A Direct Role for the Macrophage Low Density Lipoprotein Receptor in Atherosclerotic Lesion Formation*

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To evaluate the contribution of the macrophage low density lipoprotein receptor (LDLR) to atherosclerotic lesion formation, we performed bone marrow transplantation studies in different mouse strains. First, LDLR(-/-) mice were transplanted with either LDLR(+/+) marrow or LDLR(-/-) marrow and were challenged with an atherogenic Western type diet. The diet caused severe hypercholesterolemia of a similar degree in the two groups, and no differences in the aortic lesion area were detected. Thus, macrophage LDLR expression does not influence foam cell lesion formation in the setting of extreme LDL accumulation. To determine whether macrophage LDLR expression affects foam cell formation under conditions of moderate, non-LDL hyperlipidemia, we transplanted C57BL/6 mice with either LDLR(-/-) marrow (experimental group) or LDLR(+/-) marrow (controls). Cholesterol levels were not significantly different between the two groups at baseline or after 6 weeks on a butterfat diet, but were 40% higher in the experimental mice after 13 weeks, mostly due to accumulation of β-very low density lipoprotein (β-VLDL). Despite the increase in cholesterol levels, mice receiving LDLR(-/-) marrow developed 63% smaller lesions than controls, demonstrating that macrophage LDLR affects the rate of foam cell formation when the atherogenic stimulus is β-VLDL. We conclude that the macrophage LDLR is responsible for a significant portion of lipid accumulation in foam cells under conditions of dietary stress.

The development of atherosclerosis involves the recruitment of monocyte-derived macrophages into the subendothelial space and their transformation into lipid-laden foam cells (1). Because foam cell transformation is a consequence of an excessive accumulation of lipid droplets in the cytoplasm, it has long been hypothesized that macrophage lipoprotein receptor expression may play a role in this process. The macrophage expresses several receptors capable of taking in native or modified lipoproteins, including the low density lipoprotein receptor (LDLR)1 (2), the LDLR-related protein, and the scavenger receptor (3). The association between elevated levels of LDL cholesterol and increased risk of atherosclerosis suggests that the LDLR might mediate the cholesterol accumulation by macrophage-derived foam cells. However, the uptake of fresh LDL by macrophages is at least one order of magnitude lower than that of acetylated LDL, suggesting that scavenger receptor expression is physiologically more relevant than LDLR expression in this cell type (4, 5). Observations from studies both in vivo and in vitro indicate that macrophage and leukocyte LDLR expression is not required for foam cell formation (4, 6, 7). Leukocytes express little LDLR activity, which is promptly down-regulated by incubation with LDL (8). Similarly, macrophage expression of LDLR is limited (2, 9) and easily inhibited by excess cholesterol, suggesting that the physiologic contribution of the LDLR to lipoprotein uptake by the macrophage may be limited in the presence of elevated LDL cholesterol levels (4).

Most importantly, individuals with homozygous familial hypercholesterolemia, who lack functional LDLR, show accumulation of cholesteryl esters in macrophages (10), a proof that the LDLR is not necessary for foam cell transformation of macrophages. However, Tabas and co-workers (11, 12) have reported that J774 cells and mouse peritoneal macrophages bind and internalize unmodified LDL. In addition, the macrophage LDLR has the ability to take up other atherogenic lipoproteins, such as β-very low density lipoprotein (β-VLDL) and chylomicron remnants (13–15). In fact, β-VLDL is the only naturally occurring (unmodified) lipoprotein that induces transformation of macrophages into foam cells (16, 17). Therefore, macrophage LDLR expression may have a relevant impact in the metabolism and clearance of β-VLDL and may modulate foam cell formation when the main atherogenic stimulus is the diet-induced remnant.

Murine bone marrow transplantation (BMT) studies have been used to examine the role of the leukocyte LDLR in lipoprotein metabolism and atherosclerosis. LDLR deficient (-/-) mice have increased plasma LDL levels and enhanced susceptibility to diet-induced atherosclerosis (18, 19). We and others (20, 21) have shown that reconstitution of wild-type LDLR expression in the hematopoietic system of LDLR(-/-) mice (LDLR(+/+)→LDLR(-/-)), has no measurable effects on plasma lipoprotein levels and turnover time. We also demonstrated that elimination of LDLR expression from the hematopoietic cells C57BL/6 mice has no effect on plasma lipid parameters on a normal chow diet (20). Based on the qualitative observation that both LDLR(-/-)→LDLR(-/-) and LDLR(-/-)→LDLR(+/+) mice developed extensive atherosclerosis

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1 The abbreviations used are: LDLR, low density lipoprotein receptor; BMT, bone marrow transplantation; VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins.
in the aortic valves after 20 weeks on a diet containing 1.25% cholesterol and 0.5% sodium cholate, Boaивет al. (21) have suggested that the leukocyte LDLR may not play a major role in lesion development. Herijers et al. (22) found similar results in LDLR(−/−) mice and LDLR(+/−)−LDLR(−/−) mice after 20 weeks on a diet containing 1.0% cholesterol. The dietary conditions in both of these studies induced severe hypercholerolema and complex atherosclerotic lesions. Therefore, a contribution of leukocyte LDLR expression to foam cell formation might have been obscured under these conditions of extreme hypercholesterolemia and advanced atherosclerosis.

The goal of the current study was to examine whether the reconstitution of macrophage LDLR activity in LDLR(−/−) mice or its elimination in C57BL/6 mice would have an impact on the extent of atherosclerosis in a setting of less severe hypercholesterolemia and during an early stage of atherosclerosis. To this end, we compared LDLR(−/−) or C57BL/6 mice reconstituted with either LDLR(+/−) or LDLR(+/+) marrow. In LDLR(−/−) mice, extreme atherosclerosis developed irrespective of the kind of marrow received, indicating that, in the presence of massive elevations in LDL levels, the macrophage LDLR is not a modulator of foam cell formation. However, C57BL/6 mice that received LDLR(+/−) marrow had a mean aortic lesion area that was 70% less compared with mice that received LDLR(+/+). This effect was evident despite a 40% higher plasma cholesterol level in LDLR(−/−)→C57BL/6 mice, which was due to the accumulation of β-VLDL. Thus, our results are compatible with a major role of macrophage LDLR in the regulation of foam cell transformation when the atherogenic stimulus is β-VLDL.

MATERIALS AND METHODS

Animals—A colony of C57BL/6J mice is established in our animal facility. The LDLR(−/−) mice were originally purchased from Jackson Laboratories (Bar Harbor, ME) and backcrossed into the C57BL/6 background. Recipient LDLR(−/−) mice were at the 7th backcross, whereas donors for the C57BL/6 study were at the 10th backcross into C57BL/6. LDLR genotype was determined by polymerase chain reaction as described previously (20). All mice were maintained in microisolation cages on a rodent chow diet containing 4.5% fat (PMI 5010, St. Louis, MO) and acidified water (pH 2.8). Atherogenic diets used included the Western diet, containing 21% milkfat and 0.15% cholesterol, and acidified water (pH 2.8). Atherogenic diets used included the Western diet, containing 21% milkfat and 0.15% cholesterol, and acidified water (pH 2.8).

Recipient LDLR(−/−) mice were lethally irradiated (9 Gy), and 4 h later, 5 × 10^6 bone marrow cells in 0.3 ml were transplanted by tail vein injection. Serum Cholesterol and Triglycerides Analysis—Nonfasting mice were anesthetized with methoxyflurane (Mallinckrodt Veterinary, Inc., St. Louis, MO), and 4 h later, 5 × 10^6 bone marrow cells in 0.3 ml were transplanted by tail vein injection.

Separation of Lipoproteins—Mouse serum was fractionated on a Superox 6 column (Amer sham Pharmacia Biotech) using an HPLC system model 600 (Waters, Milford, MA). A 100-µl aliquot of serum was injected onto the column and separated using a buffer containing 0.15 M NaCl, 0.01 M NaHPO₄, 0.1 M EDTA (pH 7.5) at a flow rate of 0.5 ml/min. Forty 0.5-ml fractions were collected, and fractions 11–40 were analyzed for cholesterol content. Fractions 13–17 contain VLDL and chylomicrons; fractions 18–24 contain intermediate density lipoproteins (IDL) and LDL; fractions 25–31 contain high density lipoproteins (HDL), and fractions 32–40 contain nonlipoprotein-associated serum proteins.

Quantitation of Arterial Lesions—Mice were sacrificed and flushed with 30 ml of saline by slow injection through the left cardiac ventricle. The heart with ascending aorta was embedded in OCT and snap-frozen in liquid N₂. Cryosections of 10-µm thickness were taken from the region of the proximal aorta starting from the end of the aortic sinus and for 300 µm distally, according to the procedure of Paigen et al. (24). Cryosections were stained with Oil-Red-O and counterstained with hematoxylin. Quantitative analysis of lipid-stained lesions was performed using an Imaging System KS 300 (Release 2.0, Kontron Elektron GmbH). Color threshold was used to delimit the Oil-Red-O stained lesion area, and the lesion area was determined as mean lesion area per section in square micrometers.

Immunocytochemical Analysis—Immunocytochemical staining of tissue samples for LDLR and macrophages was performed on 5-µm thick serial cryosections from the proximal aortas. Sections were fixed in acetone and incubated with either rabbit antibodies to bovine LDLR, which cross-react with mouse LDLR (Rb. 455, a gift from Dr. Innerarity, Gladstone Institute, San Francisco, CA) and Ab683, a gift from Dr. Herz, University of Texas, Southwestern Medical Center, Dallas, TX) or a mouse monoclonal antibody to mouse macrophages (GALM, Accurate Chemicals, Westbury, NY). Primary antibodies were used at dilutions of 1:250, 1:300 and 1:30, respectively, and incubated overnight at 4 °C. After washing, the sections were treated with goat biotinylated antibodies to rabbit and rat IgGs (both from PharMingen, San Diego, CA) and incubated with avidin-biotin complex labeled with alkaline phosphatase (Vector Laboratories, Burlingame, CA). The enzyme activity was visualized with Fast Red TR/naphthol AS-NX substrate (Sigma). Sections were counterstained with hematoxylin. Nonimmune rabbit and rat sera were used as negative controls in the place of primary antibodies. Photomicroscopy was performed on a Zeiss Axioskop with Plan-Neofluar objectives (Zeiss, Thornwood, NY).

In Situ Hybridization—A 167-base insert consisting of nucleotides 2106–2273 of the mouse LDLR cDNA (primers were gift from Dr. Ishibashi, University of Tokyo, Japan) was cloned into pBluescript II SK phagemid (Stratagene, La Jolla, CA). Another 59-base fragment consisting of nucleotides 735–794 of the mouse LDLR gene was amplified using primers (CAGTGCTCCTCTACGTGTTGC and GCTG-TAGCCTGATGTATTC), and cloned into pGEM-T vector (Promega, Madison, WI). Antisense and sense riboprobes for LDLR were labeled with 35S-uridine (BNA transcription kit, Stratagene). Cryosections (5-µm thick) were fixed for 30 min in 4% paraformaldehyde-phosphate-buffered saline, treated for 15 min with proteinase K (5 µg/ml), prehybridized for 1 h at 55 °C in a mixture (0.3 M NaCl, 20 mM Tris, pH 8.0, 5 mM EDTA, 1 × Denhardt’s solution, 10 mM dithiothreitol, 10% dextran sulfate, 50% formamide) and, after addition of the riboprobes, incubated overnight at 55 °C. The sections were then treated for 30 min with RNase A (20 µg/ml), washed, coated with autodiagnostic emulsion (Kodak NTB-2) and exposed for 2–3 weeks. After development, the slides were counterstained with hematoxylin. The sense probe was used in parallel as a negative control.

RESULTS

The role of the macrophage LDLR in foam cell formation and atherosclerosis was examined in two different murine bone marrow transplantation models, using dietary conditions which differed significantly in ambient levels of plasma lipids and lipoproteins. The duration of the atherogenic diet in each model was selected to induce lesions consisting primarily of macrophage-derived foam cells. For a model of severe hypercholesterolemia, lethally irradiated (9 Gy) male LDLR(−/−) mice were transplanted with either LDLR(+/−) marrow (experimental group; n = 5) or LDLR(−/−) marrow (controls; n = 14). Eight weeks post-BMT, the mice were challenged with an atherogenic diet containing 21% milkfat and 0.15% cholesterol diet for 9 weeks. To examine the contribution of the macrophage LDLR to foam cell formation under conditions of more moderate hypercholesterolemia, 8-wk-old lethally irradiated (9 Gy) female C57BL/6 mice were transplanted with either LDLR(−/−) marrow (experimental group; n = 11) or LDLR(+/+) marrow (controls; n = 11). Eight weeks post-BMT.
the mice were challenged with an atherogenic diet containing 19.5% butterfat, 1.25% cholesterol, and 0.5% cholic acid for 13 weeks.

In the LDLR(−/−) mice transplanted with either LDLR(+/+) or LDLR(−/−) marrow, there were no significant differences in serum cholesterol or triglyceride levels between the two groups at baseline or after 6 weeks on a chow diet or after 6 or 9 weeks on the Western-type diet (Table I). We have previously reported that on a chow diet the lipoprotein profiles in the LDLR(+/+)→LDLR(−/−) mice and LDLR(−/−)→LDLR(−/−) mice are indistinguishable, with HDL as the predominant lipoprotein class and a significant accumulation of LDL cholesterol (20). After 6 weeks on the Western-type diet, examination of the distribution of cholesterol among the serum lipoprotein fractions by size-exclusion chromatography in the LDLR(−/−) mice revealed a massive accumulation of cholesterol in the VLDL/IDL/LDL range, with a relative decrease in the HDL cholesterol compared with the lipoprotein profile on a normal chow diet (Fig. 1A). A similar pattern was seen in the LDLR(++)/→LDLR(−/−) mice (data not shown). Thus, in the LDLR(−/−) mice, the Western-type diet induced severe hypercholesterolemia due to an accumulation of both LDL cholesterol and VLDL/IDL-sized remnant lipoproteins.

Consistent with our previous results, examination of serum cholesterol and triglyceride levels in the C57BL/6 mice transplanted with either LDLR(+/+) or LDLR(−/−) marrow revealed no significant differences on a chow diet 8 weeks post-BMT (Table II) (20). After 6 weeks on the atherogenic diet, no significant differences in serum cholesterol or triglyceride levels existed between the two groups, although the serum cholesterol levels had doubled from baseline (Table II). However, after 13 weeks on the butterfat diet, the mean serum cholesterol level in the LDLR(−/−)→C57BL/6 mice was significantly higher than in the LDLR(+/+)→C57BL/6 mice (Table II). Examination of the distribution of cholesterol among the serum lipoprotein fractions by size-exclusion chromatography after 13 weeks on the atherogenic diet revealed an accumulation of cholesterol in the VLDL/IDL/LDL range, with a relative decrease in the HDL cholesterol compared with the lipoprotein profile on a normal chow diet (Fig. 1B). Levels of HDL cholesterol in 8 LDLR(−/−)→C57BL/6 and 7 LDLR(+/+)→C57BL/6 mice were 68.6 ± 10.0 and 67.4 ± 9.37 (mg/dl ± S.D.), respectively, (p = 0.820), and the ratio of total cholesterol to HDL cholesterol was higher in the LDLR(−/−)→C57BL/6 mice than in the controls (5.01 versus 3.63). Thus, in the C57BL/6 mice, the butterfat diet induced a moderate hypercholesterolemia due to an accumulation of remnant lipoproteins.

The aortas were collected for quantitative analysis of the extent of atherosclerosis from the LDLR(−/−) recipient group after 9 weeks on the Western-type diet. The mean lesion area in the proximal aorta was not significantly different in the LDLR(+/+)→LDLR(−/−) mice and LDLR(−/−)→LDLR(−/−) mice (42.815 ± 6.358 versus 39.032 ± 8.700 μm² ± S.E.; p = 0.76) (Fig. 2A). In contrast, the mean lesion area in the experimental LDLR(−/−)→C57BL/6 mice was significantly less than in the control LDLR(+/+)→C57BL/6 mice (1,158 ± 260 μm² ± S.E.; p = 0.031) after 13 weeks on the butterfat diet (Fig. 2B). Additionally, there was not a significant correlation between individual serum cholesterol levels and the extent of lesion area in the experiment using C57BL/6 recipient mice (r = 0.45; 0 < 0.16).

Immunocytochemical studies using a macrophage specific antibody, MOMA2 (25), revealed that the lesions consisted almost exclusively of macrophage-derived foam cells in both

**Table I**

| Group | Serum lipid | Baseline | 6 weeks post-BMT | 6 weeks on Western diet | 9 weeks on Western diet |
|-------|-------------|----------|------------------|------------------------|-----------------------|
| LDLR(+/+)→LDLR(−/−) | Cholesterol | 278 ± 8 | 251 ± 5 | 1,114 ± 69 | 1,058 ± 73 |
| n = 15 | Triglycerides | 159 ± 11 | 153 ± 7 | 494 ± 51 | 246 ± 49 |
| LDLR(−/−)→LDLR(−/−) | Cholesterol | 259 ± 8 | 273 ± 8 | 1,005 ± 67 | 962 ± 75 |
| n = 14 | Triglycerides | 181 ± 16 | 145 ± 6 | 432 ± 43 | 369 ± 51 |

**Fig. 1. Lipoprotein distribution in LDLR(−/−) or C57BL/6 recipient mice on an atherogenic diet.** Lipoprotein distribution was determined by fast performance liquid chromatography followed by cholesterol analysis of each fraction. Fractions 13–17 contain VLDL; fractions 18–24 contain IDL/LDL; and fractions 25–31 contain HDL. Fractions 32–40 are the bottom nonlipoprotein-associated proteins. Panel A shows the average lipoprotein distribution of LDLR(−/−)→LDLR(−/−) mice 8 weeks after transplantation (post-BMT) on a normal chow diet (n = 5), compared with the profile after 6 weeks on the Western type diet (post-BMT) (n = 4). Panel B compares the average lipoprotein distribution of LDLR(+/+)→C57BL/6 mice (n = 4) and LDLR(−/−)→C57BL/6 mice (n = 4) after 13 weeks on the atherogenic diet.
TABLE II

| Group of animals | Serum lipid | Baseline | 8 weeks post-BMT | 13 weeks on butyrate diet |
|------------------|-------------|----------|-----------------|--------------------------|
| LDLR(+/+)→C57BL/6 | Cholesterol | 115±1    | 102±2           | 244±5                    | 234±9                    |
| n = 12           | Triglycerides | 86±5     | 91±5            | 44±3                     | 45±4                     |
| LDLR(−/−)→C57BL/6| Cholesterol | 122±3    | 105±3           | 284±46                   | 330±21*                  |
| n = 12           | Triglycerides | 105±6    | 109±6           | 39±3                     | 54±3                     |

*p < 0.001.

Fig 2. Mean lesion area in proximal aorta of LDLR(−/−)→LDLR(+/+)→LDLR(−/−) versus LDLR(+/+)→LDLR(−/−)→C57BL/6 versus LDLR(+/+)→C57BL/6 mice (A) and LDLR(+/+)→LDLR(−/−)→LDLR(+/+)→C57BL/6 versus C57BL/6 mice (B). The analysis was performed on 300 μm of the proximal aorta starting at the end of the aortic sinus using alternate 10-μm cryosections. The sections were stained with Oil-Red-O and the lesion area was measured by digitizing morphometry in a blinded fashion. Panel A shows the data from 14 LDLR(+/+)→LDLR(−/−)→C57BL/6 mice versus 9 LDLR(+/+)→LDLR(−/−)→C57BL/6 mice after 9 weeks on the Western-type diet, and panel B shows the data from 11 LDLR(+/+)→C57BL/6 versus 11 LDLR(+/+)→C57BL/6 mice. Data are expressed as mean lesion area per section in square micrometers. Significant difference was determined by Student’s t test.

The current studies provide strong evidence for a direct role of the macrophage LDLR in foam cell formation and atherogenesis in vivo. The macrophage LDLR has been implicated in the binding and internalization of β-VLDL and chylomicron remnants by a number of in vitro studies (13–15). On an atherogenic diet, C57BL/6 mice develop relatively modest hypercholesterolemia due to an accumulation of β-VLDL, providing an attractive model for testing the hypothesis that the macrophage LDLR influences foam cell formation and atherogenesis in vivo. Therefore, female C57BL/6 mice were transplanted with either LDLR(−/−) marrow or LDLR(+/+) marrow and challenged with the butterfat diet. As expected, the mice in both groups developed moderate hypercholesterolemia (Table II). Although serum cholesterol levels were not significantly different between the two groups at baseline or after 6 weeks on the butterfat diet, the serum cholesterol levels were 40% higher in the experimental LDLR(−/−)→C57BL/6 mice compared with controls after 13 weeks. The main lipoprotein class accumulating under conditions of dietary stress is a β-VLDL remnant, and the higher cholesterol levels observed in the experimental mice were due primarily to higher levels of the β-VLDL in this group. However, this significant additional accumulation of a potentially atherogenic lipoprotein did not have the expected consequences on the artery wall. In fact, quantitative analysis of the extent of atherosclerosis in the proximal aorta revealed that C57BL/6 mice reconstituted with LDLR(−/−) marrow developed 63% smaller lesion area than the LDLR(+/+) marrow recipients. Thus, our results are compatible with a major role of macrophage LDLR in foam cell formation when the atherogenic stimulus is β-VLDL.

It is noteworthy that no correlation was detected between individual serum cholesterol levels and the extent of lesion area in the experiment using C57BL/6 recipient mice, suggesting that the effect of intervention was not mediated by plasma lipoprotein changes. After 13 weeks on the atherogenic diet, the LDLR(−/−)→C57BL/6 mice had higher serum total cholesterol levels and a higher total cholesterol to HDL cholesterol ratio.
than the control group. Levels of serum triglycerides did not differ between the two groups, and the lipoprotein distributions were qualitatively similar as determined by size-exclusion chromatography. Subtle changes in HDL or apolipoprotein B composition are unlikely to explain the difference in atherosclerosis. A 75% decrease in HDL cholesterol due to knockout of the apoAI gene does not effect the extent of diet-induced atherosclerosis in 129xC57BL/6 hybrid mice (28). In apoE-deficient mice expressing only apoB100 or apoB48, serum cholesterol levels predict lesion area but the differences in apoB containing lipoproteins do not (29). In the current study, despite the presence of an apparently more atherogenic lipoprotein profile, the LDLR(−/−)→C57BL/6 mice developed significantly less atherosclerosis. Therefore, the lack of macrophage LDLR expression in these mice was apparently protective, resulting in less foam cell formation.

In the current studies, the role of macrophage LDLR expression in foam cell formation was examined in LDLR(+/+)→LDLR(+/−) mice and LDLR(+/−)→LDLR(−/−) controls under dietary conditions resulting in less severe hypercholesterolemia than in the studies of Boisvert et al. (21) and Herijgers et al. (22). The mice in both groups developed severe hypercholesterolemia due to accumulation of VLDL, IDL, and LDL cholesterol, but there were no significant differences in serum cholesterol between the two groups at baseline or after 6 or 9 weeks on the atherogenic diet. The extent of atherosclerosis was examined when the lesions had not progressed beyond fatty streak lesions. Quantitative analysis of the extent of atherosclerosis demonstrated that there were no differences between the two groups. Thus, in the presence of extremely high levels of serum cholesterol, macrophage LDLR expression did not influence the extent of foam cell lesion formation. Our results extend the findings of Boisvert et al. (21) and Herijgers et al. (22) by showing that reconstitution of LDLR expression in leukocytes and macrophages of LDLR(−/−) mice during the foam cell-rich fatty streak stage of atherogenesis does not influence the extent of atherosclerosis.

The contribution of leukocyte LDLR expression to foam cell formation and atherogenesis was examined in two different murine bone marrow transplantation models, which differed dramatically with respect to the levels of plasma lipids and lipoproteins. Although the studies in LDLR deficient mice seem
to indicate that the macrophage LDLR does not influence foam cell formation, a different picture emerges when one looks at the effect of eliminating macrophage LDLR from C57BL/6 mice on a high fat diet. In this experimental model of moderate hypercholesterolemia due predominantly to the accumulation of β-VLDL, macrophage expression of the LDLR does play a physiologic role in foam cell formation in vivo, as evidenced by the significant (p = 0.031) 70% reduction in lesion area shown by mice transplanted with LDLR(-/-) marrow. Although there is inherent variation in the diet-induced model of atherosclerosis in C57BL/6 mice, the result is clearly statistically significant, and the extent of aortic atherosclerotic lesion area obtained in C57BL/6 mice under similar dietary conditions has been shown to be highly reproducible (30). Overall, our results emphasize the importance of genetic background, dietary conditions, and stage of atherosclerosis in designing experiments to elucidate the physiologic role of expression of a gene by the macrophage in atherosclerosis.

We and others (20, 21) have previously reported that plasma lipid and lipoprotein levels do not differ in LDLR(-/-) mice reconstituted with LDLR(+/+) or LDLR(-/-) marrow on a chow diet. Based on these studies, we concluded that leukocyte LDLR activity does not play a significant role in the clearance of LDL in plasma. In contrast, Herijgers et al. (22) reported that 4 weeks post-BMT LDLR(+/+)→LDLR(−/−) mice have significantly lower levels of total serum cholesterol level and LDL cholesterol than control LDLR(−/−)→LDLR(−/−) mice, but the decrease in LDL was less prominent by 12 weeks post-BMT, suggesting it was a transient effect (22). Consistent with our current results, Herijgers et al. (22) did not see any significant differences in total serum cholesterol levels between LDLR(+/+)→LDLR(−/−) and LDLR(−/−)→LDLR(−/−) mice on a diet containing 1% cholesterol, demonstrating that the leukocyte LDLR does not influence plasma cholesterol levels in the presence of severe hypercholesterolemia. In addition, we have reported that plasma lipid and lipoprotein levels do not differ in C57BL/6 mice reconstituted with LDLR(+/+) or LDLR(−/−) marrow on a chow diet (20). In C57BL/6 mice on a chow diet, the majority of cholesterol is found in HDL, making it unlikely that a contribution of leukocyte LDLR expression to the clearance of LDL from plasma would be detected. Yet, in the current studies we found that after 13 weeks on the butterfat diet the serum cholesterol levels were 40% higher in the LDLR(−/−)→C57BL/6 mice than LDLR(+/+)→C57BL/6 mice because of accumulation of β-VLDL, van Berkel and co-workers (31, 32) have shown that Kupffer cells contribute significantly to the clearance of LDL from plasma in the rat. Therefore, a lack of Kupffer cell LDLR expression in the LDLR(−/−)→C57BL/6 may be responsible for the increased level of serum cholesterol relative to the LDLR(+/+)→C57BL/6 mice. These results suggest that the leukocyte LDLR expression can significantly influence plasma cholesterol levels under conditions of moderate hypercholesterolemia due to an accumulation of β-VLDL.

We have previously reported that six weeks after bone marrow transplantation in LDLR−/− recipient mice both the myeloid and lymphoid cells were essentially completely reconstituted by cells of donor origin (20). In addition, we have previously demonstrated that bone marrow transplantation results in reconstitution of the arterial wall with macrophages of donor origin (26). In the current study, we report that macrophages in the atherosclerotic lesions of LDLR(+/+)→LDLR(−/−) mice stain positive for the LDLR, a finding consistent with results reported by Boisvert et al. (21). These findings indicate that the LDLR is being expressed by macrophage-derived foam cells even in the setting of extreme hypercholesterolemia with high levels of LDL cholesterol. However, the results of our in situ hybridization studies indicate that the level of macrophage-derived foam cell LDLR expression in the LDLR(+/+)→LDLR(−/−) mice was down-regulated relative to the level of expression noted in the LDLR(+/+)→C57BL/6 mice. Thus, it is possible that the LDL receptor participates in foam cell formation when levels of LDL or total cholesterol are not high enough to completely down-regulate the macrophage LDLR.

In conclusion, the wider relevance of these results lay in the proof that expression of LDLR from macrophages in the artery wall directly mediates the progression of atherosclerosis and that protective changes in the macrophage can overcome atherogenic changes such as diet-induced hyperlipidemia in the plasma compartment. The contribution of macrophage LDLR to foam cell formation and atherosclerosis may be substantial given the large body of evidence implicating triglyceride-rich remnant lipoproteins in human atherosclerotic disease (33, 34). In addition, the majority of people who die of coronary heart disease have normal to modestly elevated levels of cholesterol (35), a setting in which the macrophage LDLR may contribute significantly to foam cell formation. This concept emphasizes the need for developing therapeutic strategies, based either on drugs or gene transfer, aimed at reducing the recruitment of monocytes in the artery wall or delaying macrophage transformation into foam cells to reduce the development and progression of coronary atherosclerosis.
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