Scavenger Receptors Class A-I/II and CD36 Are the Principal Receptors Responsible for the Uptake of Modified Low Density Lipoprotein Leading to Lipid Loading in Macrophages*

Vidya V. Kunjathoor‡, Maria Febbraio‡, Eugene A. Podrez‡, Kathryn J. Moore‡, Lorna Andersson‡, Stephanie Koehn‡, Jeongmi S. Rhee‡, Roy Silverstein§, Henry F. Hoff¶, and Mason W. Freeman‡

From the ‡Lipid Metabolism Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114, §Department of Medicine, Division of Hematology and Medical Oncology and Center for Vascular Biology, Weill Medical College of Cornell University, New York, New York 10021, and the ¶Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195

Received for publication, September 19, 2002, and in revised form, October 8, 2002

Modification of low density lipoprotein (LDL) can result in the avid uptake of these lipoproteins via a family of macrophage transmembrane proteins referred to as scavenger receptors (SRs). The genetic inactivation of either of two SR family members, SR-A or CD36, has been shown previously to reduce oxidized LDL uptake in vitro and atherosclerotic lesions in mice. Several other SRs are reported to bind modified LDL, but their contribution to macrophage lipid accumulation is uncertain. We generated mice lacking both SR-A and CD36 to determine their combined impact on macrophage lipid uptake and to assess the contribution of other SRs to this process. We show that SR-A and CD36 account for 75–90% of degradation of LDL modified by acetylation or oxidation. Cholesteryl ester derived from modified lipoproteins fails to accumulate in macrophages taken from the double null mice, as assessed by histochemistry and gas chromatography-mass spectrometry. These results demonstrate that SR-A and CD36 are responsible for the preponderance of modified LDL uptake in macrophages and that other scavenger receptors do not compensate for their absence.

Receptor-mediated endocytosis of modified LDL by macrophages has been implicated in the pathogenesis of atherosclerosis. The uptake of modified lipoproteins by macrophages leads to lipid-laden foam cells and fatty streak development in the arterial wall, one of the earliest steps in the progression of the atherosclerotic plaque. Scavenger receptor family members SR-A and CD36 have been identified as receptors for modified lipoproteins on macrophages, and their relevance to lipid uptake has been demonstrated in vitro and in vivo. Studies with SR-A or CD36 knockout mice show that disruption of either receptor pathway partially inhibits uptake of acetylated LDL (AcLDL) or oxidized LDL (OxLDL) in macrophages and retards atherosclerotic progression in hypercholesterolemic mice (1–3). It is not known, however, whether the major pathways of modified lipid uptake in macrophages comprise just these two receptors or whether other, more recently identified, scavenger receptors can contribute significantly to that process.

Since the cloning of the first two macrophage scavenger receptors (now called SR-A type I and type II (4, 5)), the broad SR family has grown considerably (reviewed in Ref. 6). The full range of scavenger receptor functions is far from clear, but these proteins have been shown to be involved in innate immune responses, cellular adhesion, and phagocytosis of apoptotic cells, in addition to their role in lipid uptake. It is evident that some functions overlap between some members, providing biological redundancy, but specialized functions are likely to characterize each receptor. On the basis of functional studies and evidence for expression in the arterial intima, only some of the SRs are good candidates for contributing to atherosclerotic foam cell formation. In addition to SR-A and CD36, these receptors include: 1) CD68 (SR-D class), 2) lectin-like oxidized LDL receptor (LOX-1, SR-E class), 3) scavenger receptor expressed by endothelial cells (SREC, SR-F class), and 4) scavenger receptor for phosphotidylserine and oxidized lipoprotein (SR-PSOX, SR-G class). In vitro studies have demonstrated that LOX-1, SR-PSOX, and SREC bind modified LDL with dissociation constants in the range of 3–36 μg/ml, comparable with that of SR-A (7–10, 11). The interaction of CD68 with modified lipoproteins is more controversial. Although OxLDL has been shown to bind CD68, this binding has been characterized primarily on the basis of ligand blot analysis (12). This methodology may overestimate the importance of the role of CD68 in lipid uptake as the majority of the protein appears to be localized in intracellular compartments that would not mediate modified lipoprotein uptake from the extracellular environment. CD68, LOX-1, and SR-PSOX have all been detected in human atherosclerotic lesions and could, therefore, be positioned to play a role in foam cell formation in the arterial intima (13–15). Their relevance to this process, however, remains uncertain.

To quantitate the relative importance of SR-A and CD36 in the macrophage response to different forms of modified LDL and to assess compensatory mechanisms for lipid uptake in their absence, we have generated mice lacking both SR-A and CD36. The binding, uptake, and degradation of AcLDL, as well as the degradation of OxLDL, have been assayed in these mice. These experiments have shown that in the absence of both SR-A and CD36, the uptake of modified LDL is significantly reduced. These results suggest that SR-A and CD36 are the major pathways of modified lipoprotein uptake in macrophages and that their combined contribution is sufficient to account for the uptake of modified LDL in vivo.

* This work was supported by Grant HL53315 (to H. F. H.) from the National Institutes of Health, a Scientist Development Grant from the American Heart Association (to E. A. P.), Grant F32HL10368 from the National Institutes of Health (to V. V. K.), and Grants HL45098 and HL06675 from the National Heart, Lung, and Blood Institute (to M. W. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

To whom correspondence should be addressed. Tel.: 617-726-5906; Fax: 617-726-2879; E-mail: freeman@frodo.mgh.harvard.edu.

1 The abbreviations used are: LDL, low density lipoprotein; AcLDL, acetylated LDL; OxLDL, oxidized LDL; SR, scavenger receptor; SREC, scavenger receptor expressed by endothelial cells; SR-PSOX, scavenger receptor for phosphotidylserine and oxidized lipoprotein; LPS, lipopolysaccharide; GC-MS, gas chromatography-mass spectrometry.
Scavenger Receptors in Modified Lipoprotein Uptake

EXPERIMENTAL PROCEDURES

Reagents—Cell culture reagents were from Invitrogen. 125I and 32P were obtained from PerkinElmer Life Sciences.

Animals—SR-A/II-deficient mice were generously provided by Dr. T. Kodama (University of Tokyo) (1). The CD36−/− mice used in these studies were generated as described (18). Both lines were backcrossed for five generations to C57BL/6J mice prior to intercrossing of the lines to generate mice lacking both SR-A and CD36. Double knockout mice (SR-A−/−/CD36−/−) were generated from heterozygote intercrosses at the expected ratio of 1:16. SR-A and CD36 genotypes were verified by PCR analyses of tail DNA as described previously (1, 16). Mice were maintained on a 12-h light/dark cycle and given free access to rodent chow and water.

Preparation of Peritoneal Macrophages—Mice were injected intraperitoneally with 3% thioglycollate broth, and elicited macrophages were collected after 4 days by peritoneal lavage as described previously (17). Macrophages were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum overnight, and non-adherent cells were removed by washing. Macrophages prepared in this manner routinely stained positively for CD11b (>95%) and F4/80 (>70%) by flow cytometry.

Lipoprotein Iodination and Modifications—Human LDL (density = 1.019–1.063) was purchased from Biomedical Technologies (Stoughton, MA). Iodination of LDL was accomplished by a modified iodine monochloride reaction (18). Acetylation of LDL and 125I-LDL was performed as described previously (19) and the specific activity of 125I-acetylated LDL ranged from 400–600 cpm/ng of LDL protein. For copper ion-mediated oxidation, LDL and 125I-LDL (250 μg/ml) were incubated with 5 μM CuSO4 at 37 °C for 8 (mildly oxidized) or 24 h (extensively oxidized) (17). Oxidation was terminated by addition of 50 μM butylated hydroxytoluene and 200 μM EDTA. Mildly OxLDL had a relative electrophoretic mobility 2.5 times that of native, unmodified LDL, whereas extensively OxLDL had a relative mobility of 3.5 times. The specific activity of 125I-copper-oxidized LDL was 300–600 cpm/ng of protein. LDL oxidized by the myeloperoxidase/hydrogen peroxide/nitrite system was prepared as described (19).

Modified LDL Binding, Degradation, and Foam Cell Formation Assays—Binding assays were performed at 4 °C using 10 μg/ml modified 125I-LDL (AcLDL or OxLDL) in the presence or absence of a 20-fold excess of unlabelled modified LDL competitor as described previously (9). Specific binding of AcLDL or OxLDL was calculated as the total binding of modified 125I-LDL minus binding in the presence of unlabelled modified LDL competitor. Degradation of modified LDL was assessed at 37 °C for 5 h using 10 μg/ml modified 125I-LDL (AcLDL or OxLDL) in the presence or absence of a 20-fold excess of unlabelled modified LDL competitor as described (7). Specific degradation was calculated as the total degradation of modified 125I-LDL minus degradation in the presence of unlabelled competitor. All measurements were performed on triplicate samples and were conducted independently at least three times. To assess foam cell formation, cells were incubated with 40 μg/ml LDL or OxLDL for 48 h, fixed in 4% paraformaldehyde, and stained with Oil Red O to visualize lipid accumulation. Staining was recorded on an Olympus X10 microscope equipped with a digital camera.

Cholesterol Content—Free and esterified cellular cholesterol were measured by gas chromatography as described (20, 21). 6 × 105 cells were incubated with 40 μg/ml OxLDL or AcLDL for 48 h and then extracted with hexane/isopropanol (3:2). Stigmastester (Sigma) was added as an internal standard, and lipid extracts were washed once with water and divided into two equal aliquots. One lipid aliquot was saponified for determination of total cholesterol, and the second aliquot was used for cholesteryl ester determination. Lipid extracts were evaporated to dryness and analyzed by GC-MS (G2613A system, Agilent Technologies, Palo Alto, CA) equipped with a J&W DB17 fused silica capillary column (15 m × 0.25 mm inner diameter × 0.5 μm; J&W Scientific, Folsom, CA). The GC temperature program was as follows: the initial temperature was 260 °C for 5 min, and then it was increased to 280 °C (5 °C/min) and held at 280 °C for 11 min. A model 5973N mass-selective detector (Agilent Technologies) was used in scan modes to identify the samples. Cholesterol measurements were obtained on triplicate samples and normalized to cellular protein content. The esterified cholesterol fraction was calculated by subtrac-

RESULTS

Mice deficient in both SR-A and CD36 (SR-A−/−/CD36−/−) were derived in the expected Mendelian ratios and appeared healthy when compared with wild type, SR-A (SR-A−/−), or CD36 (CD36−/−) single null mice. Macrophages from all genotypes (wild type; SR-A−/−; CD36−/−; and SR-A−/−/CD36−/−) expressed similar levels of the macrophage cell surface markers, CD11b and F4/80, as assessed by flow cytometry (Fig. 1A). Furthermore, macrophages from all genotypes produced similar levels of tumor necrosis factor-α and IL-6 in response to bacterial LPS (Fig. 1B). These findings indicate that the absence of both SR-A and CD36 does not appear to alter the expression of myeloid differentiation markers, nor does it affect the cytokine responses to the prototypical stimulator of macrophage inflammatory pathways, bacterial endotoxin.

Degradation and Binding of Modified LDL—To define the contributions of SR-A and CD36 to modified lipid uptake, we performed binding and degradation studies on macrophages derived from wild type, SR-A−/−, CD36−/−, or SR-A−/−/CD36−/− mice using LDL modified by acetylation or oxidation, using either copper ions or a myeloperoxidase/hydrogen peroxide/nitrite oxidizing system. Fig. 2A shows that during a 5-h incubation with 125I-AcLDL, SR-A−/−/CD36−/− macrophages degraded 70% less ligand than did wild type macrophages (p < 0.0001). CD36, whose role in AcLDL degradation is not well understood, appears to play a relatively minor role in AcLDL degradation, as evidenced by the very modest decline (13%) measured in the CD36−/− macrophages. In macrophages lacking both SR-A and CD36, AcLDL degradation fell ~80%. Thus, the effect of the loss of both receptors appears to reflect the sum-

In contrast to the effect on degradation of the ligand, specific binding of AcLDL was decreased by only 44% in SR-A−/− macrophages (p < 0.0001, Fig. 2B). Somewhat surprisingly, CD36 contributed more to AcLDL binding than to its degradation as a 28% decrease in binding to CD36−/− macrophages was observed (Fig. 2B). In macrophages taken from SR-A−/−/CD36−/− mice, AcLDL binding fell by 54%. These results indicate that alternative mechanisms can contribute to AcLDL binding to macrophages, accounting for nearly half of the total AcLDL bound. Nevertheless, relatively little of this alternatively bound ligand is internalized and degraded,
FIG. 1. Macrophages lacking SR-AI/II and CD36 express myeloid markers and respond to inflammatory stimuli. As shown in A, wild type (WT), SR-AI/II−/−, CD36−/−, and SR-AI/II−/−/CD36−/− (DKO (double knock-out)) macrophages were stained for CD11b, CD36, and CD68. Histograms of each staining (solid line) are overlaid with isotype control (dashed and dotted lines). As shown in B, macrophages of all genotypes were stimulated with 10 ng/ml LPS for 6 h, and supernatants were assayed for tumor necrosis factor-α and IL-6 by enzyme-linked immunosorbent assay assay. Data are expressed as the mean of triplicate samples ± standard deviation.
as evidenced by the 80% decrease in degradation in the SR-A⁻/⁻/CD36⁻/⁻ mouse.

The evidence demonstrating that both SR-A and CD36 can bind and degrade oxidized LDL is substantial. The relative affinities of this ligand for the two receptors appear to be determined by the degree of oxidation of the LDL. Extensive oxidation of LDL appears to be required for rapid uptake via SR-A, whereas mildly oxidized LDL is preferentially internalized via CD36 (2, 3, 22). To assess the contributions of other receptors to the process of oxidized LDL uptake and degradation, we performed binding and degradation studies using LDL that had been subjected to mild or extensive oxidizing conditions. A third form of oxidation, myeloperoxidase-hydrogen peroxide-nitrite modification, which may represent a more physiologically relevant pathway of LDL oxidation, was also tested (3, 19, 23).

The mildly oxidized LDL used in these experiments had a relative electrophoretic mobility that was twice that of native LDL (data not shown). This corresponds to a derivatization of 25–30% of its lysine residues (24, 25). A 68% decrease in degradation and a 90% decrease in binding, relative to that in wild type macrophages, was observed when this modified LDL was incubated with the CD36⁻/⁻ macrophages (Fig. 3A). In contrast, binding and degradation of mildly oxidized LDL were reduced by only 25 and 40%, respectively, in the SR-A⁻/⁻ macrophages. A further reduction in binding and degradation (up to 90%) was observed in macrophages lacking both SR-A and CD36 (Fig. 3A). The more extensively oxidized LDL had an electrophoretic mobility 3.5 times that of native LDL, corresponding to 40–50% derivitization of lysine residues. At this level of oxidation, receptor specificity was altered with the ligand degraded preferentially through SR-A rather than CD36 (Fig. 3B). A 47% decrease in degradation of this ligand was measured in macrophages lacking SR-A as compared with a 26% decrease in CD36⁻/⁻ macrophages. Interestingly, a similar discordance between binding and degradation, as noted for AcLDL, was again observed. CD36⁻/⁻ macrophages showed a much greater decrease in binding (62%) than did the SR-A⁻/⁻ cells (13%) despite the reverse rank order for degradation (Fig. 3B). When both receptors were inactivated, a 69% decrease in binding and a 78% decrease in degradation were measured.

The results obtained with LDL oxidized by the myeloperoxidase/hydrogen peroxide/nitrite system were concordant with that of LDL mildly oxidized by copper ions. CD36 was the preferred receptor for this ligand (Fig. 4). Degradation of this form of oxidized LDL was reduced by 60%, and binding was reduced by 74% in CD36⁻/⁻ macrophages. The corresponding changes in SR-A⁻/⁻ macrophages were 30 and 23% decreases for degradation and binding, respectively. A 75% decrease in both binding and degradation was measured in the SR-A⁻/⁻/CD36⁻/⁻ macrophages (Fig. 4). Taken together, these experiments clearly demonstrate that SR-A and CD36 account for the vast majority of lipid uptake and degradation involving modified LDL that has been altered either by acetylation or by varying degrees of oxidation. No more than 25%, and as little as 10%, of these modified lipoproteins appears to be taken up and degraded by pathways not involving SR-A or CD36.

**Foam Cell Formation**—To directly assess the potential of the alternative scavenger receptor pathways to contribute to foam cell formation, we incubated SR-A⁻/⁻/CD36⁻/⁻ macrophages with oxidized LDL and assessed lipid accumulation morphologically by Oil Red O staining and quantitatively by GC-MS. Macrophages were incubated with AcLDL or mildly oxidized LDL for 48 h and then stained with Oil Red O to detect cholesteryl ester accumulation (Fig. 5). As would be predicted by our binding and degradation results, the intensity of Oil Red O staining was dramatically reduced in both the CD36⁻/⁻ and SR-A⁻/⁻/CD36⁻/⁻ macrophages. To better quantitate this decline, GC-MS was utilized to measure cholesterol and cholesteryl ester mass in macrophages incubated with modified lipoprotein or with AcLDL or OxLDL (Table I). In wild type cells incubated with OxLDL, total cholesterol mass increased ~30% with an increase in cholesteryl ester mass from undetectable levels to 20 μg/mg of cellular protein. For AcLDL, the comparable numbers were a 70% increase in total cholesterol mass and the accumulation of 25 μg of cholesteryl ester from a previously undetectable level. Strikingly, in SR-A⁻/⁻/CD36⁻/⁻ macrophages, incubation with OxLDL led to no change in the total cholesterol mass and no accumulation of cholesteryl ester. Incubation of these cells with AcLDL produced a 30% increase in total cholesterol mass, but again, no cholesteryl ester accumulation was detected. These findings demonstrate that in the absence of both SR-A and CD36, no cholesteryl ester accumulation occurs in response to incubation with either AcLDL or OxLDL, confirming the histologic impression of the absence of any foam cell formation.
FIG. 3. Degradation and binding of Cu²⁺-modified OxLDL is significantly decreased in macrophages lacking SR-AI/II and CD36. Peritoneal macrophages were incubated with 10 μg/ml ¹²⁵I-Cu²⁺-oxidized LDL in the presence or absence of excess unlabeled competitor, using two different preparations of OxLDL: mildly oxidized (A) and extensively oxidized (B). Degradation was performed at 37 °C for 5 h, and binding was performed at 4 °C for 2 h as for AcLDL (see the legend for Fig. 2). Samples were measured in triplicate. Data are representative of at least two independent experiments. *, p < 0.05 versus wild type (WT); **, p < 0.05 versus CD36⁻⁻⁻⁻.

FIG. 4. Degradation and binding of LDL oxidized by a myeloperoxidase/hydrogen peroxide/nitrite system is significantly decreased in macrophages lacking SR-AI/II and CD36. Peritoneal macrophages were incubated with 10 μg/ml ¹²⁵I-myeloperoxidase nitrite-modified LDL in the presence or absence of excess unlabeled competitor. As shown in A, degradation was performed at 37 °C for 5 h. WT, wild type. As shown in B, binding was performed at 5 °C for 2 h. Samples were measured in triplicate. Data are representative of at least two independent experiments. *, p < 0.05 versus wild type.
Scavenger Receptors in Modified Lipoprotein Uptake

DISCUSSION

Although macrophage foam cell formation is the earliest histologic hallmark of the atherosclerotic lesion, the mechanism by which intimal macrophages accumulate cholesterol remains controversial (26–28). As the family of macrophage scavenger receptors provides a high affinity uptake mechanism for the accumulation of lipid derived from modified LDL, it is widely believed that they play a critical role in foam cell formation. The number of SR family members has grown substantially in recent years, but because of their redundancy, it has been difficult to assess the relative contribution of each of these proteins to the lipid uptake process. We, therefore, sought to characterize the receptor pathways in macrophages that could mediate modified LDL uptake and determine their contribution to cholesteryl ester accumulation and foam cell formation.

Mice lacking SR-A or CD36 were intercrossed, producing animals lacking both receptors. Comparison of these animals with wild type mice and single null progenitors of the double knockouts permitted us to quantitate the amount of lipid uptake mediated by each of the two pathways, as well as all alternative pathways not involving SR-A or CD36. The results demonstrate that SR-A and CD36 are the critical contributors to modified lipoprotein uptake in macrophages in vitro. In the absence of both receptors, lipoprotein uptake and degradation were reduced by 75–90%, using four different types of modified LDL. Using LDL mildly oxidized by copper ions, we demonstrate that lipid accumulation can occur in sufficient quantities to account for the histologic conversion of macrophages to foam cells. In addition, gas chromatography-mass spectrometry measurements directly quantitated this lipid accumulation and found ~200 nmol of total cholesterol/mg of cellular protein in macrophages incubated with OxLDL for 48 h. 30 nmol of that total represented esterified cholesterol. When acetylated LDL was employed as the ligand, the total cholesterol content was 263 nmol/mg of cellular protein, of which 38 nmol was esterified cholesterol.

The alternative receptor pathways, whose contributions could only be quantitated in the absence of both CD36 and SR-A, account for a very small percentage of the lipoprotein degradation measured. Moreover, we were unable to demonstrate histologic conversion of SR-A−/−/CD36−/− macrophages to foam cells when exposed to OxLDL. This finding was confirmed by GC-MS, which showed no accumulation of cholesteryl ester in the SR-A−/−/CD36−/− cells. These results provide strong evidence that more recently identified SRs, including CD68, LOX-1, SR-PSOX, and SREC, play a minor role, if any, in modified LDL uptake by macrophages.

Of interest, the process of lipid uptake mediated by SR-A and CD36 appears to be distinct in that SR-A binding of ligand results in a greater degradation rate than does CD36 binding. Previous studies of OxLDL and AcLDL uptake have shown trafficking of these lipids to different intracellular compartments, and Lougheed et al. (29) have suggested that this difference could arise from the involvement of separate receptors in the uptake process. Our results suggest this to be the case with AcLDL uptake predominantly driven by the action of SR-A, whereas mildly OxLDL uptake is primarily mediated by CD36 (3). The degree of oxidation, however, strongly influences the pathway of OxLDL degradation. With more extensively oxidized LDL, the contribution of the SR-A pathway is enhanced. Interestingly, the reduction in binding of extensively oxidized LDL to CD36−/− macrophages, relative to wild type macrophages, was greater than that seen with SR-A-deficient cells despite the larger decline in degradation in the latter. Although this result could be a consequence of a higher recycling rate of internalized SR-A molecules back to the plasma membrane, it is also possible that SR-A links to a distinct and more efficient pathway of ligand degradation. Studies are currently underway to explore these differences in greater detail.

The studies reported here utilized in vitro modified lipoproteins. By employing several forms of oxidized LDL, as well as acetylated LDL, we have tried to examine the full spectrum of modified lipoproteins that could interact with scavenger receptors. Whether different forms of modified lipoproteins exist in vivo remains unknown. Thus, our conclusion concerning the limited role of the alternative SRs does not preclude the possibility that they participate in the uptake of other forms of lipoproteins or that they could affect atherogenesis via altogether different mechanisms. Of note, however, quantitative real-time reverse transcription-PCR measurements of the

Table I

Accumulation of total and esterified cholesterol in peritoneal macrophages

| Treatment    | Cellular Cholesterol | Wild type | SRA−/−; CD36−/− | Wild type | SRA−/−; CD36−/− |
|--------------|----------------------|-----------|-----------------|-----------|-----------------|
|              |                      | Total     | Esterified      | Total     | Esterified      |
| None         | 97.07 ± 4.3          | 108.75 ± 7.4 | Not detected | 108.75 ± 7.4 | Not detected |
| OxLDL        | 129.08 ± 2.7         | 103.86 ± 11.2 | 20.32 ± 4.9 | 20.32 ± 4.9 |
| AcLDL        | 170.79 ± 21.7        | 131.28 ± 13.9 | 25.07 ± 2.6 | 25.07 ± 2.6 |

*p < 0.05 versus no treatment.
mRNA encoding CD68, LOX-1, SR-PSOX, or SREC showed no increase in the SR-A*−/−CD36*−/− macrophages, indicating that the transcription of these receptors is not up-regulated in response to the loss of any function that SR-A or CD36 performs in a macrophage (data not shown). The generation of mice lacking these alternative SRs will be required to shed light on their other potential functions, as well as any contribution they may make to in vivo lipid uptake. In addition, the recent report of Kruth et al. (30), demonstrating that native LDL can promote lipid accumulation in vitro in phorbol ester-treated human monocyte-macrophages, suggests that lipid accumulation and foam cell formation might also arise from non-scavenger receptor-mediated pathways.

The generation of mice lacking both of the critical receptor pathways necessary for modified lipoprotein uptake should facilitate future studies of atherogenesis. By intercrossing the SR-A*−/−CD36*−/− mice with hyperlipidemic mouse strains, it should be possible to determine whether these receptors are, in fact, required for macrophage foam cell formation in vivo. These studies are currently in progress. Although both single null mice showed decreases in atherosclerosis in previous studies, foam cell formation was not completely abrogated. Our in vitro data, demonstrating the absence of foam cell formation and cholesteryl ester accumulation in the double null macrophages, suggest that the hyperlipidemic SR-A*−/−CD36*−/− mouse might indeed fail to generate macrophage foam cells in vivo. The results of these in vitro studies should therefore clarify both the utility of the in vitro ligands in predicting in vivo lipid uptake and the potential role of alternative pathways in macrophage foam cell formation. Through such experimental approaches, it should be possible to directly test the hypothesis that foam cell formation plays a causal role in the pathogenesis of atherosclerosis and is not simply a marker of lipid deposition in the arterial intima. Should that hypothesis prove correct, the delineation of the major pathways of lipid uptake in macrophages provides potential targets for therapies designed to ameliorate atherosclerotic heart disease.

REFERENCES

1. Suzuki, H., Kurihara, Y., Takeya, M., Kamada, N., Kataoka, M., Jishage, K., Ueda, O., Sakaguchi, H., Higashi, T., Doi, T., Matsumoto, A., Azuma, S., Noda, T., Toyoda, Y., Ikakura, H., Yasaki, Y., Horuchi, S., Takahashi, K., Kar Krujit, J., van Berkel, T. J. C., Steinbrecher, U. P., Ishibashi, S., Maeda, N., Gordon, S., and Kodama, T. (1997) Nature 386, 292–296
2. Febbraio, M., Podrez, E. A., Smith, J. D., Hajjar, D. P., Hazen, S. L., Hoff, H. E., Sharma, K., and Silverstein, R. L. (2000) J. Clin. Invest. 105, 1049–1056
3. Podrez, E. A. (2000) J. Clin. Invest. 105, 1105–1108
4. Kodama, T., Freeman, M., Rohrer, L., Zabrecky, J., Matsuda, P., and Krieger, M. (1990) Nature 343, 531–535
5. Rohrer, L., Freeman, M., Kodama, T., Penman, M., and Krieger, M. (1990) Nature 343, 570–572
6. Peiser, L., and Gordon, S. (2001) Microbes Infect. 3, 149–159
7. Freeman, M. W., Ekkcl, Y., Rohrer, L., Penman, M., Freedman, N. J., Chisolm, G. M., and Krieger, M. (1991) Proc. Natl. Acad. Sci. USA 88, 4931–4935
8. Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1997) J. Biol. Chem. 272, 31217–31220
9. Andersson, L. P., and Freeman, M. W. (1998) J. Biol. Chem. 273, 19592–19601
10. Moriwaki, H., Kume, N., Sawamura, T., Ayasami, T., Hoshikawa, H., Ochi, H., Nishi, E., Masaki, T., and Kita, T. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 1541–1547
11. Shimaoka, T., Kume, N., Minami, M., Hayashida, K., Kataoka, H., Kita, T., and Yonehara, S. (2000) J. Biol. Chem. 275, 40666–40666
12. Ramprasad, M. P., Fischer, W., Wittum, J. L., Sambrano, G. R., Quehenberger, O., and Steinberg, D. (1995) Proc. Natl. Acad. Sci. USA 92, 9528–9534
13. Geng, Y. J., Holm, J., Nygren, S., Brzezinski, M., Stemme, S., and Hansson, G. K. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 1995–2002
14. Kataoka, H., Kume, N., Minami, M., Moriwaki, H., Sawamura, T., Masaki, T., and Kita, T. (2000) Ann. N. Y. Acad. Sci. 902, 328–335
15. Minami, M., Kume, N., Shimao, T., Kataoka, H., Hayashida, K., Akiyama, Y., Nagata, I., Ando, K., Nobuyoshi, M., Hanyu, M., Komea, M., Yonehara, S., and Kita, T. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 1796–1800
16. Febbraio, M., Abumrad, N. A., Hajjar, D. P., Sharma, K., Cheng, W. P., Pearce, S. F., and Silverstein, R. L. (1999) J. Biol. Chem. 274, 19055–19062
17. Moore, K. J., Fabunmi, R. P., Andersson, L. P., and Freeman, M. W. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 1647–1654
18. Goldstein, J. L., Basu, S. K., and Brown, M. S. (1983) Methods Enzymol. 98, 241–260
19. Podrez, E. A., Schmitt, D., Hoff, H. E., and Hazen, S. L. (1999) J. Clin. Invest. 103, 1547–1560
20. Jacobs, N. L. (1997) J. Lipid Res. 38, 1973–1987
21. Moore, K. J., Rosen, E. D., Fitzgerald, M. L., Randow, F., Andersson, L. P., Altshuler, D., Milstone, D., Mortensen, R. M., Speigelman, B. M., and Freeman, M. W. (2001) Nat. Med. 7, 41–47
22. Loughheed, M., Lunn, C. M., Ling, W., Suzuki, H., Kodama, T., and Steinbrecher, U. P. (1997) J. Biol. Chem. 272, 12938–12944
23. Podrez, E. A., Batyreva, E., Shen, Z., Zhang, R., Deng, Y., Sun, M., Finton, P. J., Shan, L., Gugiu, B., Hoff, H. F., Salomon, R. G., and Hazen, S. L. (2002) J. Biol. Chem. 8, 8
24. Steinbrecher, U. P., Witzum, J. L., Parthasarathy, S., and Steinberg, D. (1987) Arteriosclerosis 7, 135–143
25. Chang, Y. H., Abdalla, D. S. P., and Sevanian, A. (1997) Free Radic. Biol. Med. 23, 202–214
26. Stary, H. C., Chandler, A. B., Glagov, S., Gunton, J. R., Insull, W., Jr., Rosenfeld, M. E., Schaffer, S. A., Schwartz, C. J., Wagner, W. D., and Wassler, R. W. (1994) Arterioscler. Thromb. 14, 840–856
27. Lusis, A. J. (2000) Nature 407, 233–241
28. Kruth, H. S. (2001) Front. Biosci. 6, D429–D455
29. Loughheed, M., Moore, E. D., Scriven, D. R., and Steinbrecher, U. P. (1999) Arterioscler. Thromb. Vasc. Biol. 19, 1881–1890
30. Kruth, H. S., Huang, W., Ishii, I., and Zhang, W. Y. (2002) J. Biol. Chem. 277, 34573–34580