Cholesterol metabolism and hematopoiesis interaction in atherothrombosis

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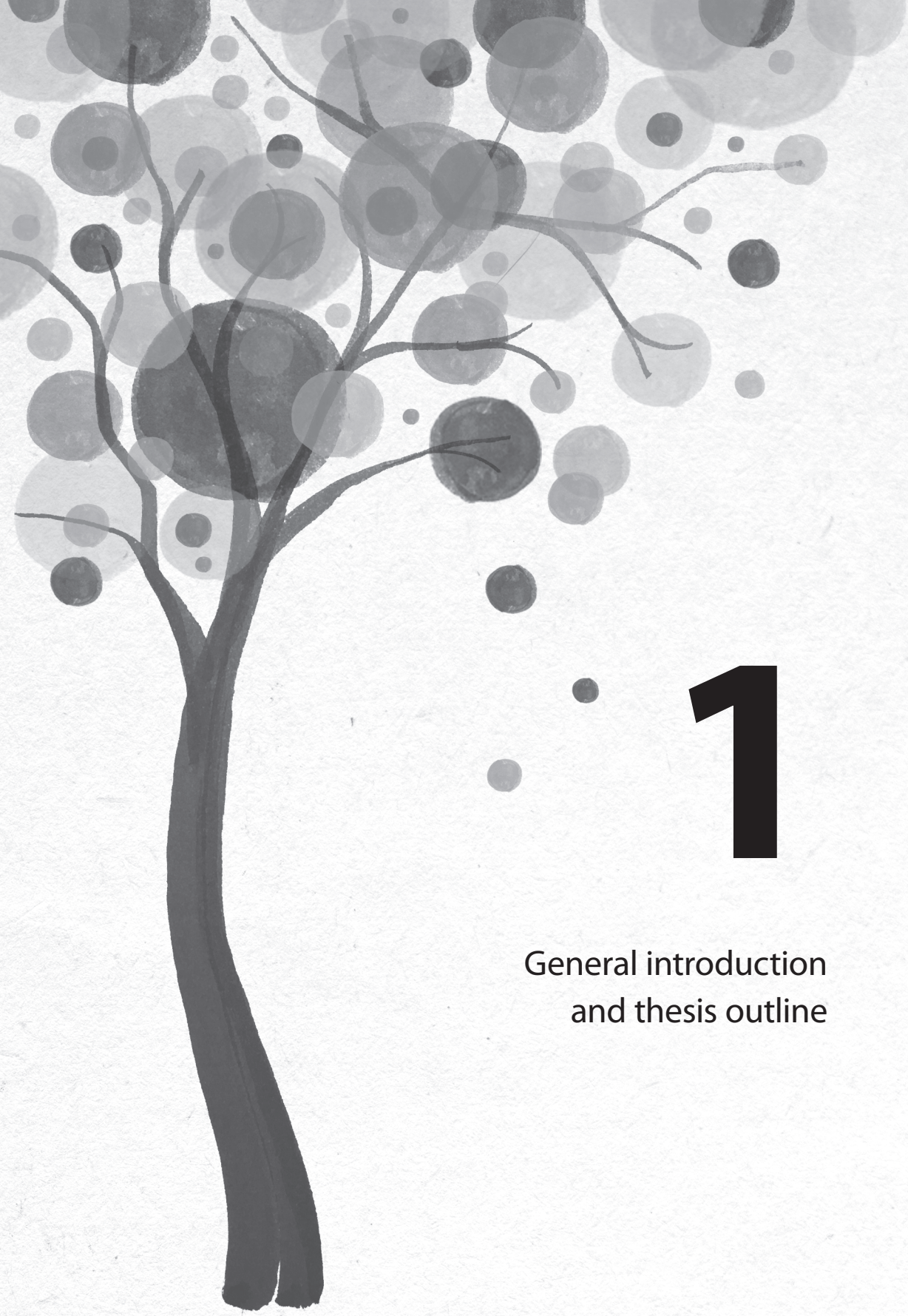

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General introduction and thesis outline

PREFACE

Atherosclerosis and its complications are the underlying cause for most cardiovascular diseases. It is a chronic, multifactorial disease in which lipid accumulates in the arterial wall, leading to a local inflammatory reaction and atherosclerotic plaque formation. Atherosclerotic disease develops largely asymptomatic over a lifetime. However, plaque rupture or erosion can cause the formation of a superimposed thrombus blocking the flow of blood. Upon rupture or erosion of an atherosclerotic plaque, platelets, the body's first responders upon endothelial disruption, initiate the formation of a platelet plug, which activates the coagulation cascade to form an arterial thrombus. Although in principle a protective response, this thrombus formation can cause acute cardiovascular events such as myocardial infarction or ischemic stroke [1,2].

Bone marrow derived cells from both the innate and adaptive immune system, especially monocytes/macrophages and T cells, and platelets have been implicated in the pathophysiology of atherosclerosis [1], The primary site of immune cell and platelet production is the bone marrow. During bone marrow hematopoiesis, hematopoietic stem cells (HSCs) mature and differentiate until the formed immune cells and platelets enter the circulation or home towards their site of action or organ for final maturation [3]. During homeostasis, hematopoiesis is tightly balanced between the different blood cell lineages. In that way, by controlling the output of immune cells and platelets, hematopoiesis plays a major role in the resolution or persistence of inflammatory diseases, such as atherosclerosis. An increased number of peripheral blood leukocytes, especially monocytes and granulocytes, as well as increased platelet counts and reactivity is associated with occurrence and outcome of cardiovascular disease [4–10]. Interestingly, defects in cholesterol metabolism and hypercholesterolemia have been shown to affect hematopoiesis, immune cell production and platelet counts and reactivity [11,12]. Therefore, bone marrow cholesterol handling is an interesting target in the battle against cardiovascular disease.

This chapter summarizes relevant information to understand the role of cholesterol metabolism in the production and reactivity of immune cells and platelets, and its effects on atherosclerosis and atherothrombosis development.

HEMATOPOIESIS

All circulating blood cells, i.e. erythrocytes, leukocytes and platelets, originate from bone marrow, in which they are formed from HSCs through hematopoiesis. Hematopoiesis is divided into three main branches: the myeloid, lymphoid and megakaryocyte-erythroid arms. HSCs constitute the top of the hematopoietic tree and give rise to committed

progenitor cells, which in turn mature into differentiated cells that home towards their site of action or organ for final maturation, Figure 1.

HSCs are self-renewing and either multi- or uni-potent. They remain quiescent to maintain their undifferentiated state. The HSC pool is divided into long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs). LT-HSCs can provide long term engraftment and can serially engraft irradiated mice, whereas ST-HSCs have more limited self-renewal capacity in a serial transplantation assay [3]. ST-HSCs are believed to give rise to multipotent progenitors (MPPs), which are capable of producing all blood cell linages, but lack the capacity for serial transplantation. These MPPs then differentiate further into either myeloid or lymphoid lineages. In the classical model of hematopoiesis, the next steps are the formation of common myeloid progenitors (CMPs), which give rise to all myeloid cells, including the megakaryo-erythroid lineage, and the common lymphoid progenitors (CLPs) [3]. However, this classical tree has been subject of debate. Recent evidence has suggested that possibly the first step from the HSC pool may not be a lymphoid-myeloid differentiation decision step, but rather one that allows the megakaryocyte-erythrocyte lineage to split off, leaving a progenitor with both lymphoid and myeloid potential, but without the capacity to form megakaryocytes or erythrocytes. Named a lymphoid-primed multipotent progenitor (LMPP), this cell then differentiates into the granulocyte-macrophage progenitor (GMP) and CLP [3]. The GMP can differentiate into a neutrophil, monocyte (which in turn can differentiate into a macrophage or dendritic cell in the periphery), or an eosinophil [13]. The CLP differentiates into a B cell, a natural killer cell (NK cell) or becomes an early thymic progenitor (ETP) that migrates from the bone marrow to the thymus to become a CD4+ T cell or a CD8+ T cell[13]. Megakaryocyte-erythroid progenitors (MEPs) differentiate further into erythrocytes (through stimulation with erythropoietin) or megakaryocytes (stimulated by thrombopoietin). Cells in the erythroid lineage are released into the bloodstream as reticulocytes that mature to erythrocytes in the circulation. Megakaryocytes mature fully in the bone marrow, after which they release platelets into the bloodstream and perish [14].

CHOLESTEROL METABOLISM

Cholesterol is an important precursor for steroid hormones and an essential component of cell membranes, in which it modulates membrane fluidity. Cholesterol can exist in eukaryotic cells in two forms: an unbound, free from in cell membranes, or stored as cholesteryl esters. Esterification for storage is important, as excess free cholesterol is cytotoxic. See Figure 2 for a schematic overview.

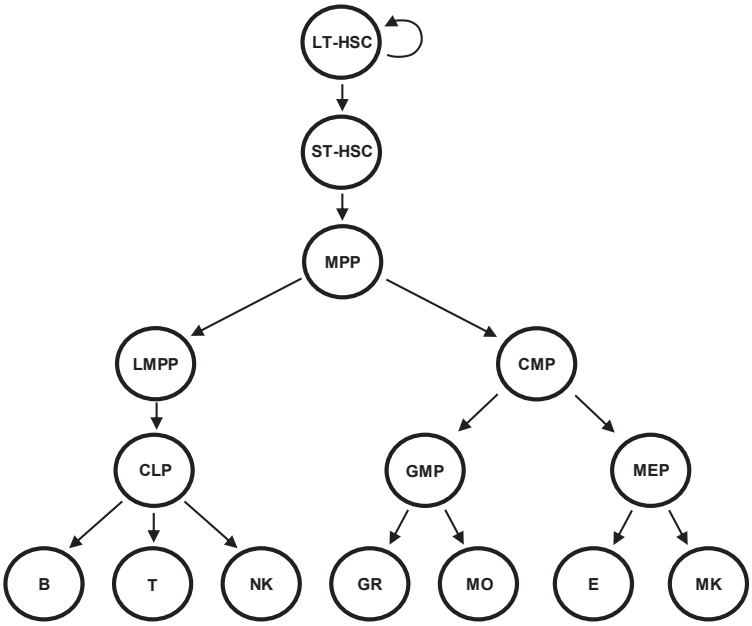

Figure 1: Schematic overview of hematopoiesis. For explanation see text. LT-HSC: long-term hematopoietic stem cell. ST-HSC: short-term hematopoietic stem cell. MPP: multipotent progenitor. LMPP: lymphoid-primed multipotent progenitor. CMP: common myeloid progenitor. CLP: common lymphoid progenitor. GMP: granulocyte-macrophage progenitor. MEP: megakaryocyte-erythroid progenitor. B: B cell. T: T cell. NK: natural killer cell. GR: granulocyte. MO: monocyte. E: erythrocyte. MK: megakaryocyte.

Lipoproteins

General structure and function

Cholesterol is a hydrophobic molecule and is insoluble in aqueous solutions. Therefore, it is transported thought the blood circulation by lipoproteins. Lipoproteins are water-soluble macromolecular complexes, comprised of a hydrophobic core containing cholesteryl esters, neutral lipids, and triglycerides, and a hydrophilic monolayered shell of phospholipids, unesterified free cholesterol and specific apolipoproteins. Lipoproteins are classified according to their size and density: chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) [15]. Chylomicrons and VLDL are the largest and least dense lipoproteins, as they consist primarily of a triglyceride rich core. LDL, a remnant of VLDL, and HDL contain a core which is comprised primarily of cholesteryl esters [15]. Moreover, the different lipoproteins can be distinguished by their apolipoprotein composition. Chylomicrons, VLDL and LDL have apolipoprotein B (ApoB) as their primary apolipoprotein, whereas apolipoprotein A1 (ApoA1) is the principle apolipoprotein of HDL.

The liver plays a central role in cholesterol metabolism, which can be divided in an exogenous, endogenous and reverse cholesterol transport pathway [6].

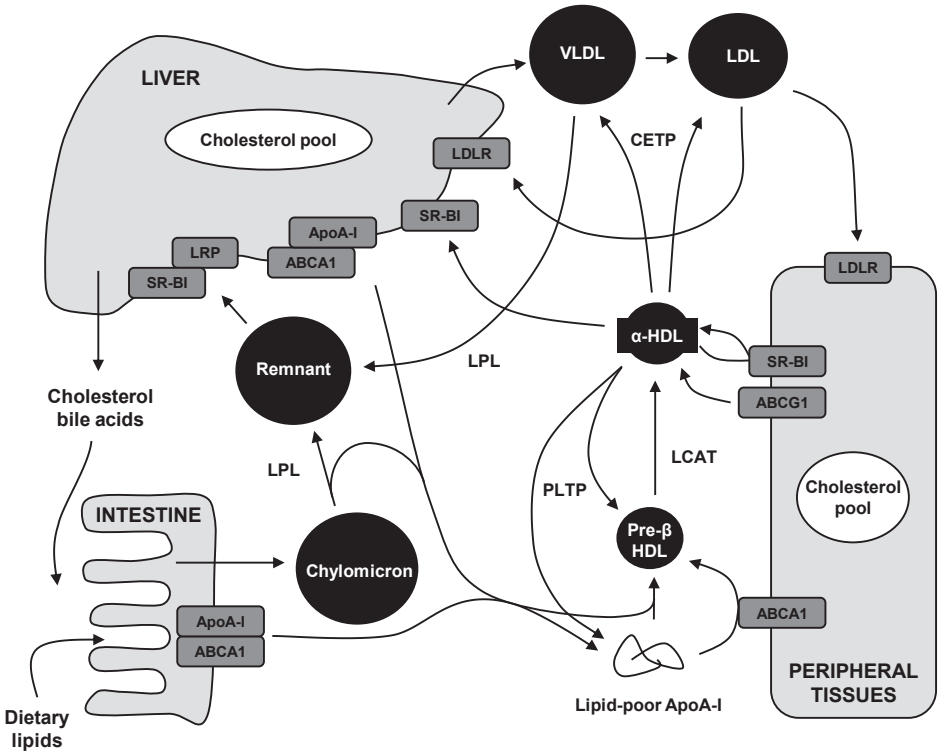

Figure 2: Schematic overview of lipid metabolism. For explanation, see text.

Exogenous lipid and cholesterol metabolism

The amount of cholesterol and triglycerides absorbed from the diet is a major contributor the lipid and cholesterol levels in the circulation. Upon absorption by the enterocytes dietary triglycerides and cholesteryl esters are, together with intestinal ApoB molecules incorporated in nascent chylomicrons. These newly synthesized chylomicrons are then exocytosed into the lymph and subsequently enter the blood circulation, where they acquire Apolipoprotein E (ApoE) and apolipoprotein C (ApoC) apolipoproteins to form mature chylomicrons [16,17]. Mature chylomicrons circulate in the blood, where their triglycerides molecules are lipolyzed by lipoprotein lipase (LPL) and taken up for energy production and storage [18,19]. The formed chylomicron remnants are then removed from the circulation through interaction of ApoE with hepatic LDL receptor related protein (LRP), remnant receptors, heparin sulphate proteoglycans, and scavenger receptor BI (SR-BI) [20–22]. In line with the importance of ApoE for removal of lipoproteins and chylomicron remnants from the circulation, targeted deletion of ApoE is associated with severe hypercholesterolemia. Hypercholesterolemia in these mice already occurs on a

standard chow diet which does not contain cholesterol, and is strongly aggravated on a high-cholesterol diet [23,24].

Endogenous lipid and cholesterol metabolism

Lipids derived from hepatic uptake of lipoprotein remnants and de novo synthesized cholesterol are packaged and (re)secreted into the circulation by the liver as ApoB100-containing nascent VLDL particles [25]. In the circulation, VLDL further acquires ApoE and ApoC [15]. Hydrolysis of triglycerides transforms VLDL to intermediate-density lipoproteins (IDL), which are partly taken up by the liver via the LDL receptor (LDLr) and LRP [26]. Further hydrolysis of IDL particles results in the formation of smaller cholesteryl ester-rich LDL, which has ApoB100 as the sole apolipoprotein [26]. Finally, LDL is recognized by the LDLr mostly on the liver as well as peripheral tissues and removed from the circulation for intracellular metabolism. In line with its important role in the removal of VLDL remnants and its ultimate remnant LDL from the circulation, mice which are genetically deficient for the LDLr have a prolonged half-life of plasma VLDL and LDL [27]. These mice display a modestly elevated plasma cholesterol level when fed a normal chow diet. However, when placed on a cholesterol-rich diet they show strongly elevated plasma cholesterol levels (hypercholesterolemia) [28,29].

Reverse cholesterol transport

Reverse cholesterol transport is the pathway through which excess cholesterol from peripheral tissues is transported back to the liver by HDL for recycling or biliary excretion.

HDL metabolism

HDL is primarily produced by the liver, and to a lesser extent by the intestine, though interaction of lipid-poor ApoA1 with the ATP-binding cassette transporter A1 (ABCA1) [30,31]. This is an essential part of HDL formation, as both genetic deficiency of ApoA1 as well as lack of ABCA1 diminishes circulating HDL levels [32,33]. Lipidation of ApoA1 results in the formation of nascent pre-β HDL particles [34]. These nascent HDL particles are potent acceptors of excess cholesterol from peripheral tissues. Upon association of, primarily, free cholesterol and phospholipids from peripheral tissues, discoidal HDL particles mature to spherical HDL_3 particles upon conversion of its free cholesterol into cholesteryl esters by lecithin:cholesteryl acyltransferase (LCAT) [35] and storage in the core of the particle. HDL_3 is subsequently converted into large α-migrating mature HDL_2 by further acquirement of phospholipids by transfer through phospholipid transfer protein (PLTP) and apolipoproteins that are released during lipolysis of triglycerides in chylomicrons or VLDL. Mature HDL circulates in the blood where it takes up excess cholesterol from tissues, excreted through cholesterol efflux transporters. The cholesteryl esters are delivered to the liver via SR-BI for recycling and biliary excretion or transferred to ApoB containing

lipoproteins by cholesteryl ester transfer protein (CETP) for redistribution through the endogenous lipid and cholesterol metabolism pathway.

Cholesterol efflux towards ApoA1 and HDL

Cholesterol efflux is an active mechanism, which is regulated by various transporters, the most important of which are members of the ATP binding cassette (ABC) transporter family. Several members of this family of transmembrane proteins mediate the transport of cholesterol between various cellular domains and eventual efflux of cholesterol to ApoA1 or HDL on the plasma membrane.

ABCA1 is present on the cellular plasma membrane, and mediates cholesterol efflux to lipid poor apolipoproteins, such as ApoA1 and ApoE, but not large HDL particles [36]. ABCA1 is highly expressed in hepatocytes and enterocytes, the two cell types that together generate the majority of the plasma HDL [37]. As a consequence, ABCA1 knockout mice are unable to lipidate circulating ApoA1, resulting in HDL deficiency [32]. In line, mutations in the ABCA1 gene in humans are the causal factor in Tangier disease, of which HDL deficiency is a major clinical manifestation [38–40]. However, ABCA1 is most known for its crucial function in lipid homeostasis and cholesterol efflux from macrophages, as the deletion of ABCA1 in bone marrow leads to a dramatic increase in foam cell formation and subsequent atherosclerosis in hypercholesterolemic mice [41].

Another important cholesterol transporter is ABCG1. ABCG1 is highly expressed in macrophages, endothelial cells, epithelial cells and brain. However, in contrast to ABCA1, not in hepatocytes or intestinal enterocytes [42]. Moreover, contrary to ABCA1, which is a full transporter, ABCG1 needs to dimerize with another ABC transporter to become functional [43]. ABCG1 mediates in cholesterol efflux to mature HDL particles, but not lipid-free apolipoproteins [42,44,45]. In line, ABCG1 does not facilitate HDL generation [42]. Interestingly, whereas ABCA1 is present on the plasma membrane and directly binds to circulating ApoA1 [46], ABCG1 is involved in intracellular sterol movement. Although the precise cellular localization of ABCG1 remains elusive, it is involved in trafficking of cholesterol over the endoplasmic reticulum membrane and endosomes. In that way it does not only increase availability of cholesterol for efflux to lipoprotein acceptors, but is also important for the regulation of endoplasmic reticulum-localized cholesterol biosynthesis genes [46,47].

Besides ABCA1 and ABCG1, ABCG4 has also been implicated as a cholesterol efflux transporter [44]. ABCG4 shows high levels of homology to ABCG1, and effluxes to HDL particles. Interestingly, it is not expressed in macrophages, but is highly expressed in megakaryocytes, the precursor cells of platelets [48].

Besides ABC transporter family members, SR-BI has also been implicated as a cholesterol efflux transporter, as it induces free cholesterol flux down a cholesterol-concentration gradient [49,50]. The exact mechanism by which SR-BI mediates cholesterol efflux

is largely unknown. Possibly, SR-BI simply mediates tethering of acceptor HDL particles closely to the cell surface [51]. Another possibility is that SR-BI reorganizes the lipids in cholesterol-rich domains in the plasma membrane, thereby facilitating the movement of cholesterol towards HDL [51].

ATHEROSCLEROSIS

Atherosclerosis is a chronic, multifactorial disease induced by lipid accumulation in the vessel wall and subsequent arterial wall inflammation, leading to atherosclerotic plaque formation. Atherosclerosis develops asymptomatic over a lifetime. However, when a plaque ruptures or erodes, a superimposed thrombus is formed causing acute cardiovascular events such as myocardial infarction or ischemic stroke.

Dyslipidemia is a major risk factor for atherosclerosis

A major risk factor for the development of premature atherosclerosis is dyslipidemia. Dyslipidemia is a broad term, which refers to a number of lipid disorders, including elevated LDL cholesterol, low HDL cholesterol, excess lipoprotein(a), hypertriglyceridemia, and mixed lipid disorders. Although familial causes account for ~20% of these disorders, most (~80%) are related to diet and lifestyle [52]. It is well established that there is a direct relationship between dyslipidemia and cardiovascular risk [53]. In healthy individuals, cholesterol levels are below 5 mmol/L. A rise of 2 mmol/L cholesterol increases the risk of death by cardiovascular disease by 50% [54]. This is most likely attributable to high LDL cholesterol, the main carrier of cholesterol in human plasma. In contrast, large population studies have consistently shown that low HDL cholesterol, as well as ApoA1 levels are independent, inverse predictors of cardiovascular disease risk [55–59]. Normalization of dyslipidemia is therefore a major target in the prevention of atherosclerosis and its clinical manifestations.

Pathophysiology of atherosclerosis

Atherosclerotic plaques form at specific arterial sites with differences in blood flow dynamics, such as arterial tree branches and bifurcations. Low shear stress, oscillatory or turbulent flow, together with dyslipidemia, which can cause lipoprotein retention in the subendothelial matrix, cause damage and endothelial dysfunction. This is generally regarded as the primary step in atherosclerosis development. Damage to the endothelium increases arterial wall permeability and increases expression of adhesion molecules, and the production of cytokines and chemokines, thereby leading to the migration of inflammatory cells such as monocytes, lymphocytes and neutrophils from the circulation into the arterial wall intima. Macrophages in the intimal wall then rapidly

engulf the accumulated lipids and the first foam cells in the nascent lesion are formed. Macrophage foam cell formation is the hallmark of early atherosclerotic lesions, also called fatty streaks. These fatty streaks can then progress towards intermediate fibro-fatty lesions, which consist of several layers of foam cells and vascular smooth muscle cells with T cells, surrounded by a relatively poor developed matrix of connective tissue. These intermediate plaques can then progress further into advanced fibrous lesions, which are characterized by a fibrous cap that covers a core of extracellular lipid and necrotic material, together with macrophages, smooth muscle cells and T cells [1]. In line with the importance of immune cell influx and inflammation in atherosclerosis development, studies in mice have indicated a causal relationship between the levels of blood monocytes and neutrophils and the extent of atherosclerosis [60–62]. Moreover, the levels of blood monocytes and neutrophils are predictive of acute cardiovascular events [4,5]. Importantly, monocytosis in the absence of hyperlipidemia is insufficient to induce atherosclerosis [11,63]. Only when the endothelium becomes inflamed in response to hypercholesterolemia, monocytosis and neutrophilia contribute to lesion development [11,64–66].

Atherothrombosis

Vulnerable plaques

Atherosclerotic plaques develop slowly, and remain largely asymptomatic as long as they remain intact. However, advanced plaques can cause complications through atherothrombosis formation. Atherothrombosis can occur in two ways: by rupture of an unstable lesion, or by erosion of the overlying endothelium. Plaques prone to rupture, so-called vulnerable plaques, are characterized by a thin fibrous cap, and a large lipid core with a relative abundance of inflammatory leukocytes [67]. In contrast, vulnerable plaques underlying areas of superficial erosion do not have thin fibrous caps. Moreover, they harbor fewer inflammatory cells, and lack large lipid pools [68]. From autopsy studies done several decades ago, it was suggested that plaque rupture most commonly led to fatal coronary atherothrombosis [67,69], whereas a minority of fatal events was caused by superficial plaque erosion. However, more recently, questions have been raised about the dominant mechanisms implicated in atherothrombosis. Recent evidence suggests that plaques with thin fibrous caps and large lipid pools seldomly rupture and cause clinical events [70,71]. Moreover, recent studies have shown that there is a shift towards plaques with significantly more fibrous, non-inflammatory characteristics [72–74]. Possibly, this shift in plaque characteristics could lead to a subsequent shift in plaque rupture versus erosion occurrence [75,76].

Pathophysiology of atherothrombosis

When a plaque ruptures or erodes, subendothelial collagen and thrombogenic plaque material, such as macrophage tissue factor, are exposed to the arterial circulation. This leads to thrombus formation on top of the ruptured or eroded plaque [2]. Upon the breach of the arterial endothelial lining, platelets are the first responders. Within one minute after rupture, platelets adhere and aggregate on collagenous plaque components. In line with their importance in atherothrombotic development, platelet characteristics such as density and volume are associated with the risk of acute coronary syndromes [6–9], and implies mean platelet volume, a determinant of platelet reactivity, as both a causal and prognostic factor [10]. Following the platelet response, approximately three minutes after breaching of the endothelial lining, the thrombus is characterized by thrombin and fibrin formation, and by tissue factor-triggered coagulation cascade activation [77]. This thrombus formation can lead to rapid occlusion of the vessel, a cause myocardial infarction, ischemic stroke and sudden death. This deadly nature of atherothrombosis has made it a critical target for investigation.

Interplay between cholesterol and bone marrow cells in atherosclerosis

Atherosclerosis is generally characterized as an inflammatory response to hypercholesterolemia. However, there is increasing evidence that the inflammatory process driving the atherogenic response to hypercholesterolemia may be initiated in the bone marrow. A large body of evidence indicates that bone marrow functioning and hematopoiesis are affected by changes in HSC cholesterol homeostasis, i.e. hypercholesterolemia and cholesterol efflux.

Hypercholesterolemia and HSCs

Several studies on the effects of hypercholesterolemia on bone marrow function have been done in high-fat, high-cholesterol (HFHC) diet -fed LDLr and ApoE knockout mice. Both HFHC diet-fed ApoE knockout, and to a lesser extent HFHC diet-fed LDLr knockout mice display and augmented expansion and proliferation of HSCs [64,78,79]. This phenotype is paralleled by an increase in myeloid cell production and increased circulating counts of monocytes and neutrophils [64,78,79].

Interestingly, hypercholesterolemia-induced hyperproliferation of HSCs, as well as the consequent leukocytosis, are also evident in recipients of transplanted bone marrow from hypercholesterolemic mice [79,80]. Thus, long-term intrinsic changes in stem cells are induced by the hypercholesterolemia.

Besides HSC proliferation and leukocytosis, hypercholesterolemia also alters platelet production and reactivity. Rabbits and guinea pigs fed a high-cholesterol diet develop megakaryocytes with increased ploidy and size [81,82]. Hypercholesterolemia in humans is also associated with higher megakaryocyte ploidy, indicative of altered megakaryocyte

maturation [83]. Notably, the change in megakaryocyte maturation in hypercholesterolemic human subjects coincided with a higher mean platelet volume [83].

Cholesterol efflux and HDL and HSCs

Most of the atherosclerosis-protective properties of APOA1 and HDL are attributed to their role in cholesterol efflux and subsequent reverse cholesterol transport from macrophage foam cells, as well as direct anti-inflammatory properties [84–86]. HSCs utilize cholesterol efflux pathways to oppose the excessive production of myeloid cells [11,64,78,87]. Genetic deletion of ABCA1 and ABCG1 leads to dramatic expansion and proliferation of HSCs and an increase in committed myeloid progenitor populations [11]. This stimulates the development of severe monocytosis and neutrophilia and infiltration of multiple organs with myeloid cells and accumulation of macrophage foam cells [11]. A similar phenotype is observed when ABCA1/ABCG1 double knockout bone marrow is transplanted into LDLr homozygous or heterozygous knockout mice [11,88]. Interestingly, increased HSC numbers and the myeloproliferative phenotype caused by combined deletion of ABCA1/ABCG1 in bone marrow could be rescued by transgenic overexpression of ApoA1 in recipient mice [11]. Infusion of ApoA1 into LDLr/ApoA1 double knockout mice also reduces the number of LSK cells in bone marrow [87], and reduces HSC proliferation in hypercholesterolemic LDLr knockout mice, and ApoE knockout mice [64,78].

Transplantation of ABCA1 knockout bone marrow into LDLr knockout mice is associated with lymphocytosis, although no such phenotype is found in total body ABCA1 knockout mice [41]. Similarly, chow-fed ApoA1 knockout mice do not display monocytosis or proliferation of HSCs [64]. As both ABCA1 and ApoA1 knockout mice are hypocholesterolemic through the inability to produce HDL, this indicates that the importance of cholesterol efflux may depend on the levels of circulating cholesterol levels. Genetic deletion of only ABCG1 has thus far not been shown to affect bone marrow hematopoiesis, neither as a total body knockout nor after transplantation to hypercholesterolemic recipient mice.

ABCG4 is specifically expressed in MEP cell populations and megakaryocyte progenitors. In line, ABCG4 knockout megakaryocyte progenitors show defective cholesterol efflux to HDL and increased MEP and megakaryocyte progenitor cell populations, which are relatively more responsive to thrombopoietin. As a consequence, ABCG4 knockout transplanted LDLr knockout mice have increased platelet counts [48].

OUTLINE OF THE THESIS

Defects in cholesterol metabolism and hypercholesterolemia have been shown to affect hematopoiesis, immune cell production and platelet counts and reactivity. Therefore,

bone marrow cholesterol handling is an interesting target in the battle against cardiovascular diseases, and acute cardiovascular events in particular.

This thesis will describe novel interactions between cholesterol metabolism and the production immune cells and platelets, and its effects on atherosclerosis and atherothrombosis development.

The first part of this thesis focusses on cholesterol metabolism in hematopoiesis.

It is well established that dyslipidemia is an important risk factor for cardiovascular disease. Circulating lipid levels are highly correlated with atherosclerotic plaque development. In addition, experimental evidence suggests that lipids also directly influence thrombosis and influence the risk and the outcome of acute cardiovascular events. In **chapter 2**, the effects of lipoproteins on three important components of atherothrombosis pathophysiology, i.e. endothelial function, platelets and secondary coagulation, are reviewed.

A substantial body of clinical evidence indicates that platelet characteristics such as density and volume are associated with increased risk of acute coronary syndromes [6–9], and implies mean platelet volume, a determinant of platelet reactivity, as both a causal and prognostic factor [10]. Therefore, modification of platelet functionality is a promising therapeutic target in the prevention and treatment of cardiovascular disease. In **chapter 3**, we show that elevated plasma unesterified cholesterol levels impairs megakaryopoiesis and platelet production in SR-BI knockout mice. Our findings suggest that impaired platelet production, in addition to the previously reported augmented platelet clearance, may explain part of the thrombocytopenic phenotype associated with SR-BI deficiency. In **chapter 4**, we describe novel and opposing roles of the cholesterol efflux transporters ABCA1 and ABCG1 in megakaryopoiesis, platelet production and effects on platelet characteristics.

In addition to effects on hematopoietic stem cell functionality and hematopoiesis, ApoA1 and HDL also directly influence inflammatory cell responses [86]. In **chapter 5**, we dissociated the direct effects of ApoA1 deficiency on immune cells in the context of atherosclerosis from effects that are generated through changes in bone marrow immune cell production.

The second part of this thesis focusses on the development of experimental animal models of atherothrombosis.

Although atherosclerosis is widely studied in experimental mouse models, experimental studies regarding atherothrombosis are hampered due to lack of suitable mouse models. In **chapter 6** recent advances in the development of mouse models for spontaneous atherothrombosis are reviewed. In **chapter 7**, we describe a novel RNA interference-based mouse model for spontaneous atherothrombosis in ApoE knockout mice. Further characterization and attempts to optimize this model through administration of phenylephrine to ApoE knockout mice are described in **chapter 8**. Murine SR-BI

deficiency is associated with increased platelet reactivity and a higher susceptibility for $FeCl_3$ damage-induced arterial thrombosis in the carotid artery [12]. In **chapter 9**, we apply the developed atherothrombosis model to hypercholesterolemic SR-BI knockout mice.

Finally, all the results obtained in this thesis and future perspectives are summarized and discussed in **chapter 10**.

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