ABC transporters and scavenger receptor BI : important mediators of lipid metabolism and atherosclerosis

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Chapter 11

General discussion and future perspectives

GENERAL DISCUSSION AND PERSPECTIVES

INTRODUCTION

Despite important progress in the treatment of atherosclerosis by widespread statin administration and reduction of risk factors, atherosclerosis is still a major cause of death due to the development of acute clinical events such as myocardial infarction and cerebral stroke.[1, 2] One of the earliest events in atherosclerosis is the adherence of monocytes to the endothelium and transmigration into the arterial intima, where they differentiate into macrophages. Upon differentiation, macrophages start to accumulate large amounts of lipids by the uptake of (modified and/or aggregated) lipoproteins, including oxidized low-density lipoproteins (oxLDL), leading to the formation of foam cells.[3-5] The transformation of macrophages into foam cells by excessive accumulation of cholesterol is a pathological hallmark of atherosclerosis. Therefore, of particular importance in the initiation of atherosclerosis is dysregulation of the balance between cholesterol influx and efflux in macrophages. Key mediators for macrophage cholesterol homeostasis include scavenger receptors and ATP-binding cassette (ABC) transporters, which facilitate the influx and efflux of lipids.[6] Macrophages, are incapable of limiting the uptake of cholesterol by scavenger receptors, including scavenger receptor class A (SR-A) and CD36.[6, 7] Therefore, mechanisms by which macrophages export cellular cholesterol have been intensively investigated in recent years. Several epidemiological studies have shown that plasma levels of high-density lipoprotein (HDL) cholesterol are inversely correlated with the risk of atherosclerosis.[8-10] The protective effect of HDL against macrophage foam cell formation and atherosclerosis is primarily attributed to its role in reverse cholesterol transport (RCT), a process by which excess cholesterol in peripheral tissues is transported to the liver for excretion. In addition to HDL-raising therapies, modulating macrophage cholesterol efflux pathways might be an interesting new approach in the treatment of atherosclerosis. Therefore, gaining more knowledge on the different efflux pathways is of prime importance for the development of new therapeutic strategies. In this thesis, bone marrow transplantation (BMT) was used to investigate the role of different ABC-transporters in lipid efflux from macrophages, lipoprotein metabolism, and atherogenesis. Additionally, novel ABC-transporters that are differentially expressed during macrophage foam cell formation and atherosclerotic lesion development were identified using microarray analysis.
Furthermore, we showed a vital role for SR-BI in both murine and human biology, indicating that SR-BI can be a promising novel therapeutic target in atherosclerosis.

The role of ABC-transporters in macrophage cholesterol homeostasis and atherosclerotic lesion development

Macrophage cholesterol efflux is a critical step in RCT and protects the cells from excess lipid accumulation and thereby free cholesterol and oxysterol-induced toxicity.[11] The principal molecule involved in cholesterol efflux from macrophage foam cells to HDL is ABCA1. ABCA1 belongs to the ABC-transporter super family, the largest family of conserved

transmembrane proteins that use the energy of ATP hydrolysis to pump a wide variety of substrates. ABCA1 promotes the first step in RCT, namely the efflux of cholesterol and phospholipids to lipid-poor apolipoproteins such as apoA-I.[12, 13] In addition to ABCA1, ABCG1 has also been implicated in cholesterol efflux from macrophages.[14] ABCG1 is highly expressed in cholesterol-loaded macrophages and plays a critical role in lipid homeostasis by facilitating cellular cholesterol and phospholipid efflux from macrophages to mature HDL.[15-17] Thus, since ABCG1 is highly expressed in cholesterol-loaded macrophages and shown to play a role in cholesterol efflux, ABCG1 was expected to protect against atherosclerosis. Nevertheless, the role of macrophage ABCG1 in the development of atherosclerosis remains uncertain, as independent studies using the technique of bone marrow transplantation have shown that ABCG1 might be anti-atherogenic[18, 19] as well as pro-atherogenic[20, 21]. The latter results were attributed to increased susceptibility of the ABCG1 knockout macrophages to apoptosis [20] or to an increase in ABCA1 expression and secretion of apoE from macrophages lacking ABCG1.[21]

In **chapter 2**, total body ABCG1/LDLr double knockout (ABCG1$^{-/-}$/LDLr$^{-/-}$) mice were used to assess the effect of ABCG1 deficiency on different stages of atherosclerotic lesion development. In this study, we demonstrated that ABCG1 deletion in LDLr$^{-/-}$ mice can both induce and attenuate atherosclerotic lesion development. ABCG1 deficiency led to a significant increase in early atherosclerotic lesion size after 10 wks Western-type diet feeding, while a significant decrease in the size of more advanced lesions was observed after 12 wks WTD feeding. These data implicate that the effect of ABCG1 deficiency on atherosclerotic lesion development in LDLr$^{-/-}$ mice depends on the stage of atherogenesis. In agreement, we showed for the first time using correlation analysis with published studies of the independent groups, the current study, and one other unpublished study of our group that the fold increase/decrease in atherosclerotic lesion size of ABCG1$^{-/-}$ mice compared to ABCG1$^{+/+}$ mice is highly correlated (R=0.92) with the atherosclerotic lesion size. In early atherosclerotic lesions, ABCG1 is primarily protective as ABCG1 deficiency causes an increase in atherosclerotic lesion development, most likely as a direct result of the impaired cholesterol efflux to HDL from ABCG1-deficient macrophages. Interestingly, in more advanced atherosclerotic lesion sizes, the role of ABCG1 in atherogenesis switches from anti-atherosclerotic to pro-atherosclerotic. In more advanced lesions, the persistently impaired cholesterol efflux from ABCG1-deficient macrophages is likely to induce accumulation of (oxy)sterols, which leads to enhanced apoptosis and/or other compensatory mechanisms and, subsequently, decreased atherosclerotic lesion size.

Although ABCA1 and ABCG1 are the major contributors to macrophage cholesterol homeostasis, also other ABC-transporters have been implicated in macrophage cholesterol efflux. To date, 52 members of the ABC-transporter family have been identified in humans, which are divided into seven distinct subfamilies (ABCA-G), based on organization of their ATP-binding domains and amino acid homology.[22] A vast majority of these ABC-transporters are expressed by macrophages and display cholesterol-responsive regulation.[23, 24] Among these ABC-transporters, ABCA5, ABCA7, and ABCB1 (MDR1 in mice) were anticipated to be novel candidates involved in macrophage cholesterol homeostasis and therefore their role in lipid homeostasis an atherosclerotic lesion development was determined using the BMT technique.

Similar to ABCA1, ABCA5 expression in macrophages is highly upregulated during *in vitro* cholesterol loading and downregulated upon cholesterol unloading. In addition, recently, we have reported that ABCA5 is highly expressed by Kupffer cells in rats and Western-type diet feeding further induced the expression of ABCA5 in these resident macrophages of the liver.[25] It was thus conceivable that ABCA5 might play a role in macrophage cholesterol homeostasis. However, the evidence for a role of macrophage ABCA5 in atherogenesis was lacking. In **chapter 3,** the BMT technique was used to specifically disrupt ABCA5 expression in macrophages, by transferring bone marrow from ABCA5$^{-/-}$ mice into LDLr$^{-/-}$ mice. Our data revealed that ABCA5 potentially influences macrophage cholesterol homeostasis. Disruption of ABCA5 in macrophages did not affect atherosclerotic lesion development in male LDLr$^{-/-}$ mice. Atherosclerotic lesion size in female LDLr$^{-/-}$ mice, which are more susceptible to atherosclerosis, was significantly increased in absence of functional macrophage ABCA5. Interestingly, expression of ABCA1 was upregulated in ABCA5 deficient macrophages, which might have limited the effects of macrophage ABCA5 deficiency on atherosclerosis. Therefore, generation of ABCA1 and ABCA5 double knockout mice will be important to clarify the exact role of macrophage ABCA5 in atherosclerosis.

ABCA7, a very close relative of ABCA1, is induced during *in vitro* differentiation of human monocytes into macrophages and it is reported to be a sterol-sensitive gene.[26, 27] Therefore, ABCA7 has been anticipated to play a role in cellular cholesterol homeostasis. Interestingly computer-based analysis of the ABCA7 genome revealed multiple potential binding sites for transcription factors with roles in hematopoiesis, indicating a possible role for ABCA7 in the developmental specification of hematopoietic cell lineages.[28] While not yet confirmed experimentally, this finding is consistent with the preferential distribution of ABCA7 in hematopoietic tissues. In addition to cholesterol homeostasis and hematopoiesis, ABCA7 was also described to play an important role in phagocytosis, as macrophage ABCA7-deficiency resulted in defective engulfment of apoptotic cells.[29] All together, ABCA7 has been implicated in several physiologically important pathways, nevertheless its role in atherosclerotic lesion development remained unknown. In **chapter 4**, the goal was to gain insight into the function of ABCA7 in atherosclerotic lesion development by the use of BMT. Selective disruption of ABCA7 in hematopoietic cells did not affect atherosclerosis susceptibility of LDLr$^{-/-}$ mice. Interestingly, ABCA7 deficiency was associated with 2-fold higher macrophage ABCA1 mRNA expression levels.

Owing to ABCA7 deficiency in macrophages led to significant upregulation of ABCA1, the effect of combined macrophage ABCA1 and ABCA7 disruption and single macrophage deficiencies of these transporters on lipid metabolism and atherosclerosis was evaluated by means of the BMT technique. Disruption of macrophage ABCA7 did not affect foam cell formation in the peritoneal cavity nor atherosclerotic lesion development, possibly due to compensatory upregulation of ABCA1 expression. In addition, ABCA1 KO and ABCA1/ABCA7 double KO (dKO) transplanted mice exhibited significantly higher amounts of foam cells in the peritoneal cavity, while also the susceptibility to atherosclerosis was increased. The observed increase in lesion size in dKO transplanted mice is thus most likely attributable to the deficiency in macrophage ABCA1 only. Notably, foam cell formation and susceptibility to atherosclerotic lesion development in the transplanted mice was less severe compared to single ABCA1 KO transplanted mice, indicating that the presence of ABCA7 augments

macrophage foam cell formation and possibly atherosclerosis in the absence of ABCA1. Interestingly, severe splenomegaly was observed in dKO transplanted mice, whereas no such phenotype was present in WT, ABCA1 KO, and ABCA7 KO transplanted mice. Furthermore, the amount of T cells was significantly reduced in spleens of dKO transplanted mice. As ABCA7 is predominantly expressed in macrophages and other lympho-myeloid cell types and it is anticipated to play a role in hematopoiesis, it is conceivable that ABCA7 is involved in processes associated with immune functions. The observed reduction in splenic T-cells might, therefore, be a direct result of impaired lymphopoiesis in absence of ABCA7. Overall, our data reveal that ABCA7 in bone marrow-derived cells does not affect atherosclerotic lesion development in LDLr KO mice, possibly due to compensatory upregulation of ABCA1 expression. In addition, no synergistic role of the transporters ABCA1 and ABCA7 in atherosclerotic lesion development was demonstrated. Nevertheless, combined deletion of ABCA1 and ABCA7 in hematopoietic cells causes severe splenomegaly and a decreased amount of splenic T cells. Considering the potential role of ABCA7 in different complex physiological processes, ABCA7 will be difficult to designate as a novel candidate for therapeutic approaches for the prevention of atherosclerosis.

Also ABCB1 is regulated in response to the cellular content of cholesterol.[24] Furthermore, ABCB1 has been suggested to be involved in the transport of free cholesterol from the plasma membrane to the endoplasmic reticulum, thereby enhancing cholesterol esterification.[30, 31] Interestingly, ABCB1 is highly expressed in human artherosclerotic lesions compared to control lesions.[32] However, it was unknown if ABCB1 expression by macrophages influences the pathogenesis of atherosclerosis. Therefore, a BMT study was performed to assess the role of macrophage ABCB1 in atherosclerotic lesion development in LDLr$^{-/-}$ mice (**chapter 5**). In this study, macrophage ABCB1 deficiency did not affect atherosclerotic lesion development, although disruption of macrophage ABCB1 resulted in a significant increase in VLDL and LDL cholesterol levels. Furthermore, macrophage ABCB1 deficiency did not affect cholesterol efflux to apoA-I and HDL in bone marrow-derived macrophages. Thus, despite its anticipated role in cholesterol esterification, which plays an important role in atherogenesis, macrophage ABCB1 does not affect atherosclerosis susceptibility in LDLr-/- mice.

In **chapter 6**, we identified novel ABC-transporters that are differentially expressed during macrophage foam cell formation and atherosclerotic lesion development using microarray analysis. Using a collar-induced carotid artery atherosclerosis model, transcriptional profiling of ABC-transporters was performed in atherosclerotic lesiond in the carotid artery of LDLr KO mice challenged with a Western-type diet for 2 weeks. Already after 2 weeks of WTD feeding, a significant increase in CD68 mRNA expression was observed, indicating rapid infiltration of macrophages into the carotid arterial wall and the presence of atherosclerotic lesion development. Out of the 46 ABC-transporters measured 6 transporters were significantly regulated during atherosclerotic lesion development in the carotid artery of LDLr$^{-/-}$ mice. Microarray analysis showed significant downregulation of the mRNA expression level of ABCD3, whereas the mRNA expression levels of ABCB1b, ABCB4, ABCC3, ABCC9, and ABCG1 were significantly induced of which ABCB1b, ABCB4, and ABCG1 showed the highest fold change. As massive macrophage infiltration in the carotid arterial wall was observed *in vivo*, we determined mRNA expression levels of ABC transporters specifically in macrophages loaded with different lipoproteins *in vitro*. Peritoneal macrophages (PM) or

bone marrow-derived macrophages (BMDM) were incubated with the pro-atherogenic lipoproteins β-VLDL or ox-LDL, after which tRNA was isolated for microarray analysis. Out of the 46 ABC-transporters 12 transporters were significantly regulated during foam cell formation compared to non-foamy cell, including ABCA3, ABCB1b, ABCB2, ABCB4, ABCB6, ABCB7, ABCC3, ABCC5, ABCC10, ABCD3, ABCF2, and ABCG1. The transporters which were significantly upregulated upon foam cell formation induced by different lipoproteins were ABCA3, ABCB1b, ABCB4, ABCB6, ABCB7, ABCC3, ABCC5, ABCF2 and ABCG1, whereas ABCB2 and ABCC10 were significantly downregulated upon foam cell formation. Interestingly, ABC-transporters which were significantly regulated to a similar extent in both cell types include ABCB2, ABCB4, and ABCB6, of which ABCB2 and ABCB4 both show a high fold-change. Interestingly, ABCB1b and ABCB4 were also significantly induced in atherosclerotic lesions in the carotid arteries. It is important to note that in **chapter 5** the effect of macrophage ABCB1 on atherosclerosis was determined. In our experimental setup, however, macrophage ABCB1 did not affect atherosclerotic lesion development. Additional experiments with different experimental setups (different diet, different time of diet feeding) could be performed to assess the role of ABCB1 in cholesterol homeostasis and atherosclerotic lesion development. This study identified ABCB1b, ABCB2 (a high fold change *in vitro*), and ABCB4 as novel candidates in macrophage cholesterol homeostasis and, therefore, these ABC-transporters might be promising novel therapeutic targets in atherosclerosis.

In addition to ABC-transporters, which are suggested to play a role in cholesterol efflux, other unidentified genes might play an important role in macrophage cholesterol homeostasis i.e. cholesterol uptake and storage. In **chaper 7**, our aim was to identify novel macrophage genes that are highly regulated during the transformation of peritoneal macrophages and bone marrow-derived macrophages into foam cells upon loading with different commonly used pro-atherogenic lipoproteins, including β-VLDL, acLDL, and ox-LDL. Our findings point out important differences in lipid loading pattern in macrophages induced by the specific lipoproteins. Lipid droplets unite and form large cytosolic intracellular lipid deposits in macrophages after βVLDL loading. AcLDL loading resulted in small cytosolic lipid droplets, distributed throughout the macrophage, while loading with oxLDL resulted in a more diffuse lipid distribution. Although lipid droplet formation was observed in both cell types after loading with the different lipoproteins, genes which are involved in lipid droplet formation and catabolism, i.e. cell death inducing DFFA-like effector (Cide) b and c, perilipin (Plin), and perilipin 4 (Plin4), showed no difference in mRNA expression levels compared to non-foamy control cells. These findings suggest posttranscriptional regulation of these lipid droplet-associated proteins. Complete profiling of significantly regulated genes after loading with the different lipoproteins in PM and BMDM identified four genes of high interest, including MRC1, CLEC4N, SORT1, and SCARF2.

The expression of MRC1 is increased by βVLDL and acLDL loading, and decreased 2.5-fold by oxLDL. CLEC4N and SCARF2 showed a highly significant decrease in expression for all three lipoproteins compared to control non-loaded cells, whereas SORT1 expression was significantly induced compared to non-loaded macrophages. Although, to date the exact molecular functions and the regulatory mechanisms of these proteins remains largely unknown, their regulated expression pattern during foam cell formation and their anticipated

role in receptor-mediated endocytosis[33, 34] suggest the involvement of these entities in the uptake of lipoproteins and macrophage cholesterol homeostasis. Overall, these entities might serve as novel targets to inhibit foam cell formation and the initiation of atherosclerotic lesion development.

The role of SR-BI in human and murine biology

Low plasma levels of HDL cholesterol are inversely correlated with the risk of atherosclerosis and one of the strongest risk factors for cardiovascular disease in man.[8] The protective effects of HDL against atherosclerosis can largely be attributed to its role in RCT, the process that describes the HDL-mediated removal of excess cholesterol from macrophages in the arterial wall and delivery to the liver for biliary excretion.[35] The delivery of HDL cholesterol to the liver is mediated by scavenger receptor BI (SR-BI), which selectively takes up cholesteryl esters from HDL without internalisation of the HDL particle.[36] Disruption of SR-BI in mice results in a 3-fold increase in free cholesterol levels in the circulation, which was mainly due to a 2-fold increase in HDL cholesterol.[37, 38] In addition, despite elevated HDL-C levels, SR-BI deficiency leads to an increased susceptibility to diet-induced atherosclerosis.[39, 40] In this thesis, we demonstrated that in addition to its role in RCT, SR-BI also play an important role in other physiological processes.

In **chapter 8**, the effect of high HDL levels induced by SR-BI deficiency on erythrocytes in mice was investigated. Disruption of SR-BI in mice caused anaemia and the accumulation of reticulocytes in the circulation. Erythropoiesis was significantly increased in SR-BI-deficient mice, which was a direct consequence of a reduced life-span of erythrocytes in SR-BI-deficient mice. The increased HDL cholesterol levels due to SR-BI deficiency induced the erythrocyte cholesterol:phospholipid ratios, resulting in a decreased deformability and increased osmotic fragility, thereby providing an explanation for the observed reduction in life-span. Thus, our data reveal that SR-BI is not only essential for HDL cholesterol homeostasis and prevention of atherosclerosis, but also for maintaining normal erythrocyte life-span. SR-BI is also expressed in megakaryocytes and platelets.[41] Interestingly, SR-BI deficiency in mice resulted in impaired platelet function (**chapter 9**). Platelets from SR-BI-deficient mice showed excessive free cholesterol accumulation. Furthermore, murine SR-BI-deficient platelets exhibited increased P-selectin expression and adherence to immobilized fibrinogen was increased, indicating that platelets circulated in an activated state. Together these data suggest that in SR-BI-deficient mice, excess platelet cholesterol may result in an increased baseline state of activation of platelets. It is also hypothesized that the activated state of the platelets *in vivo* led to desensitization of receptors that are essential for positive feedback mechanisms to enhance the platelet response. In agreement, *ex vivo* aggregation of murine SR-BI-deficient platelets was inhibited. Additionally, murine SR-BI deficiency resulted in increased susceptibility to arterial thrombosis in the $FeCl_3$-induced acute arterial injury model.

Together these findings show that SR-BI plays an important role in cholesterol homeostasis in mice. However, whether SR-BI is important for human biology was still unknown. The study described in **chapter 10**, is the first study to report on a family with a loss-of-function mutation in SR-BI. Unrelated Caucasian subjects (n=162) with hyperalphalipoproteinemia were sequenced and 1 subject was identified as a heterozygous carrier of a novel SR-BI

mutation (P297S). Subsequently, 19 SR-BI^{P297S} carriers were identified in the proband's family, who were characterized by high HDL-C levels and a reduced capacity for efflux of cholesterol from macrophages. However, the carotid artery intima–media thickness was similar in carriers and in family noncarriers. Furthermore, the mutation in SR-BI was associated with impaired adrenal steroidogenesis.

In agreement with the platelet studies performed in SR-BI-deficient mice (**chapter 9)**, the loss-of-function P297S mutation in SR-BI resulted in impaired platelet function. Platelets from SR-BI^{P297S} carriers showed excessive free cholesterol accumulation and increased P-selectin expression and adherence to immobilized fibrinogen. These findings suggest that, like in SR-BI-deficient mice, in SR-BI^{P297S} carriers a, excess platelet cholesterol may result in an increased baseline state of activation of platelet. As a consequence of the activated state of SR-BI^{P297S} platelets, *ex vivo* aggregation of human SR-BI^{P297S} platelets was inhibited.

Together, this study, for the first time, provided evidence for an essential role for SR-BI in human physiology.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Upon review or the various aspects of this thesis, the question raises which therapeutic approach/targets will eventually become most promising for use in a clinical setting, modulation of macrophage efflux by influencing ABC-transporter expression and/or activity or modulation of HDL reverse cholesterol transport by targeting SR-BI. Currently, the treatment of atherosclerosis is mainly based on reducing serum LDL levels by the use of statins, inhibitors of the novo cholesterol synthesis. Since numerous epidemiological studies have established an inverse correlation between HDL levels and the risk for atherosclerosis[8-10], various therapies have been designed to increase HDL levels with the aim to promote macrophage RCT. For example, therapeutic inhibition of CETP, which promotes the transfer of CE from HDL to other lipoproteins, raises HDL-C and decreases LDL-C levels. CETP inhibition might thus be a promising therapeutic approach to prevent the development of atherosclerosis-induced cardiovascular events. In the ILLUMINATE trial it was investigated whether torcetrapib, a potent CETP inhibitor, might reduce major cardiovascular events. Unfortunately, the ILLUMINATE trial was prematurely stopped, as torcetrapib plus atorvastatin caused a 25% increase in major cardiovascular events and a 40% increase in death from cardiovascular causes compared to patients treated with atorvastatin plus placebo.[42] Despite the highly favorable changes in lipid profile (increased HDL levels associated with reduced LDL levels), torcetrapib also caused off-target effects, including elevated blood pressure and changes in electrolyte levels. Although many difficulties have to be defeated, novel CETP inhibiting compounds under development may still provide a potential therapeutic way to raise HDL, promote macrophage RCT, and reduce atherosclerosis.

Another example by which atherogenesis can be attenuated is through HDL apoA-I induction.[43] In humans, infusion of a single dose of pro-apoA-I increased fecal sterol excretion[44], and a weekly infusion of recombinant apoA-I Milano/phospholipid complexes for 5 weeks appeared to induce regression of coronary atherosclerosis in a small study.[45].

Enhancing macrophage RCT is thus still a promising strategy to inhibit atherosclerotic lesion development or even induce regression of existing atherosclerotic lesions.

This thesis showed for the first time that also in humans SR-BI plays an important role in the selective uptake of cholesteryl esters from HDL. Therefore, increasing hepatic SR-BI expression will be a viable therapeutic approach to increase macrophage RCT and possibly retard or regress atherosclerosis. The farnesoid X receptor (FXR)[2] and liver receptor homolog 1 (LRH-1)[46] are important transcription factors controlling SR-BI expression. Activation of these nuclear receptors might thus enhance SR-BI expression and designate them as promising targets to establish therapeutic strategies for the treatment of atherosclerosis.

In addition to HDL-raising therapies, specifically targeting macrophage cholesterol efflux might also be an interesting therapeutic approach for the prevention of atherosclerosis. This thesis suggests that upregulation of ABCG1 in macrophages may be a beneficial therapeutic strategy, as ABCG1 deficiency resulted in impaired cholesterol efflux to HDL. Upregulation of ABCG1 can be achieved by pharmacological activation of the liver X receptors (LXR). Upon stimulation by endogenous oxysterols, LXR (LXRα and β) forms a heterodimer with the retinoid X receptor (RXR), which subsequently induce ABCG1 transcription.[17] An advantage of this activation pathway is that simultaneously ABCA1 expression, a key transporter involved in cholesterol efflux, is enhanced.[47]

Several studies have shown that synthetic LXR agonists (TO901317 and GW3965) induce cholesterol efflux from macrophages *in vitro*, promote macrophage RCT *in vivo* and reduce atherosclerosis in mice, illustrating the therapeutic potential of these LXR ligands. [48-50] However, the possibilities for LXR agonists as new therapeutics are counterbalanced by one significant side effect. As LXRs are also expressed in the liver and regulate genes involved in fatty acid, triglyceride and cholesterol metabolism, activation of hepatic LXRs results in stimulation fatty acid biosynthesis, which induces hepatic steatosis.[51] Interestingly, a novel synthetic LXR agonist has been identified which selectively activates LXR target gene expression in macrophages rather than in the liver, without inducing liver steatosis.[52] Thus, assuming problems of increased fatty liver can be solved, modulating ABCG1 expression levels by LXR activators might be ideal for enhancing macrophages cholesterol efflux and protection against atherosclerosis. In this thesis we showed that the effect of ABCG1 on lesion development depends on the stage atherogenesis whereby the absence of ABCG1 leads to increased lesions in early atherosclerotic lesion development, while in more advanced stages of atherosclerosis enhanced apoptosis and/or compensatory mechanisms lead to retarded lesion progression.[21] Therefore, modulation of ABCG1 expression as a therapy in the prevention against atherogenesis, might be complex due to the dual role of ABCG1 in atherosclerotic lesion development. Compensatory mechanisms included upregulation of ABCA1 in absence of ABCG1. Mice deficient for both ABCA1 and ABCG1 exhibited extreme lipid accumulation and foam cell formation in tissue macrophages.[53-55] Several studies have indicated that the observed massive accumulation of lipid in tissue macrophages of ABCA1/ABCG1-deficient mice is a direct result of an impaired efflux capacity of macrophages lacking both ABCA1 and ABCG1. These findings would suggest a dramatic enhancement of the susceptibility to atherosclerosis in ABCA1/ABCG1-deficient mice. Interestingly, total-body ABCA1/ABCG1-deficient mice showed no atherosclerotic lesion development, despite extreme foam cell formation. A possible explanation for the absence of lipid accumulation in

the arterial wall would be that the severe hypocholesterolemic conditions observed in these animals might be unable to provide the stimulus to attract macrophages to the arterial wall. Studies using mice overexpressing both ABCA1 and ABCG1 are essential to clarify whether ABCG1 would be an interesting therapeutic target in prevention against atherogenesis.

Other ABC-transporters which are shown to be cholesterol-responsive and highly expressed by macrophages included ABCA5, ABCA7, and ABCB1. Using BMT, we identified a role for ABCA5 in macrophage cholesterol homeostasis. Therefore, ABCA5 can be considered as a promising novel candidate for modulating macrophage lipid metabolism and, subsequently, atherogenesis. However, as ABCA1 expression is induced in the absence of functional ABCA5 in macrophages, generation of ABCA1/ABCA5 double knockout mice will be essential to clarify the exact role of ABCA5 in atherosclerotic lesion development. Furthermore, as the ABC-transporter ABCA7 is anticipated to be involved in many different and complex physiological processes and a prominent role of macrophage ABCA7 in cholesterol efflux and atherosclerotic lesion development is not confirmed in this thesis, ABCA7 is considered not to be a good candidate for therapy in the prevention of atherosclerosis.

Interestingly, although macrophage MDR1 deficiency resulted in a significant increase in cholesterol transported by VLDL and LDL, atherosclerotic lesion development was not affected. Clarification of the mechanism responsible for the increase in VLDL and LDL cholesterol levels is necessary to determine whether increasing macrophage MDR1 expression/activity might be useful in a clinical setting to reduce pro-atherogenic VLDL and LDL levels.

In addition, the ABC-transporters ABCB1b, ABCB2, and ABCB4 which are suggested to play a role in lipid transport across membranes, and MRC1, CLEC4N, SORT1, and SCARF2, which are anticipated to be involved in receptor-mediated endocytosis, have been identified as new genes involved in macrophage lipid homeostasis. However, further research is necessary to determine whether these proteins will be an efficient therapeutic target for the prevention of atherosclerosis

In conclusion, modulation of macrophage foam cell formation by affecting ABC-transporter expression levels and enhancement of RCT by augmenting SR-BI expression levels in the liver are both attractive therapeutic approaches for prevention of atherosclerosis.

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