Remnant Lipoproteins and Atherogenesis

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Recent clinical studies have revealed that increased serum triglyceride (TG) levels are closely related to atherosclerosis, independently of serum levels of high-density lipoproteins (HDL) and low-density lipoproteins (LDL). Among triglyceride-rich lipoproteins (TRLs), remnant lipoproteins (RLPs) are considered to be atherogenic and an independent coronary risk factor. We previously reported that monocytes cultured in the presence of RLPs increased their adhesion to vascular endothelial cells. The underlying mechanism involved activation of RhoA, a member of small GTP binding proteins, resulting in activation of focal adhesion kinase (FAK) and α1-integrin. It is also known that RLPs enter vessel walls. In another study, we reported that RLPs induced smooth muscle cell (SMC) proliferation, independently of oxidative stress. Recently, we identified the molecular mechanisms, in which RLPs from hypertriglyceridemic patients stimulated SMC proliferation via epidermal growth factor (EGF) receptor transactivation and heparin-binding EGF-like growth factor (HB-EGF) shedding. More recently, we reported that apoB48 receptor was involved in RLP-induced foam cell formation in macrophages. The current review focused on the molecular mechanisms for the atherogenicity of RLPs. J Atheroscler Thromb, 2005; 12: 73–76.

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Introduction

Since the notion of “postprandial hyperlipidemia” has been proposed (1), attention has been focused on the significance of triglyceride (TG)-rich lipoproteins (TRLs) in atherogenesis. Recent clinical studies have revealed that serum TG levels are closely related to atherosclerosis (2). Among TRLs, remnant lipoproteins (RLPs), produced by hydrolysis of chylomicrons (CMs) and very low-density lipoproteins (VLDLs), are considered to be highly atherogenic and an independent coronary risk factor (3, 4), independently of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. It has been also known that RLPs are associated with metabolic syndrome (5, 6). As reported in J-LIT study (7, 8), it is suggested that metabolic syndrome plays a pivotal role in the progression of coronary artery disease (CAD) not only in western countries, but also in Japan. Recently, a prospective study that followed up coronary events in type 2 diabetic patients with CAD reported that RLP-cholesterol is the most significant and independent risk factor of CAD and predicts future coronary events among these patients (9). As shown above, many epidemiological studies have revealed that RLPs promote atherosclerosis, however, the underlying mechanism(s) have not been fully elucidated.

As nascent TRLs are converted to RLPs after undergoing gradual hydrolysis in the circulation by lipoprotein lipase, RLPs are heterogeneous in size, density, lipid/apolipoprotein composition, charge etc. (10), and there has been no standardized method to separate RLPs due to their heterogeneity. Recently, immunoaffinity gel containing apoB100 antibody and apoA-I antibody has been developed to isolate RLPs (11). Using RLPs separated with immunoaffinity gel, we tried to elucidate the role of RLPs in atherogenesis. In this review, we will focus on
the direct effect of RLPs on vascular cells.

**RLP and Smooth Muscle Cells**

It is known that RLPs enter vessel walls. In fact, TRL remnants are found in human atherosclerotic plaques (12). These findings may support a potential role of RLPs in subendothelial spaces. However, the direct effects of RLPs on smooth muscle cell (SMC) have remained almost unknown. We reported for the first time that RLPs induced SMC proliferation, regardless of its oxidative stress (13), and more recently identified the molecular mechanisms by which RLPs induce proliferation of vascular SMCs (14). In the latter, we reported that RLPs from hypertriglyceridemic (HTG) patients stimulated SMC proliferation via epidermal growth factor (EGF) receptor transactivation and heparin-binding EGF-like growth factor (HB-EGF) shedding. RLPs induced rat aortic SMC proliferation at pathophysiological concentrations (> 7.5 mg cholesterol/dl) in a dose-dependent manner. RLPs sequentially activated Raf-1-MEK1/2-ERK1/2. PD98059, a specific MEK1 inhibitor, and dominant negative Raf-1 significantly inhibited RLP-induced SMC proliferation. We also found that upon RLP treatment the EGF receptor was phosphorylated prior to MAPK activation. Further AG1478, a specific EGF receptor inhibitor, significantly inhibited RLP-induced ERK activation and SMC proliferation. Since several lipoproteins have been reported to induce the migration and proliferation of SMCs via G protein-coupled receptor (GPCR)-dependent protein kinase C (PKC) activation (15), we examined the potential involvement of GPCR and PKC in RLP-induced SMC proliferation. RLPs activated PKCδ and PKCα in SMCs. Rottlerin, a specific PKCδ inhibitor, significantly reduced RLP-induced EGF receptor transactivation and SMC proliferation, as did Go6976, a specific PKCα inhibitor, though to a lesser extent. Pretreatment of SMCs with pertussis toxin, a Gi protein inhibitor, attenuated PKCa activation. In contrast, pertussis toxin treatment resulted in a partial inhibition of PKCδ activation. In contrast, when cell surface lipoprotein lipase (LPL) and heparan-sulfate proteoglycan (HSPG), which are known to facilitate the binding RLPs to their B/E receptors, were removed by heparin and heparitinase treatment, RLP-induced PKCδ activation was significantly inhibited. Therefore, we speculated that a GPCR-independent pathway might be involved in PCK activation. We next examined whether HB-EGF is involved in RLP-induced SMC proliferation. Recently, the potential cleavage of membrane-anchored HB-EGF (pro HB-EGF) or “HB-EGF shedding” by matrix metalloproteinase (MMP), a disintegrin and metalloproteinase (ADAM) family has been demonstrated in EGF receptor transactivation in vitro and in vivo (16, 17). Most of the soluble HB-EGF binds to HSPG on the surface of SMCs, then to the EGF receptor (18). Treatment with RLPs increased the amount of soluble HB-EGF in SMC membranes, supporting the occurrence of the HB-EGF shedding. Pretreatment of SMCs with rottlerin and Go6976 inhibited HB-EGF shedding in RLP-treated SMCs. We also examined the involvement of the MMP/ADAM family in this process. MMP3 inhibitor, tissue inhibitor metalloproteinase-1 (TIMP-1) and, to a lesser extent, TIMP-2, reduced HB-EGF shedding in RLP-treated SMCs and attenuated RLP-induced SMC proliferation. Pretreatment of SMCs with anti-HB-EGF neutralizing antibody significantly reduced RLP-induced SMC proliferation, indicating that HB-EGF shedding is involved, at least in part, in RLP-induced EGF receptor transactivation. To confirm the relevance of the observed RLP-induced-SMC proliferation in vivo, we attempted to determine whether activation of EGF receptor and HB-EGF shedding occurred in the aortas of apoE knockout mice, a model of hyper-remnant lipoproteinemia. HB-EGF shedding and tyrosine-phosphorylation of the EGF receptor were detected in the apoE knockout mouse aortas, but not in those of wild-type mice. These results suggest that some of the in vitro observations regarding RLP-induced SMC proliferation may be operative in vivo as well.

Recently, Oi et al. reported that RLPs from patients with sudden cardiac death enhanced coronary vasospasm (19). RLP treatment exacerbated serotonin-induced SMC contraction by up-regulating Rho-kinase, suggesting that several signaling pathways are involved in the action of RLPs on SMCs.

**RLP and Monocyte/Macrophages**

It is known that monocyte-endothelial interaction plays a causative role in the early stage of atherosclerosis. We reported that human monocytic U937 cells cultured in the presence of RLPs increased their adhesion to human umbilical vein endothelial cells (HUVECs) under physiological laminar flow conditions. The treatment of U937 cells with RLPs activated RhoA, a member of small GTP binding proteins, via PKC, which resulted in activation of focal adhesion kinase (FAK) as well as β1-integrin. RLP treatment also increased the expression of CD49d on the surface of U937 cells. Interestingly, pretreatment of U937 cells with atorvastatin significantly reduced RLP-induced U937 cell adhesion. The inhibitory effect of atorvastatin was mainly dependent on the inhibition of RhoA activation. Our study suggested that RLPs contribute to atherogenesis via modulation of monocyte-endothelial interactions.

As described above, RLPs enter vessel walls, where they are taken up by macrophages and induce foam cell formation (20). Recently, a novel macrophage remnant receptor has been cloned, and named as “apoB48 receptor” by Gianturco et al. (21, 22). It recognizes apoB48 or its equivalent domain of apoB100 of TRLs, and takes
up CM, CM remnant, HTG-VLDL, and VLDL remnant, but not nascent VLDL or LDL. We examined the involvement of apoB48 receptor in RLP-induced foam cell formation in macrophages (23). In our experiments, siRNA against apoB48 receptor significantly suppressed their expression in THP-1 macrophages, and inhibited RLP-induced foam cell formation. Interestingly, pitavastatin, a novel HMG Co-A reductase inhibitor, was found to inhibit the expression of apoB48 receptor via inhibition of RhoA. In fact, pretreatment of THP-1 macrophage significantly reduced RLP-induced foam cell formation, independently of other remnant receptors. As for the action of RLPs on macrophages, further investigations will be required to elucidate it.

**RLP and Endothelial Cells**

Several studies have reported the effect of RLPs on endothelial cells. Incubation with RLPs induced the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and tissue factor in HUVEC in part through a redox-sensitive mechanism (24). Incubation of RLP with isolated rabbit aortas showed inhibition of arterial relaxation by acetylcholine in a dose-dependent manner. It was also shown that postprandial RLPs were correlated with the decrease of flow-mediated dilatation (FMD), indicating that RLPs contribute significantly to the endothelial dysfunction occurring during the postprandial hyperlipidemia (25). Recently, it was reported that RLPs induce apoptosis in endothelial cells by NAD(P)H Oxidase-mediated superoxide and cytokine production of via lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) (26).

**Conclusion**

Our observations have suggested that RLPs contribute to atherogenesis by directly affecting the vascular cells such as monocytes, endothelial cells, and SMCs. It was also made clear that the effects of RLPs on these cells were in part mediated via novel receptors such as GPCR and apoB48 receptor. More careful and extensive examinations will be required to fully elucidate the role of RLP in atherogenesis.

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**References**


