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Role of the macrophage very-low-density lipoprotein receptor in atherosclerotic lesion development

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Abstract

Objectives: The very-low-density lipoprotein receptor (VLDLr) is highly expressed in macrophage-rich areas of atherosclerotic lesions. The exact role of the macrophage VLDLr in atherosclerotic lesion development, however, is presently unclear.

Methods and results: To assess the role of the macrophage VLDLr in atherosclerotic lesion development in vivo, we used the technique of bone marrow transplantation to selectively disrupt or reconstitute the VLDLr in macrophages in VLDLr+/+ and VLDLr−/− mice, respectively. After 10 weeks high-cholesterol diet feeding, the lesion area in control transplanted wild-type mice was $17 \pm 4 \times 10^3 \pm \mu m^2$. Disruption of the macrophage VLDLr by transplanting bone marrow from VLDLr−/− mice to wild-type VLDLr+/+ littermates resulted in a tendency to a slight reduction in lesion size to $12 \pm 3 \times 10\,\mu m$. The mean atherosclerotic lesion area, measured in control transplanted VLDLr−/− mice, lacking the VLDLr in all tissues was $12 \pm 3 \times 10^3\ \mu m^2$. Interestingly, reconstitution of the macrophage VLDLr in VLDLr-deficient recipients resulted in a 2.7-fold increase ($P < 0.05$) in the mean atherosclerotic lesion area to $32 \pm 3 \times 10^3\ \mu m^2$.

Conclusions: The macrophage VLDLr facilitates atherosclerotic lesion development, probably by mediating the accumulation of atherogenic lipoproteins.

Keywords: VLDL receptor; Lipoproteins; Macrophage; Atherosclerosis; Bone marrow transplantation

1. Introduction

The very-low-density lipoprotein (VLDL) receptor is a member of the low-density lipoprotein (LDL) receptor superfamily [1–4]. The VLDL receptor (VLDLr) is a multiligand receptor that binds β-VLDL [1,5,6], chylomicron remnants [7], and lipoprotein lipase (LPL) [6,8]. In addition, it binds several ligands that are not directly linked to lipoprotein metabolism, including receptor-associated protein (RAP) [9,10], urokinase-plasminogen activator (uPA)/plasminogen activator inhibitor type 1 (PAI-1) complexes [8], serine proteinase/serpin complexes [11], and reelin [12].

The VLDLr is abundantly expressed in heart, skeletal muscle, and adipose tissue, but not in liver [1,13,14]. Since the VLDLr is primarily expressed in tissues with an active triglyceride metabolism, it was hypothesised to play a pivotal role in the delivery of free fatty acids from VLDL-triglycerides to these tissues as an energy source [13–15]. Disruption of the gene in mice leads to a reduction in adipose tissue mass [16,17] and an increase in serum triglycerides upon fasting [18]. Furthermore, Tacken et al. showed that on an LDL receptor-deficient background, VLDLr deficiency

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results in a significant increase in serum triglyceride levels on a high fat diet, while overexpression significantly decreased serum triglycerides [19].

In healthy human vascular tissue, the VLDLr is expressed by endothelial and smooth muscle cells. In addition, in atherosclerotic lesions the VLDLr is also found in macrophage-derived foam cells [20–23]. The abundant expression of the VLDLr in atherosclerotic lesions does suggest a potential role for this receptor in atherosclerosis. In vitro studies have suggested a role for the VLDLr in smooth muscle cell migration [24,25]. In agreement, Tacken et al. demonstrated an increased smooth muscle cell-mediated intimal thickening of the femoral artery in absence of the VLDLr after placement of a non-restrictive cuff [26]. VLDLr deficiency, however, did not affect atherosclerotic lesion development in LDL receptor-deficient mice [26] nor in human apoB transgenic mice [18].

In addition to its role in smooth muscle cell migration, the VLDLr has been implicated in macrophage foam cell formation. Transfection of the rabbit VLDLr in LDL receptor negative Chinese hamster ovary cells increased β-VLDL-induced lipid accumulation [27]. Moreover, Kosaka et al. recently showed that transformation of PMA-induced THP-1 macrophages to foam cells by β-VLDL correlated with VLDL receptor expression [28].

To specifically assess the biological role of the macrophage VLDLr in foam cell formation and atherosclerotic lesion development in vivo we used the technique of bone marrow transplantation. Using this technique, chimeras were created that specifically lack or express the VLDLr in monocytes and macrophages. Interestingly, reconstitution of macrophage VLDLr expression in VLDLr-deficient mice markedly increased atherosclerotic lesion development, indicating a facilitating role for the macrophage VLDLr in atherosclerotic lesion development.

2. Methods

2.1. Mice

VLDL receptor (VLDLr) deficient mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The mice were hybrids between the C57Bl/6 and 129Sv strains (F3 generation of backcrosses to C57Bl/6). Heterozygous VLDLr-deficient mice were cross-bred to generate homozygous knockout (VLDLr−/−) mice and non-transgenic wild-type (VLDLr+/+) littermates. The presence of the targeted and wild-type VLDLr alleles was assessed by PCR amplification of DNA extracted from tail biopsies (primers 5′-GAC TTC AGC TGA CAT GCA ATA GC-3′, 5′-GGC TGA CGG CCA CAC TGC TC-3′, and 5′-GAT TGG GAA GAC AAT AGC AGG CAT GC-3′). Mice were housed in sterilised filter-top cages and given unlimited access to food and water. Mice were maintained on sterilised regular chow, containing 4.3% (w/w) fat and no cholesterol (RM3, Special Diet Services, Witham, UK), or were fed a semi-synthetic high-cholesterol diet, containing 15% (w/w) fat, 1% (w/w) cholesterol, and 0.5% cholate (Diet N, Hope Farms, Woerden, The Netherlands). Drinking water was supplied with antibiotics (83 mg/L ciprofloxacin and 67 mg/L polymyxin B sulphate) and 6.5 g/L sucrose. Animal experiments were performed at the Gorlaeus laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with the National Laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

2.2. Bone marrow transplantation

To induce bone marrow aplasia, female VLDLr+/+ and VLDLr−/− mice were exposed to a single dose of 9 Gy (0.19 Gy/min, 200 kV, 4 mA) total body irradiation, using an Andrex Smart 225 Röntgen source (YXLON International, Copenhagen, Denmark) with a 6 mm aluminium filter, 1 day before transplantation. Bone marrow was isolated by flushing the femurs and tibias from female VLDLr+/+ and VLDLr−/− donor mice with phosphate-buffered saline. Single-cell suspensions were prepared by passing the cells through a 30 μm nylon gauze. Irradiated recipients received 0.5×10^7 bone marrow cells by intravenous injection into the tail vein.

2.3. Assessment of chimerism

The hematologic chimerism of the transplanted mice was determined in genomic DNA from bone marrow by PCR. Three oligonucleotides were used for PCR amplification to detect both the wild-type and the null mutant VLDLr gene simultaneously, as described above.

2.4. Serum lipid analyses

After an overnight fasting-period, approximately 100 μl blood was drawn from each individual mouse by tail bleeding. The concentrations of free cholesterol and cholesteryl esters in serum were determined using enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany). The distribution of lipids over the different lipoproteins in serum was determined by fractionation of 30 μl serum of each mouse using a Superose 6 column (3.2 mm × 30 mm, Smart-system, Pharmacia, Uppsala, Sweden). Total cholesterol content of the effluent was determined using enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany).

2.5. Histological analysis of the aortic root

To analyse the development of atherosclerosis at the aortic root, transplanted VLDLr+/+ and VLDLr−/− mice were sacrificed after 10 weeks high-cholesterol diet feeding. The arterial tree was perfused in situ with phosphate-buffered saline (100 mm Hg) for 20 min via a cannula in the left ventricular apex. The heart plus aortic root and descending