The pathogenesis of atherosclerosis

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According to the World Health Organization, cardiovascular diseases are the leading cause of death worldwide, accounting for approximately 30 of all global deaths in 2008. A large proportion of CVDs is attributable to atherosclerosis, which is a major cause of myocardial infarction or stroke (1).

The pathogenesis of atherosclerosis

Over the past two decades, the inflammatory hypothesis of atherosclerosis has gained strong footing through multiple lines of supportive evidence (reviewed in (2)). Nowadays, atherosclerosis is considered a complex chronic inflammatory disease of medium- and large-sized arteries.

Atherosclerosis occurs predominately at sites of disturbed laminar flow, in particular, arterial branch points and bifurcations. Human and animal studies indicate that the key initiating step is subendothelial accumulation of apolipoprotein B-containing lipoproteins (apoB-LPs). ApoB-LPs are secreted by the liver as very low-density lipoproteins, which are converted in the circulation to atherogenic low-density lipoproteins (LDL). In addition, apoB-

LPs are secreted by the intestines as chylomicrons, which are converted by lipolysis into atherogenic particles, called remnant lipoproteins (3).

Subendothelial apoB-LPs are believed to initiate an early inflammatory response, which may be enhanced by oxidative modification of LPs, through activation of overlying endothelial cells in a manner that leads to the recruitment of monocytes. Activated endothelial cells express adhesion molecules (e. g. intracellular adhesion molecule-1 and vascular adhesion molecule-1) and secrete cytokines and chemoattractants, or “ chemokines” (e. g. monocyte chemoattractant protein-1 and RANTES), that act on monocytes and promote directional migration towards and into the artery wall.

Once resident in the arterial intima, monocytes acquire the morphological characteristics of macrophages and increase their expression of scavenger receptors, including scavenger receptor A and CD36. Excessive uptake and internalization of modified lipoproteins via their scavenger receptors leads to the accumulation of cholesteryl esters in cytoplasmic droplets. These lipid-laden macrophages, known as foam cells, characterize the early atherosclerotic lesion (figure 1).

As the atherosclerotic lesions further develops, macrophage and foam cells predominate, and further serve to alter the plaque environment, changing extracellular matrix composition and decreasing smooth muscle cell content, predisposing to plaque rupture (2, 4).

Atherosclerosis and high-density lipoprotein therapy

Although the development of atherosclerosis is dependent on a complex interplay between many factors and processes, a clear association has been established between elevated levels of plasma cholesterol and increased atherosclerotic disease (6). To attenuate the risk of atherosclerotic complications, primary and secondary prevention strategies seek to correct aberrant blood cholesterol levels. Actively reducing low-density lipoprotein (LDL) cholesterol through lipid-modifying therapies, such as statins, yield a proportional decrease in CVD risk (7). However, despite their potency, only 25-50% of cardiovascular events are prevented with highly potent statins, which highlights the importance of seeking for additional treatments for the optimal management of cardiovascular risk (8).

Besides reducing LDL, improving HDL levels has gained a considerable amount of attention during the last decade. Epidemiological studies have shown that plasma levels of high-density lipoproteins (HDL) are inversely associated with clinical events resulting from atherosclerosis (9). Human and animal intervention studies have shown that increasing HDL results in a reduced atherosclerotic plaque size, suggesting that HDL may be an effective therapy for the regression of atherosclerosis (10).

The mechanisms for plaque regression have been primarily attributed to the ability of HDL to promote cholesterol efflux from peripheral tissues, including macrophages, to the liver for excretion in the bile and feces. This process, called reverse cholesterol transport, is widely believed to account for much of the inverse relationship between plasma HDL levels and CVD revealed by population studies (11).

HDL components can remove cellular cholesterol by four distinct processes. The presumed major precursor for this pathway is lipid-poor apoA-I, which is initially synthesized and secreted by the liver. Once in plasma, it rapidly acquires phospholipids and cholesterol from cell membranes in a reaction mediated by the ATP-binding cassette A1 (ABCA1) that results in the formation of pre-Î² HDL particles (figure 2). A second mechanism involves ATP-binding cassette G1 (ABCG1), with pre-Î² and large spherical HDL acting as the main acceptor. A third involves scavenger receptor B1 (SR-B1), which has the same acceptors as ABCG1. Lastly, cholesterol can be removed from cell membranes to HDL particles through passive diffusion. The latter three mechanisms are dependent on the presence and activity of Lecithin: Cholesterol AcylTransferase (LCAT). LCAT can esterify any unesterified cholesterol entering the outer surface of HDL, after which it will move into the intensely hydrophobic central core, leaving the outer surface of the HDL particle able to accept more unesterified cholesterol (12).

Next to promoting cholesterol efflux, studies have shown that HDL is able to protect against cardiovascular diseases through a variety of additional functions, including anti-oxidant, anti-thrombotic, anti-apoptotic (reviewed in (12)). HDL has been shown to exert anti-inflammatory effects, which are mainly investigated in endothelial cells, and to a lesser extent in vascular smooth muscle cells (13, 14).

Macrophages in the pathology of atherosclerosis

It is generally accepted that macrophages play a pivotal role in the pathophysiology of atherosclerosis. The accumulation of macrophages and their conversion into foam cells, through the uptake of excessive amounts of

lipids and cholesterol from modified apoB-LP, are considered hallmarks of atherogenesis. By expressing various effector molecules, including inflammatory cytokines, chemokines and extracellular matrix degrading enzymes, macrophages have a great impact on the activation, migration and survival of other cells in the plaque and ultimately affect plaque stability. However, within atherosclerotic plaques, macrophages represent a heterogeneous cell population, which may consist of several subsets that have distinct phenotypic and functional characteristics, ranging from large quiescent lipid-laden foam cells to a small active inflammatory cell. Furthermore, macrophages demonstrate a high degree of plasticity, which depend on the environmental cues they are exposed to. In general, macrophages are skewed by interferon-Î³ or lipopolysaccharide towards a pro-inflammatory or M1 phenotype, which produce mediators that have a pro-atherogenic effect. On the other hand, anti-inflammatory or M2 macrophages are polarized by interleukin (IL)-4, IL-10 and IL-13, which are believed to be of an anti-atherogenic nature. A phenotypical distinction can be made between these subsets based on their differential expression of cell surface expression (15, 16).

High-density lipoproteins in modulating macrophages

To date, HDL is considered to be the “ good cholesterol”, because of its protective effects in atherosclerosis, such as anti-inflammatory properties (14). However, the effects of HDL on macrophages, which are major players in atherosclerosis, have yet to be established.

The majority of the research conducted on the effects of HDL on macrophages have mainly been performed on cholesterol- or lipid-loaded

macrophages. Here, HDL exerts anti-inflammatory effects by decreasing NF-ÎºB activation and secretion of pro-inflammatory cytokine, including tumor necrosis factor Î± (TNFÎ±), while increasing anti-inflammatory cytokines, like IL-10.

To date, however, it is not known how HDL affects non-cholesterol or -lipid-loaded macrophages.

Cholesterol is an important structural lipid that modulates

Perturbations in cellular cholesterol levels has been shown to affect

NF-ÎºB is an essential regulator of inflammatory processes in mammalian cells, including macrophages. When the NF-ÎºB pathway is activated, NF-ÎºB translocates to the nucleus and activates transcription of its target genes, including genes involved in cytokine production and secretion (17).

In addition to NF-ÎºB, A Disintegrin And Metalloproteinases (ADAMs) are also implicated in numerous cellular processes, including inflammation. ADAMs are a group of enzymes that cleave the extracellular domains of various cell surface molecules, of which ADAM 10 and 17 are the best studied family members. ADAM 10 and 17 are closely related proteases and share many substrates, including TNF-Î± and its receptor. ADAM activity can be regulated at various levels, including localization within the plasma membrane, where lipid rafts are thought to play a role in. Lipid rafts are cholesterol- and sphingolipid-enriched microdomains within the cell membrane, which are able to include or exclude proteins to variable extents. This dynamic process regulates protein interactions and influences their functions. Lipid rafts can

be decreased and disrupted by cholesterol depletion, e. g. by HDL (18). Tellier et al. showed in vitro that TNFÎ± shedding was increased in fibroblasts and ECs after incubation with HDL. This was attributed to an increased activity of ADAM 17, which was due to lipid raft disruption by cholesterol depletion (19).

Study aim and design

The purpose of this study is to investigate the effects of HDL on macrophage phenotype and whether NF-kB signaling, lipid raft disruption and increased activity of ADAM10 and 17 are involved in this.

First, we will determine the effects of HDL on M1 and M2 macrophage polarization by exposing bone marrow-derived macrophages (BMDMs) from C57BL/6 mice to HDL. Here, M1 and M2 polarization markers will be determined using quantitative PCR and ELISA.

Second, we will examine whether NF-ÎºB signaling is involved in the pro-inflammatory effects induced by HDL in macrophages. ?????????

Lastly, we will investigate whether HDL skews macrophages towards a pro-inflammatory state by increasing ADAM10 and 17 activity through lipid raft disruption. ?????????? activity assay??

We hypothesize that HDL polarizes macrophages towards a pro-inflammatory phenotype due to activation of the NF-ÎºB signaling pathway and an increased ADAM10 and 17 activity, through lipid raft disruption

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