppression of low density lipoprotein receptor on the cells of human monocytic leukemia cell line (THP-1 cell). *Biochem. Biophys. Res. Commun.* **146:** 802–808.
40. Akeson, A. L., K. Schroeder, C. Woods, C. J. Schmidt, and W. D. Jones. 1991. Suppression of interleukin-1β and LDL

Downloaded from www.jlr.org by guest, on June 18, 2012

scavenger receptor expression in macrophages by a selective protein kinase C inhibitor. *J. Lipid Res.* **32:** 1699–1707.
41. Moulton, K. S., H. Wu, J. Barnett, S. Parthasarathy, and C. K. Glass. 1992. Regulated expression of the human acetylated low density lipoprotein receptor gene and isolation of promoter sequences. *Proc. Natl. Acad. Sci. USA.* **89:** 8102–8106.
42. 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.* **343:** 531–535.
43. Geng, Y., T. Kodama, and G. K. Hansson. 1994. Differential expression of scavenger receptor isoforms during monocyte-macrophage differentiation and foam cell formation. *Arterioscler. Thromb.* **14:** 798–806.
44. Basheeruddin, K., C. Rechtoris, and T. Mazzone. 1992. Transcriptional and post-transcriptional control of apolipoprotein E gene expression in differentiating human monocytes. *J. Biol. Chem.* **267:** 1219–1224.
45. Auwerx, J. H., S. Deeb, J. D. Brunzell, R. Peng, and A. Chait. 1988. Transcriptional activation of the lipoprotein lipase and apolipoprotein E genes accompanies differentiation in some human macrophage-like cell lines. *Biochemistry.* **27:** 2651–2655.
46. Ziegler-Heitbrock, H. W., G. Fingerle, M. Strobel, W. Schraut, F. Stelter, C. Schutt, B. Passlick, and A. Pforte. 1993. The novel subset of CD14+/CD16+ blood monocytes exhibits features of tissue macrophages. *Eur. J. Immunol.* **23:** 2053–2058.
47. Fleit, H. B., and C. D. Kobasiuk. 1991. The human monocyte-like cell line THP-1 expresses Fc gamma RI and Fc gamma RII. *J. Leukocyte Biol.* **49:** 556–565.
48. Fraser, I., D. Hughes, and S. Gordon. 1993. Divalent cation-independent macrophage adhesion inhibited by monoclonal antibody to murine scavenger receptor. *Nature.* **364:** 343–346.
49. Fais, S., V. L. Burgio, M. Silvestri, M. R. Capobianchi, A. Pacchiarotti, and F. Pallone. 1994. Multinucleated giant cells generation induced by interferon-gamma. Changes in the expression and distribution of the intercellular adhesion molecule-1 during macrophages fusion and multinucleated giant cell formation. *Lab. Invest.* **71:** 737–744.
50. Geng, Y., and G. K. Hansson. 1992. Interferon-gamma inhibits scavenger receptor expression and foam cell formation in human monocyte-derived macrophages. *J. Clin. Invest.* **89:** 1322–1330.
51. Lopes-Virella, M. F., R. L. Klein, and H. C. Stevenson. 1987. Low density lipoprotein metabolism in human macrophages stimulated with microbial or microbial-related products. *Arteriosclerosis.* **7:** 176–184.
52. Nicholson, A. C., S. Frieda, A. Pearce, and R. L. Silverstein. 1995. Oxidized LDL binds to CD36 on human monocyte-derived macrophages and transfected cell lines: Evidence implicating the lipid moiety of the lipoprotein as the binding site. *Arterioscler. Thromb.* **15:** 269–275.
53. Nozaki, S., H. Kashiwagi, S. Yamashita, T. Nakagawa, B. Kostner, Y. Tomiyama, A. Nakata, M. Ishigami, J. I. Miyagawa, K. Kameda-Takemura, Y. Kurata, and Y. Matsuzawa. 1995. Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36-deficient subjects. *J. Clin. Invest.* **96:** 1859–1865.
54. Kotake, H., S. Oikawa, T. Naito, K. Hayasaka, and T. Toyota. 1992. T lymphocytes increase the synthesis of esterified cholesterol in human monocyte-derived macrophages by activation of the scavenger pathway. *Biochim. Biophys. Acta Mol. Basis Dis.* **1138:** 327–333.
55. Van Lenten, B. J., and A. M. Fogelman. 1992. Lipopolysaccharide-induced inhibition of scavenger receptor expression in human monocyte-macrophages is mediated through tumor necrosis factor-α. *J. Immunol.* **148:** 112–116.
56. Vijayagopal, P. 1993. Enhanced synthesis and accumulation of proteoglycans in cholesterol-enriched arterial smooth muscle cells. *Biochem. J.* **294:** 603–611.
57. 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.
58. Bottalico, L. A., G. A. Keesler, G. M. Fless, and I. Tabas. 1993. Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein[a] and apoprotein[a] internalization and degradation. *J. Biol. Chem.* **268:** 8569–8573.
59. 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.
60. De Villiers, W. J. S., I. P. Fraser, D. A. Hughes, A. G. Doyle, and S. Gordon. 1994. Macrophage-colony-stimulating factor selectively enhances macrophage scavenger receptor expression and function. *J. Exp. Med.* **180:** 705–709.
61. Schaub, R. G., M. P. Bree, L. L. Hayes, M. A. Rudd, L. Rabbani, J. Loscalzo, and S. K. Clinton. 1994. Recombinant human macrophage colony-stimulating factor reduces plasma cholesterol and carrageenan granuloma foam cell formation in Watanabe heritable hyperlipidemic rabbits. *Arterioscler. Thromb.* **14:** 70–76.
62. Huang, Y., A. Von Eckardstein, S. Wu, N. Maeda, and G. Assmann. 1994. A plasma lipoprotein containing only apolipoprotein E and with gamma mobility on electrophoresis releases cholesterol from cells. *Proc. Natl. Acad. Sci. USA.* **91:** 1834–1838.
63. Shimano, H., N. Yamada, M. Katsuki, M. Shimada, T. Gotoda, K. Harada, T. Murase, C. Fukazawa, F. Takaku, and Y. Yazaki. 1992. Overexpression of apolipoprotein E in transgenic mice: marked reduction in plasma lipoproteins except high density lipoprotein and resistance against diet-induced hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **89:** 1750–1754.
64. Tso, J. Y., X-H. Sun, T. Kao, K. S. Reece, and R. Wu. 1985. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. *Nucleic Acids Res.* **13:** 2485–2502.
65. Paik, Y., D. J. Chang, C. A. Reardon, G. E. Davies, R. W. Mahley, and J. M. Taylor. 1985. Nucleotide sequence and structure of the human apolipoprotein E gene. *Proc. Natl. Acad. Sci. USA.* **82:** 3445–3449.
66. Ravetch, J. V., and B. Perussia. 1989. Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. *J. Exp. Med.* **170:** 481–497.

Downloaded from www.jlr.org by guest, on June 18, 2012