Leukocytes and complement in atherosclerosis

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c. Review: Cell-mediated lipoprotein transport: a novel anti-atherogenic concept

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ABSTRACT

Lipoprotein transport is thought to occur in the plasma compartment of the blood, where lipoproteins are modulated by various enzymatic reactions. Subsequently, lipoproteins can migrate through the endothelial barrier to the subendothelial space or are taken up by the liver. The interaction between pro-atherogenic (apoB-containing) lipoproteins and blood cells (especially monocytes and macrophages) in the subendothelial space is well known. This lipoprotein-inflammatory cell interplay is central in the development of the atherosclerotic plaque. In this review, a novel interaction is described between lipoproteins and both leukocytes and erythrocytes in the blood compartment. This lipoprotein-blood cell interaction may also be related to the process of atherosclerosis by inducing inflammatory changes in the case of leukocytes (pro-atherogenic) and as an anti-atherogenic transport-system by adherence to erythrocytes. Triglyceride rich lipoprotein (TRL)-mediated leukocyte activation can lead to an inflammatory situation with generation of oxidative stress and the production of cytokines, ultimately resulting in acute endothelial dysfunction. Binding of apoB containing lipoproteins to erythrocytes may be a potential antiatherogenic mechanism protecting the vessel wall from the pro-inflammatory effects of these lipoproteins and also playing a role in the removal of these particles from the circulation. One of the proposed mechanisms of this interaction implies complement activation on the lipoprotein surface and binding to the Complement Receptor 1 (CR1) on erythrocytes and leukocytes, followed by clearance by the liver.

INTRODUCTION

Cardiovascular disease is the major cause of death in the general population. The classical risk factors for developing atherosclerosis are smoking, dyslipidemia, diabetes mellitus, hypertension, obesity and a family history of premature cardiovascular disease (1).
Atherosclerosis can progress very slowly over decades without clinical manifestations. The accumulation of LDL and remnants in the subendothelial space is recognized as one of the main contributors to atherogenesis. The initial steps in the process of lipid-mediated atherosclerosis are thought to be modification of lipoproteins, migration to the subendothelial space and binding to the scavenger receptors on monocytes and macrophages (2,3). This leads to the generation of oxidative stress and production of cytokines, eventually causing a local and generalized inflammatory response resulting in endothelial dysfunction (4).
The endothelium actively controls the trafficking of lipoproteins between the intra- and extravascular compartments. Small lipoproteins like remnants, LDL and HDL migrate to the subendothelium through a non-receptor-mediated process of transcytosis (5). Alternatively, these lipoproteins may enter the sub-intimal space through leaky junctions over the endothelium or fluxes across fenestral pores. Remnants of TRL's increase the permeability of the endothelium and are cytotoxic for endothelial cells (6).
Recent studies have suggested that apoB-containing lipoproteins may interact with the leukocytes in the blood leading to inflammatory changes which may be related to endothelial damage (7). In theory, apoB-containing particles may also bind to erythrocytes, leading to a lower concentration of free apoB-containing lipoproteins in the plasma compartment and therefore less interaction with the endothelium. This mechanism could represent an anti-atherogenic process.

CURRENT CONCEPT OF LIPOPROTEIN TRANSPORT

The current concept of lipid metabolism consists of two major pathways, the exogenous and the endogenous pathway. The exogenous pathway starts in the intestinal cells with the synthesis and secretion of chylomicrons after absorption of dietary fat. Chylomicrons enter the systemic circulation at the site of the thoracic duct, after having been transported in the chyle. Until recently, it was thought that apoCII and lipoprotein lipase (LPL) where necessary and sufficient for the hydrolysis of chylomicron triglycerides (TG) (8,9). Recent work from Stephen Young's laboratory has elegantly shown that glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) plays a critical role in the lipolytic process of chylomicrons. GPIHBP1 is located on the luminal face of the capillary endothelium and appears to be an important platform for the LPL-mediated processing of chylomicrons in capillaries (10,11).

The endogenous pathway depends on the hepatic production of VLDL. These lipoproteins are secreted into the circulation and degraded to LDL by LPL and hepatic lipase (HL). This modification of lipids by lipolytic enzymes leads to the generation of atherogenic remnants and HDL particles (4,5,12. This process takes place in the plasma where other enzymes like cholesterol ester transfer protein (CETP), lecithin cholesterol acyl transferase (LCAT) and phosolipid transfer protein (PLTP) are involved in the modulation of these lipoproteins (4,13). The liver and the intestine can also synthesize and secrete HDL directly.
Besides this well accepted concept, it has been proposed that apoB-containing lipoproteins are not only present in the plasma compartment, but may also be located in a so called "marginated pool" attached to various cells. This marginated pool is thought to be located mainly (but not solely) on the surface of endothelial cells (14). Other candidates for lipoprotein binding in the blood are leukocytes and erythrocytes.

LIPOPROTEIN BINDING TO BLOOD CELLS

The finding that lipoproteins bind to blood cells in human is not new. Binding of LDL to lymphocytes was reported by Hui and Harmony three decades ago (15,16). Tertov et al. described intracellular accumulation of triglycerides and cholesterol esters in freshly isolated leukocytes of patients with coronary heart disease (17). Recent work from our laboratory has shown that apoB-containing lipoproteins can bind to leukocytes in the blood and that meal-derived fatty acids can be taken up by these cells in vivo (18). The fact that different types of leukocytes showed a different content of apoB on their surface (with neutrophils carrying the largest number of apoB), suggested that a specific receptor could be involved. Furthermore, it was proposed that this interaction may lead to activation of leukocytes, especially neutrophils and monocytes (7). It was also suggested that triglyceride rich lipoprotein (TRL)-mediated leukocyte activation could lead to an inflammatory situation with generation of oxidative stress and the production of cytokines (19-21), ultimately resulting in acute endothelial dysfunction. Intuitively, all these effects on leukocytes and endothelial cells could be related to the generation of endothelial cell damage and atherosclerosis. The mechanism whereby lipoproteins bind to leukocytes has not been elucidated yet.
Binding of LDL to erythrocytes has also been reported by Hui and Harmony. These authors proposed that the binding site for LDL on the erythrocyte membrane was not the LDL-R (22,23). Arbustini et al. found that the cholesterol content of erythrocyte membranes was more than 2 times higher in patients presenting with acute coronary syndrome than in patients with stable angina (24). It is not known how this cholesterol-enrichment of the erythrocyte membrane occurs. Membrane-bound cholesterol may be incorporated in the phospholipid layer of the membrane. Alternatively, cholesterol carrying lipoproteins may also be attached to the erythrocyte and contribute to the cholesterol in the membrane.

Preliminary data from our laboratory suggest that apoB can be detected on erythrocytes in humans. Flowcytometric measurements, with specific anti-apoB antibodies, detected apoB at different concentrations on all blood cells in humans (Figure 1).

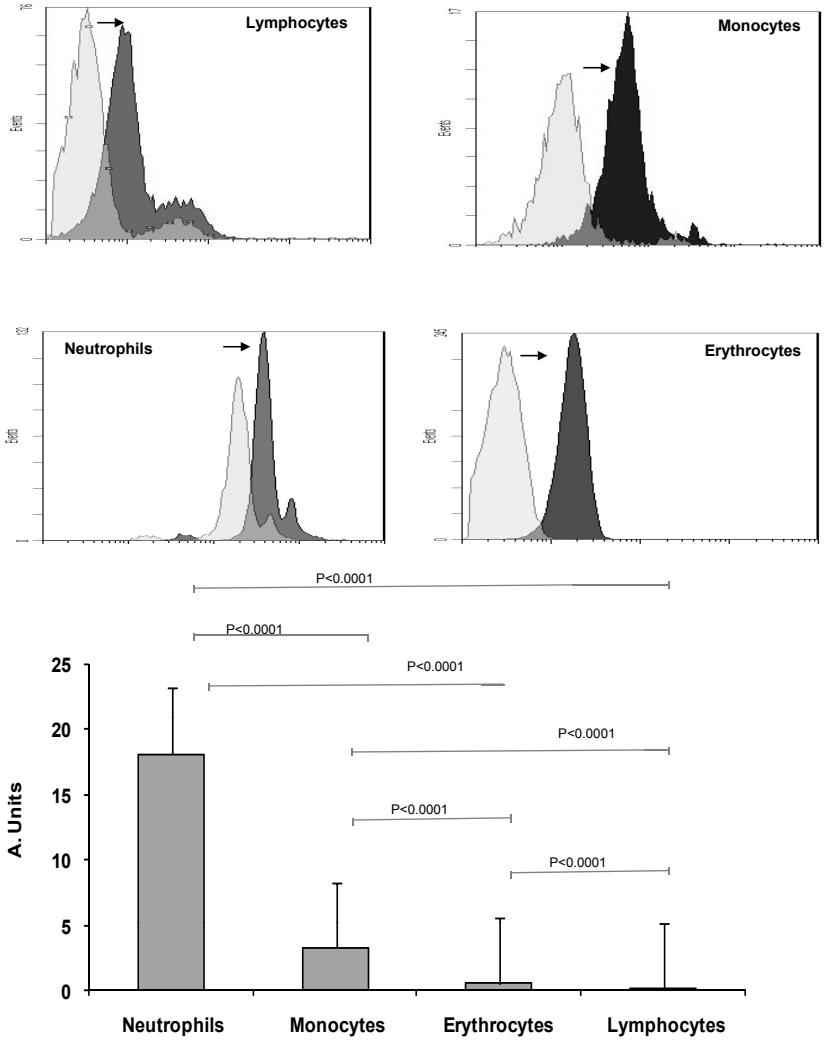

Figure 1. Histograms showing overlay of fluorescence for samples with and without apo B antibodies (panel a). The gray graphs represent the background signal due to binding of the FITC-labeled secondary antibody only. The colored graphs depict ApoB signal. Panel (b) shows fluorescence of background staining (Auto) and ApoB signal (ApoB) on the different types of leukocytes and erythrocytes. The differences of the apoB levels on all cell types are statistically significant ($P < 0.0001$). Data are given in arbitrary units ($x \pm SD$).

PROPOSED MECHANISM OF LIPOPROTEIN BINDING TO ERYTHROCYTES

If both leukocytes and erythrocytes can carry apoB-containing lipoproteins, it seems logical to assume one common mechanism. The LDL-R seems to be one logical candidate, but erythrocytes do not express these receptors under normal conditions (2).

An alternative candidate is the complement receptor 1 (CR1). This receptor is present in both blood cell types in primates (25–27). The average number of CR1 receptors of erythrocytes is small (mean < 2000/cell) compared with that of circulating B lymphocytes, monocytes and neutrophils (20,000–150,000/cell). The circulating erythrocytes however, outnumber the leukocytes approximately 1000-fold, and therefore the vast majority of all CR1 receptors present in the circulation of humans is located on the erythrocyte (28).

Erythrocytes play an important role in the removal of immune complexes in the bloodstream. Primate erythrocytes can bind soluble, as well as complement opsonised immune complexes (IC) in the circulation (28,29). CR1 facilitates attachment and clearance of bound IC, either via the natural ligand C3b, or via the hexopolymer (HP) construct, without lysis or destruction of the erythrocyte (30) (a phenomenon also known as "immune adherence"). In theory, binding of apoB-containing lipoproteins to erythrocytes can be an anti-atherogenic mechanism by preventing these atherogenic particles to interact with the endothelium. In the liver, erythrocyte-apoB may be cleared from the circulation without erythrocyte destruction in a similar way as immune complexes attached to the CR1 on erythrocytes (31).

Preliminary data from our laboratory suggest that subjects with coronary artery disease (CAD) have a lower signal of apoB on erythrocytes than subjects without CAD. Moreover, apoB binding to erythrocytes does not correlate to classical plasma lipid levels like cholesterol or apoB concentrations. This suggests that apoB binding to blood cells is not merely a reflection of plasma concentrations.

Finally, in patients with atherosclerosis (32,33), and in asymptomatic adults (34), monoclonal and polyclonal antibodies against lipoproteins can be detected and explain the presence of immune complexes (IC). This formation of LDL-IC and the binding to red cells can affect the cholesterol homeostasis and LDL metabolism (28,35).

APOLIPOPROTEIN B AND COMPLEMENT ACTIVATION

C3 concentrations increase significantly in the postprandial situation reflecting complement activation (20,36,37). C3 activation may occur on the surface of lipoproteins resembling the immune-adherence phenomenon. Since complement activation can only take place on proteins and glycoproteins, we propose that mannose binding lectin (MBL) binds to certain sugars on the surface of the apoB containing lipoproteins (38).

It is well known that the oligosaccharides necessary to bind MBL, mannose (17.8%), N-acetyl-glucosamine (16.8%), galactose (13.4%) and fructose (3.4%) are all present on the apoB molecule (39). This binding of MBL could be the start of the activation of a part of the complement system leading to the activation of C4, C2 and resulting in a C3 convertase (C4C2a). C3b generated in thisway may bind to the surface of the lipid particle and to the CR1 molecule on the erythrocytes.

The C3a molecule is immediately inactivated by carboxypeptidase N (CPN) becoming the well known C3adesArg also known as acylation stimulating protein (ASP) which plays a role in triglyceride and free fatty acid metabolism (40,41).

Recent work form our laboratory has provided supporting evidence for this concept by demonstrating that the MBL pathway is involved in TRL metabolism in humans (42). Figure 2 provides a schematic representation of the model developed in our laboratory. This model could integrate the findings of several groups showing that lipoprotein metabolism and the complement system are closely connected.

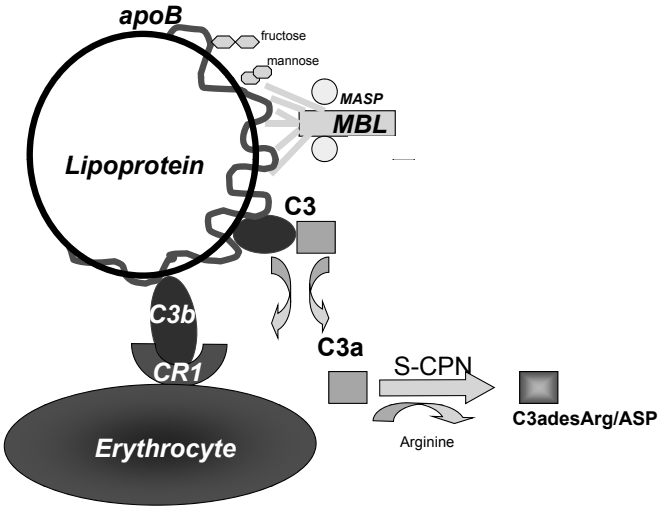

Figure 2. Triglyceride lipoproteins (TRP) may bind with mannose binding lectin (MBL) and C3. MBL binds to certain sugars on the apoB molecule. This binding of MBL is the start of the activation of C4, C2 and resulting in C3 convertase. C3b remains attached to the surface of the lipid particle. This C3b is also able to bind to the CR1 molecule on the erythrocyte (immune adherence), ultimately resulting in binding of the lipoprotein to the erythrocyte.

In conclusion, the transport of atherogenic lipoproteins in humans is not only a process that occurs in the plasma compartment but it seems to depend in part on cellular transport by erythrocytes and leukocytes. While the exact mechanism whereby binding to blood cells takes place is not known, complement activation on the lipoprotein surface by the MBL pathway and binding to CR1 is one of the candidate mechanisms.

CONFLICT OF INTEREST

None.

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