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Review

Triglyceride Rich Lipoprotein -LPL-VLDL Receptor and Lp(a)-VLDL Receptor Pathways for Macrophage Foam Cell Formation

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Very low-density lipoprotein (VLDL) receptor is a member of the low-density lipoprotein (LDL) receptor family. It binds triglyceride rich lipoprotein (TGRL) but not LDL, because it recognizes apolipoprotein (apo)E only but not apoB. The VLDL receptor functions as a peripheral lipoprotein receptor in concert with lipoprotein lipase (LPL) in heart, muscle, adipose tissue and macrophages. In contrast to the LDL receptor, VLDL receptor binds apo E2/2 VLDL and apoE3/3 VLDL particles, and its expression is not down-regulated by intracellular lipoproteins. It has been reported that both LDL-cholesterol (LDL-C) and postprandial triglyceride (chyromicron and VLDL remnants) are risk factors for human atherosclerotic cardiovascular disease (ASCVD). True ligands such as lipoprotein particles of the VLDL receptor are chyromicron remnant (CMR) and VLDL remnant (postprandial hyperlipidemia). Although the oxidized LDL (oxLDL)-scavenger receptors pathway is considered to be the main mechanism for macrophage foam cell formation, it seems that the TGRL-LPL-VLDL receptor pathway is also involved. Since Lp(a) is one of the ligands for the VLDL receptor, the Lp(a)-VLDL receptor pathway is another potential alternative. The expression of VLDL receptor protein in mouse macrophages is modest compared to that in rabbit and human macrophages, both *in vitro* and *in vivo*. Therefore, we need to elucidate the mechanism of human ASCVD not by using the mouse model and scavenger receptors pathway but instead using the rabbit model and VLDL receptor pathway, respectively.

Key words: VLDL receptor, Lipoprotein lipase, Lp(a), Atherosclerosis, Macrophage foam cell formation

Introduction

Macrophage foam cell formation is a critical step in the initial stages of the atherosclerotic process. The "Response to injury" hypothesis indicates that injury of endothelial cells (ECs) results in a series of reactions that include overexpression of adhesion molecules and secretion of various chemokines, which in turn result in the recruitment of circulating monocytes and T lymphocytes[1]. The alternative "response to retention" hypothesis argues that LDL enters the intimal space, where it is retained by subendothelial extracellular matrix molecules, predominantly proteoglycans. Proteoglycan-bound LDL increases the susceptibility of LDL to oxidation. The latter (oxLDL) enhances the synthesis of monocyte chemoattractant protein (MCP-1) by ECs and smooth muscle cells (SMCs), and acts directly as a chemoattractant to monocytes[2]. The atherosclerotic process starts with the transmigration of monocytes into the arterial intima, where they differentiate into macrophages. Simultaneously, macrophages take up oxLDL through the scavenger receptors (SR-A, CD36, and Lox1)[3]. However, evidence suggests that the SR-A and CD36 are not involved in macrophage foam cell formation since targeted deletion of SR-A and CD36 does not abrogate macrophage foam cell formation or reduces the atherosclerotic area in apoE knockout (KO) mice[4, 5]. Although LDL-C remains the primary treatment target to reduce the risk of ASCVD, epidemiological studies have shown that high triglyceride levels (remnant lipoprotein or non-fasting triglycerides) are independently associated with increased incidence of cardiovascular events in Japanese patients[6, 7]. Both LDL-C and postprandial triglycerides (CMR and VLDL remnant) are risk factors for human ASCVD on a global basis[8-10]. But

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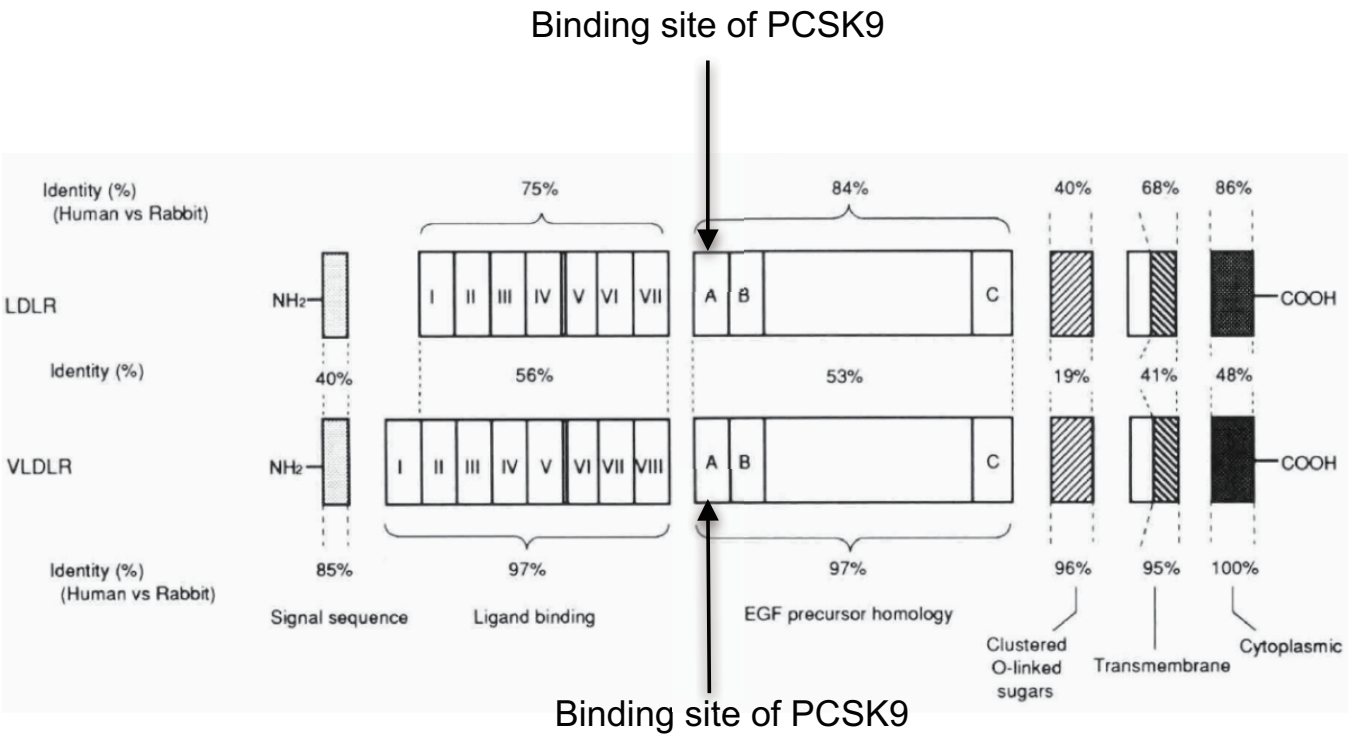

Fig. 1. Structure of the VLDL receptor and the LDL receptor[10].
PCSK9 binds the EGF-A site of both LDL receptor and VLDL receptor.

there remains some uncertainty regarding the direct causal role of TGRL in ASCVD. True ligands such as lipoprotein particles of the VLDL receptor are CMR, VLDL remnant, and Lp(a). In addition macrophages express both VLDL receptor and LPL. In this review, I want to advocate the importance of TGRL-LPL-VLDL receptor and Lp(a)-VLDL receptor pathways for macrophage foam cell formation.

Difference between VLDL and LDL Receptors

We first cloned and characterized the VLDL receptor and found structural domains that were similar to those of the LDL receptor, including: (i) an amino-terminal ligand binding domain composed of multiple cysteine-rich repeats, (ii) an epidermal growth factor (EGF) precursor homology domain, (iii) an O-linked sugar domain with clustered serine and threonine, (iv) a single transmembrane domain, and (v) a cytoplasmic domain with an FDNPVY sequence described in rabbit and human VLDL receptor cDNA[11, 12] (**Fig. 1**). The exon-intron organization of the VLDL receptor gene is almost identical to that of the LDL receptor gene, except for an extra exon that encodes an additional repeat in the ligand binding domain (LDL receptor contains a 7-fold repeat while the VLDL receptor has an 8-fold repeat) (**Fig. 1**). The two genes are located on different chromosomes; the VLDL receptor gene on chromosome 9 (9p24) and the LDL receptor gene on chromosome 19 (19p13.2). Subsequent studies indicated that LDL receptor mutation causes familial hypercholesterolemia (FH)[8]. In 2005, a human VLDL receptor mutant was discovered and homozygous deletion of the VLDL receptor gene was found to be the cause of autosomal recessive cerebellar hypoplasia with cerebral gyral simplification[13] (**Table 1**).

VLDL receptor cDNA overexpressing ldlA-7 (LDL receptor-deficient CHO) cells bound apoE-containing lipoproteins, including VLDL, intermediate-density lipoprotein (IDL) from Watanabe heritable hyperlipidemic (WHHL) rabbits, and β-VLDL (β-migrating VLDL) from cholesterol-fed rabbits, but did not bind LDL from WHHL rabbits[11]. On the other hand, ldlA-7 cells transfected with the LDL receptor cDNA bound both apoB- and apoE-containing lipoproteins, including VLDL, IDL, LDL from WHHL rabbits, and β-VLDL from cholesterol-fed rabbits[11]. Notably, VLDL from fasted normal human subjects bound with lower affinity compared to VLDL prepared from WHHL rabbits[11]. The VLDL from WHHL rabbits was recognized by VLDL receptor cDNA overexpress-

Table 1. Differences between VLDL receptor and LDL receptor

	VLDL receptor	LDL receptor
Gene location	9p24	19p13.2
Phenotype of human mutant	Cerebellar hypoplasia with cerebral gyral simplification	Familial hypercholesterolemia (FH)
Ligands	ApoE, LPL, Lp(a), RAP, PCSK9, Reelin, Thrombospondin-1, uPA/plasminogen activator inhibitor-1 complex, Serine protease-serpin complex, Tissue factor pathway inhibitor (TFPI), Fibrin, Hepatitis C virus	ApoE, ApoB, RAP, PCSK9, Hepatitis C virus
Main expression sites	Heart, Muscle, Adipose tissue, Macrophages, Endothelial cells, Brain	Liver, Adrenal gland
Binding capacity of apoE2/2	Equal to apoE3/3	Less than 1%
Sterol regulation	None	Negative feedback
Alternative splicing	+	–

Apo: apolipoprotein, LPL: lipoprotein lipase, RAP: receptor-associated-protein, PCSK9: proprotein convertase subtilisin/kexin type 9, uPA: urokinase plasminogen activator
It is controversial that whether LDL receptor binds Lp(a). It seems that Lp(a) is recognized by LDL receptor *in vitro*, but most of the *in vivo* data including human have shown that LDL receptor does not involved in the catabolism of Lp(a).

ing ldlA-7 cells because LDL receptor deficiency induced the accumulation of remnant particles in VLDL. More than 25 years ago, I thought the new cloned gene producing protein was not a lipoprotein receptor even though the new gene was structurally similar to LDL receptor gene. Over a period of six months, I examined the ligand binding properties using my own fasted-VLDL and LDL, but could not obtain clear binding data. My fasted-VLDL and LDL particles were never recognized by new gene overexpressing ldlA-7 cells (data not shown). Accidentally, I once obtained my own postprandial VLDL and LDL after eating curry rice in the Co-op café at Tohoku University. I found that postprandial VLDL, but not LDL, was bound and internalized by the new gene overexpressing ldlA-7 cells. At that stage, I was sure that the true ligands of VLDL receptor for lipoprotein particles were CMR and VLDL remnant (postprandial lipoproteins). To ensure the ligand specificity of VLDL receptor *in vivo*, we generated the VLDL receptor and LDL receptor double KO mice and compared them to LDL receptor KO mice. Non-fasting serum total cholesterol was not different between the two, but non-fasting serum triglyceride was elevated in the double KO mice. High-performance liquid chromatography (HPLC) analysis found high LDL-cholesterol fraction during all fasting times in LDL receptor KO mice. When VLDL receptor deletion was induced in LDL receptor KO mice, the VLDL-cholesterol fraction was higher than the LDL-cholesterol fraction at all fasting times (**Fig. 2**). These data indicate that the VLDL receptor acts on TGRL metabolism both quantitatively and qualitatively. On the other hand, the low affinity binding of fasting human VLDL to the VLDL receptor could be overcome by enriching VLDL with either apoE or LPL[14]. Niemeier *et al.*[15] reported that the same mechanism was the case for chylomicron particles. The VLDL receptor mediated the uptake of CMR, and this uptake was further increased by the addition of apoE and inactivated LPL. Argraves *et al.* found that LPL itself bound with high affinity to purified VLDL receptor[16]. *In vivo*, VLDL receptor KO mice have reduced LPL activity[17]. Taking into account that the VLDL receptor and LPL are expressed in the same tissues (heart, muscle, adipose tissue, and macrophages), these findings suggest that CMR and VLDL remnant are true ligands for the VLDL receptor in concert with LPL. In contrast to the LDL receptor, VLDL receptor bound apoE2/2 VLDL and apoE3/3 VLDL identically *in vitro*[18]. Further adenovirus-mediated VLDL receptor expression in the liver of apoE2/2 and apoE3-Leiden transgenic mice resulted in lowering plasma cholesterol levels, indicating that the VLDL receptor recognized apoE2/2 and apoE3-Leiden. The reduction in plasma cholesterol was mainly due to a reduction in VLDL levels[19]. Ruiz *et al.* showed that the VLDL receptor recognizes all apoE isoforms (apoE2, apoE3, and apoE4) and avidly binds lipid-free apoE[20].

The VLDL receptor expression is highly abundant in heart, muscle, adipose tissue, macrophages, ECs and brain, but barely detectable in the liver, in which the LDL receptor is expressed abundantly.

It is currently understood that the LDL receptor is down-regulated by intracellular lipoproteins. We

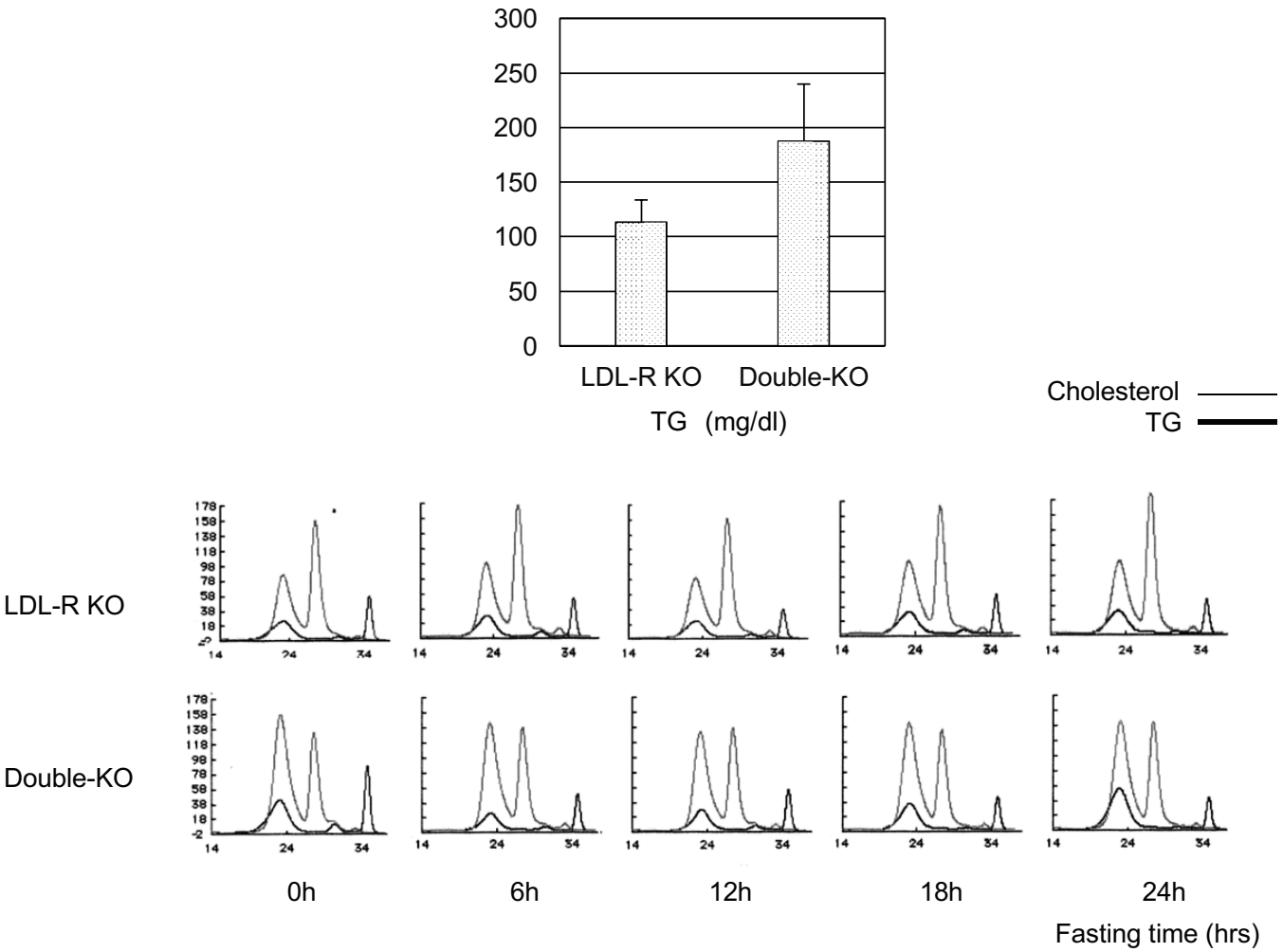

Fig. 2 ***Top*****:** Non-fasting serum triglyceride (TG) in LDL-R KO and double KO mice ($n=6$).
Bottom**:** HPLC profile during 24 h fasting times in LDL-R KO and double KO mice.

were the first group to report that the VLDL receptor is not down-regulated by sterols in THP-1 and rabbit resident alveolar macrophages[12, 21]. Western blots also showed the disappearance of LDL receptor protein following the addition of 100 μg/ml of β-VLDL to the medium for 48 h, though the VLDL receptor protein level did not change in THP-1 cells[22]. The human LDL receptor gene contains a sterol regulatory element (SRE)-1 while the VLDL receptor gene includes two SRE-1-like sequences[12]. SRE-1 contains a direct repeat of the nucleotide sequence CAC on the same DNA strand separated by two Cs. The sequences of the two CAC are believed to be the target of the SRE-binding protein 1 that controls transcription of the LDL receptor gene[23]. The SRE-1-like sequences in the VLDL receptor contain single nucleotide substitutions that disrupt the direct CAC repeats. This could be the reason why the VLDL receptor expression is not regulated by intracellular lipoproteins.

The VLDL receptor mRNAs produce mainly two kinds of VLDL receptor proteins by alternative splicing; type 1 VLDL receptor and type 2 VLDL receptor that lacks O-linked sugar domain encoded by exon 16[12].

Multiple Ligands for VLDL and LDL Receptors

In addition to apoE and LPL, the VLDL receptor binds Lp(a)[24], receptor-associated protein (RAP)[25], proprotein convertase subtilisin/kexin type 9 (PCSK9)[26-28], reelin[29], thrombospondin-1[30-32], urokinase plasminogen activator (uPA)/plasminogen activator inhibitor-1 complex[16, 33-36], serine protease-serpin complex[37], and tissue factor pathway inhibitor (TFPI)[36]. It seems that ECs are important sites for the action of the VLDL receptor because the movement of active LPL across

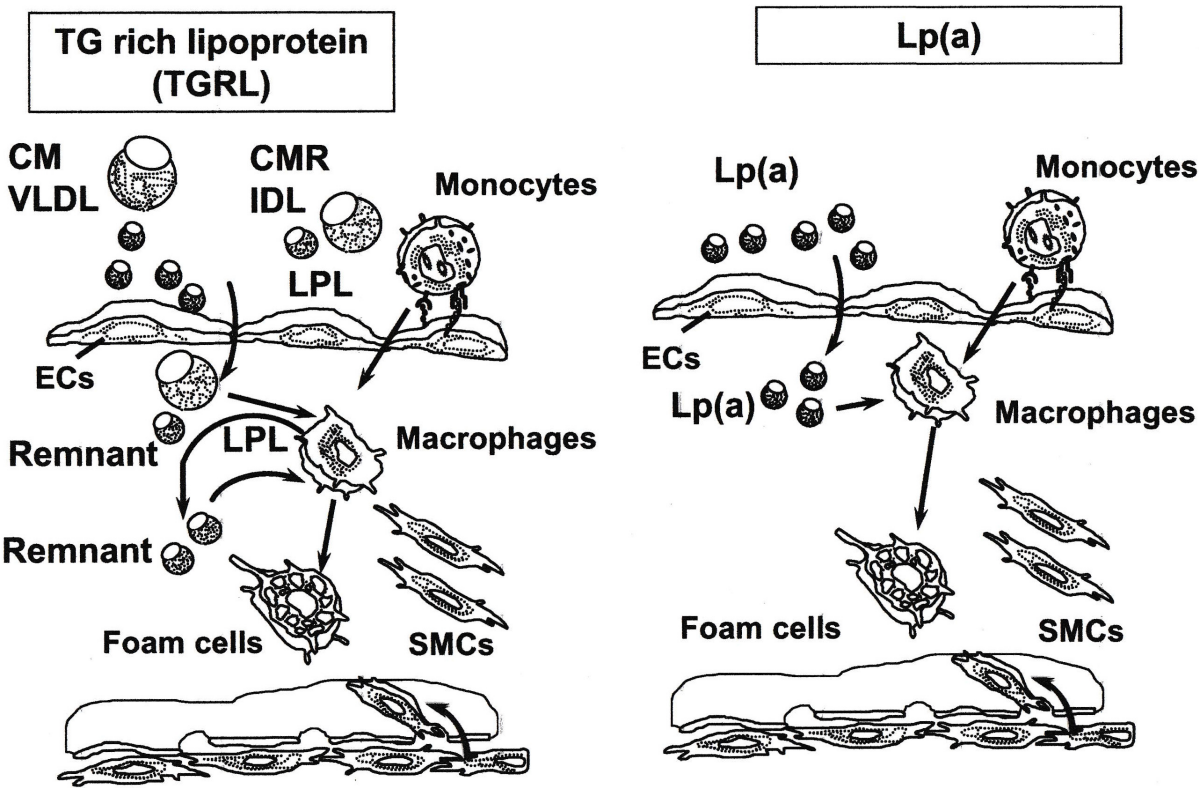

Fig. 3. Schematic diagram of the putative TGRL-LPL-VLDL receptor and Lp(a)-VLDL receptor pathways for macrophage foam cell formation.

CM: chyromicron, CMR: chyromicron remnant, VLDL: very low-density lipoprotein, IDL: intermediate-density lipoprotein, LPL: lipoprotein lipase, ECs: endothelial cells, SMCs: smooth muscle cells

ECs involves both heparan sulfate proteoglycan and the VLDL receptor[38]. In addition, it is intriguing that fibrin is one of the ligands for the VLDL receptor. Interaction of fibrin with the VLDL receptor promotes transendothelial migration of leukocyte as in the case for LPL and thereby enhances inflammation[39]. Anti-VLDL receptor antibodies inhibit fibrin-VLDL receptor interaction and significantly reduce myocardial injury induced by ischemia-perfusion[40]. The common ligands of the VLDL and LDL receptors are apoE, RAP, PCSK9 and hepatitis C virus[41-43]. Because anti-PCSK9 monoclonal antibody (evolocumab and alirocumab) increases the protein expression of both VLDL and LDL receptors[44], patients with hepatitis C treated with anti-PCSK9 monoclonal antibody should be watched carefully (**Table 1**, **Fig. 1**).

TGRL-LPL-VLDL Receptor and Lp(a)-VLDL Receptor Pathways for Macrophage Foam Cell Formation

The expression of VLDL receptor primarily in macrophages has been confirmed in human and rabbit atherosclerotic lesions[24, 45-47]. *In vitro*, we reported that IFN-γ inhibited VLDL receptor expression and foam cell formation in three types of human macrophages (PMA-induced THP-1, PMA-induced HL-60, and human monocyte-derived macrophages) by β-VLDL, a representative lipoprotein in the metabolic syndrome and type Ⅲ hyperlipoproteinemia[48]. These results suggest that the VLDL receptor could be a macrophage β-VLDL receptor, which is one of the receptors involved in macrophage foam cell formation. However, controversial *in vivo* findings using a mouse model were reported. Atherosclerotic lesions were not different between HuB (human apoB) transgenic mice and VLDL receptor-deficient HuB transgenic mice fed atherogenic diet for 4 months[17]. Tacken *et al.* also showed that both VLDL receptor deficiency and endothelial VLDL receptor overexpression did not affect the size of atherosclerotic lesions. Interestingly, they indicated that deficiency of the VLDL receptor profoundly increased intimal thickening after vascular injury[49]. We also compared the area of atherosclerotic lesions in double KO and LDL receptor KO mice, but found no difference in the area even though we

showed clear difference in lipoprotein profile (**Fig. 2**). Fortunately, we were able to obtain rabbit polyclonal anti-VLDL receptor antibody that recognized human, rabbit, rat, and mouse VLDL receptors. A synthetic peptide, CASVGHTYPAISVVSTDDDL, which corresponds to the carboxy-terminus of the human, rabbit, rat, and mouse VLDL receptors, was synthesized and injected into Japanese White rabbits to obtain polyclonal antibody (named VR2). VR2 reacted only with human VLDL receptor, but not with human LDL receptor or human ApoER2 cDNA transfected ldlA-7 cells. Furthermore, VR2 specifically recognized the human and wild-type mouse heart VLDL receptor while it did not detect VLDL receptor bands in hearts of VLDL receptor KO mice. Western blots showed that although VLDL receptor protein was detected in PMA-treated THP-1 human macrophages and wild-type mouse heart, it was not detected in cell lines derived from mouse macrophages (Raw264.7 and J774.2) and also mouse peritoneal macrophages. The VR2 antibody detected rabbit VLDL receptor protein in heart but not in liver by immunohistochemistry. VLDL receptor proteins were clearly detected in some of the RAM11-positive macrophages in the thoracic aorta of WHHLMI rabbits, which are indicative of atherosclerotic lesion. In contrast to the atherosclerotic lesions in WHHLMI rabbit thoracic aorta, no VLDL receptor protein was observed in BM8-positive mouse macrophages in aortic atherosclerotic lesions in chow-fed apoE KO mice and LDL receptor KO mice whose diet had been supplemented with 1.25% cholesterol for 12 weeks[50]. We detected abundant amounts of VLDL receptor protein in human atherosclerotic coronary arteries but not in non-atherosclerotic coronary arteries, using the same VR2 antibody (data not shown). Argraves *et al*. have already detected the VLDL receptor protein in human atherosclerotic plaque and the VLDL receptor protein was co-located with plaque KP-1-positive macrophages and foam cells[24]. TGRL has also been isolated from human artery segments[51]. Recently Matsuo *et al*. reported that serum remnant lipoprotein levels were positively correlated with the necrotic components of the coronary plaques and negatively correlated with the fibrotic components evaluated by intravascular ultrasound (IVUS) in patients with stable angina[52] and it is known that both LDL-C and TGRL are independent risk factors for human ASCVD[8-10]. Therefore, I consider that the mechanisms of macrophage foam cell formation are somewhat different between mice and humans or rabbits. Finally, I want to call up the TGRL-LPL-VLDL receptor pathway for macrophage foam cell formation, especially in rabbit and human. Since Lp(a) is one of the ligands for the VLDL receptor[24], the Lp(a)-VLDL receptor pathway may be considered as another alternative pathway (**Fig. 3**). Since both rabbit and human macrophages express VLDL receptor protein, studies on the importance of VLDL receptor signaling for TGRL should focus on these species rather than on the mouse systems (mouse peritoneal macrophages, apoE KO mice, and LDL receptor KO mice).

Conclusions

The VLDL receptor could be a so-called macrophage β-VLDL receptor which is involved in macrophage foam cell formation. Notably researchers in atherosclerosis should be known that macrophage VLDL receptor protein expression is different among species. I want to see more detailed researches about TGRL-LPL-VLDL receptor and Lp(a)-VLDL receptor pathways for macrophage foam cell formation in the future.

Conflict of Interest

The author declares no conflict of interest.

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