Identification of therapeutic targets in coronary artery disease: from patient to mice and back

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CHAPTER 1

General introduction

CARDIOVASCULAR DISEASE AND ATHEROSCLEROSIS

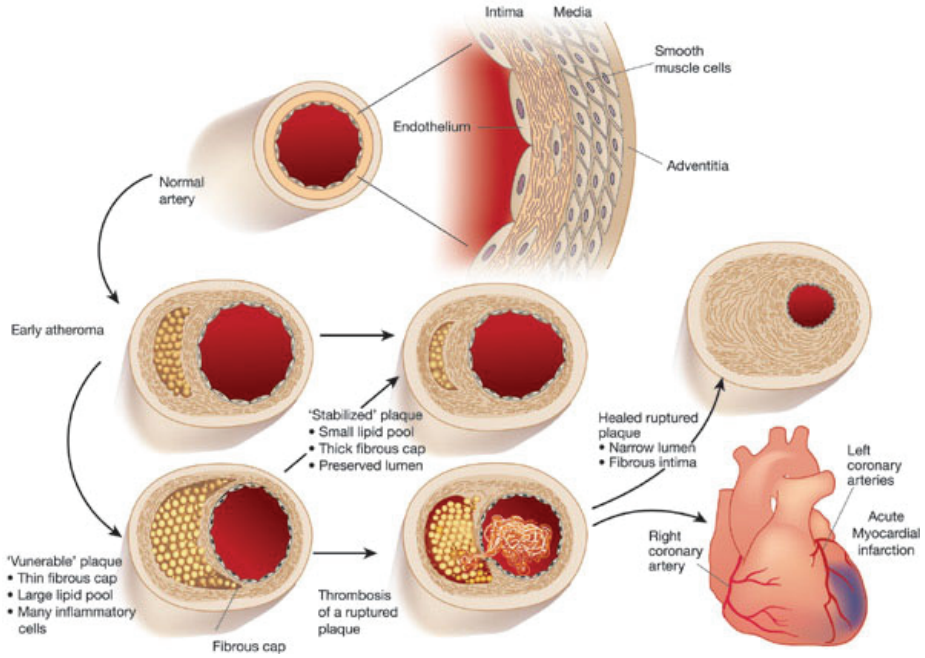
Cardiovascular disease remains to be the leading cause of morbidity and mortality in the world, despite advances in its detection and in (drug) treatment. In 2005, the World Health Organization (WHO) reported 17.5 million deaths related to cardiovascular disease, accounting for 30% of that year's global causes of death, and this number is expected to rise to 20 million deaths each year by 2015 [1]. In the Netherlands, cardiovascular mortality has steadily dropped since the early 1970's. Despite this drop, in 2006 41.720 registered deaths were still attributable to cardiovascular disease [2].

The majority of all cardiovascular disease is caused by the process of atherosclerosis, and its clinical consequences. Atherosclerosis, derived from the Greek *athere* – meaning gruel – and *skleros* – meaning hardness, can be defined as the gradual thickening and obstruction of the arterial wall in middle and large sized arteries. The ensuing arterial stenosis can lead to ischemia of down-stream organs, or in case of plaque rupture, to life threatening thrombus formation with subsequent myocardial or cerebral infarction. While a few relatively rare genetic disorders have been identified to lead to excessive (premature) atherosclerosis like familial hypercholesterolemia [3], the main risk factors for the development of atherosclerosis are age, hypertension, diabetes mellitus, hypercholesterolemia and life style choices like smoking, diet and physical inactivity. Adaptation of a detrimental life style into a healthier one is, not surprisingly, still the most important and effective therapeutic strategy to delay the onset, progress and consequences of atherosclerosis. However drug therapies like statin treatment, blood-glucose lowering drugs, anti-hypertensive medication and surgical interventions such as angioplasty with (drug eluting) stents, have profoundly contributed to a reduction of cardiovascular morbidity and mortality, but are usually only prescribed after the disease has become clinically manifest. Despite the apparent effectiveness of current drug therapy, it does not prevent a sizable portion of patients to suffer from cardiovascular diseases. Hence the search for more effective drug therapies still continues and many therapeutic targets and therapies, like gene therapy, are being studied and/or are under (pre)clinical investigation. This introduction presents a synopsis on the pathophysiological background of atherosclerosis, as well as on the identification of therapeutic targets in (unstable) atherosclerotic plaques, like chemokines and specific genes.

PATHOGENESIS OF ATHEROSCLEROSIS

Although atherosclerosis is traditionally thought to be mainly driven by lipid accumulation in the arterial wall [4], over the last two decades atherosclerosis has been increasingly regarded and is currently viewed as an inflammatory disease [5, 6]. As the consequences of atherosclerosis generally become manifest in a person over the age of 50, atherosclerosis already initiates in childhood and presumably even *in utero* [7]. Deposits of lipids like cholesterol as well as fibrous material and cellular debris accumulate within the arterial wall as a "response to injury" of the arterial endothelial lining, preferably at sites with low shear stress like branch points or

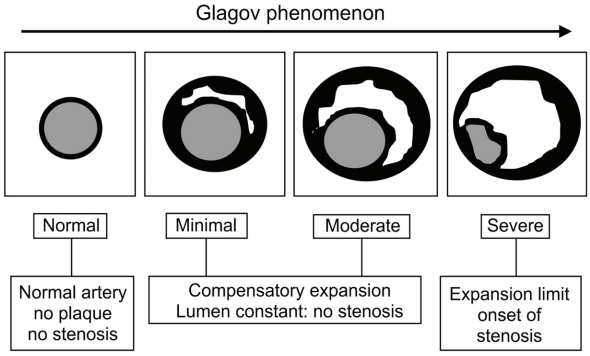
Figure 1.



The vascular wall gradually thickens due to the uptake of lipoproteins within the intima. This lesion grows and can turn into a stable atherosclerotic plaque, characterized by a thick fibrous cap, or a vulnerable plaque, characterized by a large lipid pool or necrotic core and a thin, rupture-prone, fibrous cap. *Adapted with permission from P. Libby; Nature 2002; 868-74.*

bifurcations [8, 9]. Figure 1 depicts the stages of disease development. Early plaque formation is characterized by subendothelial retention of lipoproteins, recruitment of monocytes, their differentiation into macrophages which ingest and accumulate retained lipoproteins, leading to an early atheroma or fatty streak. This atheroma further develops as inflammatory cells, like monocytes and T-cells, infiltrate into the fatty streak and the atherosclerotic plaque gradually enlarges. Now, the first clinical symptoms start to occur, as the growing atherosclerotic plaque can jeopardize the patency of the lumen, leading to symptoms of ischemia in down-stream organs, like stable angina pectoris or intermittent claudication. However, in response to the obstructing process of atherosclerosis the vessel wall will expand outward, a process referred to as vascular remodelling or Glagov's phenomenon, in order to restore lumen size (figure 2) [10]. The ability of the artery to adapt is partly mediated by hemodynamical parameters, like shear stress, heart rate and pulse pressure, and partly by biochemical pathways like enhanced proteolysis via the MMP9 and TIMP2 proteins as well as pathways regulating inflammation via TLR4 activity [11]. Functionally important lumenal stenosis therefore usually occurs when the plaque occupies >40% of the area within the internal elastic lamina (IEL). A decreased remodelling response or vascular failure to remodel will more rapidly lead to luminal narrowing and obstruction [12].

Figure 2.



An overview of the vascular outward remodelling response; upon plaque expansion, the original lumen diameter is preserved, until the expansion limit is reached. *Adapted with permission from S Glagov et al; New England Journal of Medicine 1987; 316:1371–1375.*

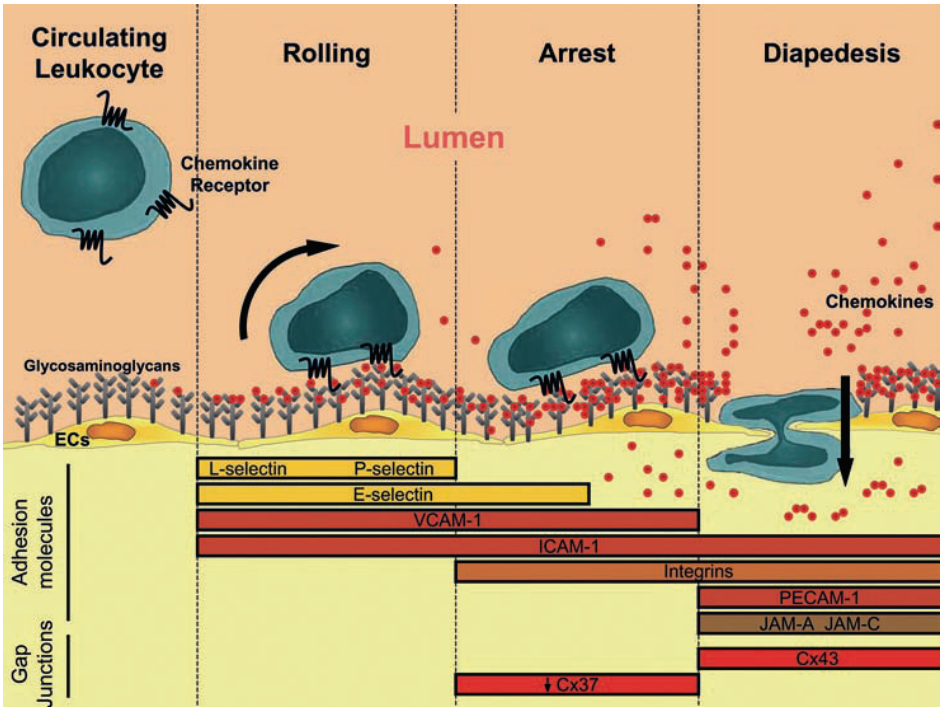
Despite the fact that the artery is able to show an outward remodelling response, the adaptive remodelling response cannot totally compensate for the lumen loss caused by progressive plaque growth, resulting in lumen stenosis.

Macrophages constitute the major pool of inflammatory cells within a plaque. After uptake of oxidized lipoproteins they transform into large foam cells, which excrete various pro-inflammatory mediators, amplifying the inflammatory cascade. These mediators, which are also excreted by attracted inflammatory T-cells, facilitate matrix degradation and promote vascular smooth muscle cell (VSMC) apoptosis, leading to cap thinning and lipid core expansion and eventually to a destabilized and vulnerable plaque with a large lipid pool and a thin fibrous cap. Such a thin cap fibroatheroma may easily rupture and expose its thrombogenic content to the bloodstream which in turn induces the formation of a thrombus via activation of the coagulation cascade [13]. Furthermore, erosion of the fibrous cap constitutes another mechanism by which thrombus formation is initiated. If the thrombus obstructs the vascular lumen, it can compromise adequate flow of the arterial oxygen-rich blood stream to the downstream organs with ischemia and ultimately infarction as, often lethal, consequences.

ATHEROSCLEROSIS: INFLAMMATION, ADHESION AND SIGNALLING MOLECULES

The endothelial cells form a continuous monolayer covering the inner surface of the entire vasculature, including atherosclerotic lesions. Under normal or non-inflamed conditions, the endothelium only expresses low levels of specific adhesion molecules and adhesion of circulating cells is a rather infrequent event. However, at sites of disrupted laminar flow and increased turbulent flow or upon injury of the endothelial layer, endothelial cells up-regulate

Figure 3.



Schematic overview of the various stages of capturing, rolling, arrest and diapedesis of circulating leukocytes on the endothelial surface. Adhesion and rolling starts with the cooperation between various chemokines and selectins. Following rolling, VCAM-1 and ICAM-1 induce firm adhesion and arrest, whereafter PECAM-1 and JAM-A initiate diapedesis into the intima. *Adapted with permission from Braunersreuther et al; Cellular and Molecular Life Sciences 2006; 63(18):2079-88.*

the expression of a variety of adhesion molecules as well as chemokines which all take part in the directing circulating inflammatory cells towards areas of injury/inflammation. This recruitment occurs in a complex, orchestrated manner of 1) tethering of the circulating cells on the endothelium 2) firm adhesion of the cells and ultimately 3) transmigration or diapedesis through the endothelial layer into the neointima (figure 3).

The family of Selectins is involved in the capturing, tethering and rolling of circulating monocytes on the inflamed arterial endothelium. L-selectin is expressed on most leukocytes and mediates leukocyte rolling via interaction with P-selectin glycoprotein ligand-1 (PSGL-1) [14]. It is currently however not entirely clear if the absence of L-selectin influences the development of atherosclerosis [15]. P-selectin is quickly released from intracellular Weibel Pallade bodies upon endothelial cell activation and binds circulating neutrophils, monocytes and lymphocytes through an interaction with PSGL-1 [16]. It is, together with E-selectin, expressed on chronic and acute inflamed endothelial cells. P-selectin deficient mice on an ApoE deficient background were seen to develop smaller atherosclerotic lesions, and the same protective effect was observed in E-selectin deficient mice, although to a lesser degree [17, 18]. However, the

most dramatic, 80%, reduction in atherosclerosis were seen with combined P- and E-selectin deficiency [19]. A second class of adhesion molecules involved in atherosclerosis are vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), members of the immunoglobin superfamily. Substantial evidence has been gathered on their function in atherosclerosis. [14]. Triggers for enhanced expression of these molecules are accumulation of cholesterol in the intima, disturbed oscillatory flow and an atherogenic diet, all pro-atherogenic factors exerting their effect via up-regulation of ICAM-1 and VCAM-1. Several mouse studies showed a decrease in atherogenesis upon genetic ICAM-1 or VCAM-1 deficiency [20, 21]. Platelet endothelial cell adhesion molecule-1 (PECAM-1), another member of the immunoglobin superfamiliy, is expressed both on endothelial cells as well as on a wide array of circulating leukocytes. It mediates endothelial responses to fluid shear stress, which is often severely altered in atherosclerosis, as it moves to the apical surface of endothelial cells upon activation, mediating attachment and diapedesis via its involvement in tight junction stabilisation. Interestingly, PECAM-1 single nucleotide polymorphisms and elevated levels of PECAM-1 in patients were associated with severe coronary artery disease [22]. PECAM-1 deficient mice show reduced atherosclerotic lesion size on a western type diet [23]. Finally, junctional adhesion molecules (JAMs) are essential for the transmigration of leukocytes through the endothelial layer. They are localized to intercellular junctions of endothelial cells as well as on circulating leukocytes. JAM-A is the most studied member in the context of atherosclerosis. It participates in monocyte diapedesis through the endothelial layer [24], and high levels were detected on atherosclerotic plaques derived from cardiovascular patients [25]. Ostermann et al. elegantly demonstrated the JAM-A dependent recruitment of T-cells and monocytes in an ex-vivo carotid artery perfusion model [26]. Furthermore, JAM-A deficiency resulted in decreased neo-intimal formation upon wire injury in carotid arteries with a concomitant decrease in lesional macrophages [27].

Together, these data show that vascular adhesion molecules are vital in normal vascular homeostasis, as their actions can mediate atherogenesis after exposure to various pro-atherogenic stimuli. Chemokines regulate the homing and adhesion of circulating leukocytes as well. They are not only produced by endothelial cells but also by VSMCs, macrophages and T-cells as they attract monocytes, induce firm adhesion and subsequent rolling of these cells along the endothelium.

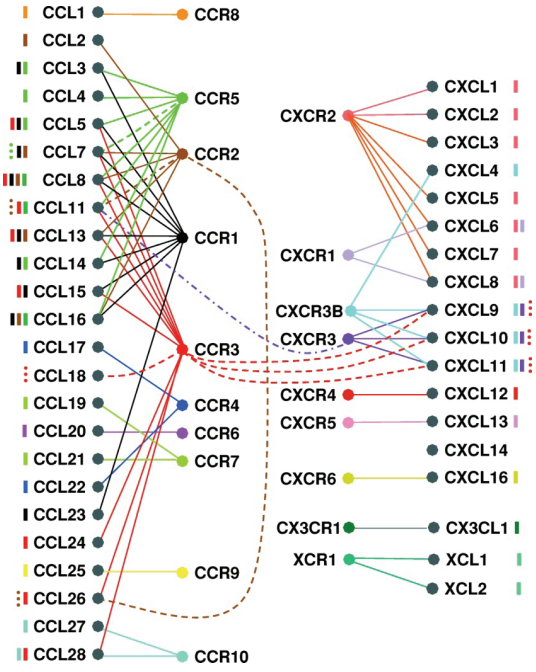
CHEMOKINES: INTRODUCTION ON GENERAL CHARACTERISTICS

Chemokines or *chemo*tactic cyto*kines* are small secreted proteins with a molecular weight of 6-16 kDa. They are, independent of their function, subdivided into four families based on their N-terminal specific cysteine motifs in their amino acid sequence (i.e. CC, CXC, XCL and CX_3C). The CC chemokine family is the largest group as it consists of over 25 chemokines, whereas the CXC chemokine family has a non-conserved amino acid in-between the cysteine residues and currently constitutes 16 members. The XCL family lacks two out of four canonical cysteines and

consists of 2 members and finally the CX_3C, with three amino acids within the cysteine residues, only has one member (CX_3CL1) [28]. It must be emphasized however that many non-chemokine molecules with chemokine-like function exist, next to various splice isoforms as well as currently non-identified chemokines [29].

Chemokines function primarily by orchestrating the trafficking of bone marrow derived cells, but also influence processes like granule exocytosis, mitogenic effects, gene transcription and apoptosis [30]. Considering their function, there are basically two types of chemokines. Inducible chemokines are released upon an inflammatory stimulus and regulate inflammatory reactions by mediating leukocyte recruitment [31]. Homeostatic chemokines are constitutively expressed in various tissues and regulate normal leukocyte homeostasis and lymphoid functions [32]. Chemokines function in a redundant and pleiotropic way, as various chemokines can bind to various seven-transmembrane G-protein coupled chemokine receptors (GPCRs) and vice versa (figure 4). Furthermore, chemokines are seen to act in concert and chemokine signalling is in the majority of cases not regulated by just one single chemokine, due to heterophilic interactions at chemokine and receptor level. This makes them ingeniously designed trafficking agents of a plethora of cellular functions and gives them a central position in the complex intercellular communication. The down-side however is that the development of therapeutics

Figure 4.

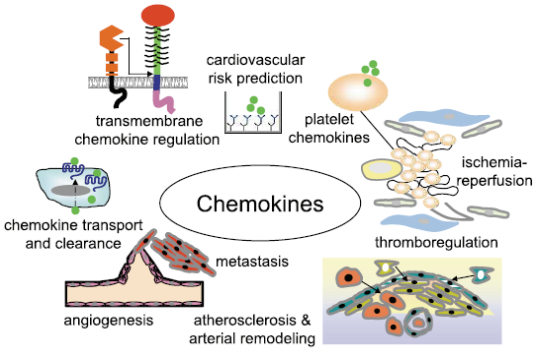


Chemokines and their receptors. Chemokines can bind to more than one receptor, while chemokine receptors in most cases can bind various chemokines. *Adapted with permission from A. Rot et al. Annual Review of Immunology 2004; vol 22; 891-928.*

directed against one single chemokine or chemokine receptor could not only interfere with the anticipated chemokine-receptor interaction. It could also shift the balance of a number of other chemokine-receptor interactions due to the mentioned redundancy, potentially impairing the systemic immune response [33].

As chemokine receptors are also expressed on endothelial cells and vascular smooth muscle cells next to leukocyte subsets [34], they were seen to be involved in various aspects of vascular disease like the post myocardial infarction/ischemia inflammatory repair mechanism, neovascularisation/angiogenesis and atherothrombosis as shown in summary in figure 5 [35]. Most of these aspects are beyond the scope of this thesis, however chemokine related activity in atherosclerosis and plaque stability is briefly highlighted in the next section and extensively reviewed in chapter 2, while the activity of chemokines in myocardial ischemia repair mechanisms is briefly discussed in the next section.

Figure 5.



Chemokines have a central function in a wide variety of physiological processes. They not only function as messengers in ischemia-reperfusion responses, thrombus formation, atherosclerosis and vascular remodelling, but also in metastasis formation. Their release into the circulation makes them attractive candidates for (cardiovascular) risk prediction. *Adapted with permission from C. Weber; Thrombosis and Haemostasis 2007 May;97(5):685-7.*

CHEMOKINES IN ATHEROSCLEROSIS

The expression of various chemokines and chemokine receptors within the atherosclerotic plaque has recently been demonstrated in a variety of publications. Chemokines not only play a pivotal role in the homing of inflammatory cells towards the atherosclerotic plaque, they also influence vascular smooth muscle cell growth and migration, as well as the process of macrophage induced matrix degradation by matrix metalloproteinases [36-38]. All these processes are instrumental in plaque (de)stabilisation. Illustratively, targeted deletion of CCR2 and CX3CR1 resulted in decreased atherosclerotic lesion formation indicating the pivotal role of these receptors and their ligands [39, 40], while Lutgens et al. demonstrated the plaque decreasing as well as stabilising potential of a murine CCL2 and CCL12 inhibiting antibody [41]. While chemokines are

associated with atherosclerosis and plaque destabilisation, the use of chemokines as inflammatory biomarkers in the identification of patients with cardiovascular disease as well as clinical outcome has been suggested [42]. In this regard, CCL2 (MCP-1) and CXCL8 (IL-8) were identified in patient cohorts as early markers of cardiovascular disease independent of other traditional risk factors, although the levels of IL-8 between groups showed considerable overlap and the association of CCL2 already diminished after correction for age [43, 44]. CCL2 was also shown to be helpful in predicting adverse cardiovascular events in patients with CAD, pointing to its diagnostic as well as therapeutic potential [45]. However, another large case-control cohort study showed that IL-8, CCL2 and CXCL10 levels were elevated in patients preceding a cardiovascular event [46]. But also in this study, the association lost its significance when the levels were adjusted for other inflammatory markers and cardiovascular risk factors. Therefore, chemokines might not be very suitable biomarkers of myocardial infarction or cardiac ischemia itself, but could serve as potential biomarkers in identification of high risk patients.

The search for other chemokine markers or even chemokine signatures in the various stages of cardiovascular disease is currently ongoing, but we have to keep the previously mentioned statistical pitfalls in mind which are applicable to other chemokines and inflammatory markers in general to draw any solid conclusions. Chapter 2 provides a more extensive overview of the various associations and influences of chemokines in atherosclerosis and plaque destabilisation [47].

CHEMOKINES IN CARDIAC ISCHEMIA

When myocardial perfusion is hampered by coronary atherosclerotic stenosis or a plaque rupture induced thrombus, the myocardium turns ischemic within minutes. If the cardiac myocytes are too long deprived of oxygen, they ultimately become necrotic and start to release their intracellular contents and initiate an innate inflammatory response which is mediated by reactive oxygen species (ROS), cytokines, the complement cascade and the NF-κB system, with an important regulatory role for chemokines[48, 49]. ROS formation causes cell injury and death, but this response is also critically involved in phagocyte function to remove cellular debris. Chemokines are shown to be increased during transient cardiac ischemia and this is mainly mediated by ROS [50]. Furthermore it has been suggested that pro-inflammatory cytokines like TNF-α and IL-1β stimulate chemokine expression in ischemic tissue as TNF-α activates the NF-kB system and thereby induces chemokine expression [51]. Chemokines also take part in the homing of stem cells following myocardial infarction towards the infarcted area. In particular, the SDF-1α/CXCR4 axis was shown to confer protection for myocardial ischemia/reperfusion injury by mobilising a specific subset of progenitor cells [52, 53]. Altogether, a variety of chemokines have been linked to myocardial ischemia, some having a cardioprotective and others a deleterious effect. A number of clinical studies focussing on chemokines in acute coronary syndromes is discussed in chapter 2. As chemokines play an important role in the post-inflammatory

ischemic response, enhancement of their function might be a therapeutical opportunity in this regard. Indeed, local myocardial delivery of proteinase resistant SDF-1α with sustained activity improved rat cardiac function as compared to native SDF-1α [54]. This shows that stem cell homing by local chemokine delivery is a promising new therapeutic option. Next to SDF-1α, various other chemokines were seen to be involved in ischemia repair mechanisms which could also serve as viable therapeutic targets. The association of a few chemokines with cardiac ischemia has already been shown in patient cohorts of angina pectoris and myocardial infarction, but as there are approximately 50 chemokines described, many other chemokines might be involved in myocardial ischemia reperfusion responses, and in that regard could serve as biomarkers or novel therapeutic targets.

GENETIC SUSCEPTIBILITY AND ATHEROSCLEROTIC DISEASE

The search for suitable therapeutic candidates in cardiovascular disease has been tremendously augmented by genome-wide association studies of large patient cohorts and genetic linkage analysis in families. Furthermore, candidate genes are regularly tested by genotyping single nucleotide polymorphisms (SNPs) to see if they are associated with atherosclerotic disease. We now know that various genetic deficiencies are linked to cardiovascular disease. For instance, genetic mutations in the gene encoding an adaptor protein are linked to autosomal recessive hypercholesterolemia [55], and genetic deficiency of the cholesterol ester transfer protein (CETP) has been shown to raise High Density Lipoprotein (HDL) levels thereby decreasing cardiovascular risk [56]. Recently, a joint analysis of two genome-wide association studies identified several genetic loci which are significantly associated with the risk of coronary artery disease [57]. In particular chromosome 9p21 has consistently been identified as a important locus in coronary artery disease, and many of the encoded proteins of this locus are members of the INK4 family, important regulators of cell-cycle regulation [58, 59]. Furthermore, genes encoding cell-growth regulators have been associated with the risk of myocardial infarction, like SMAD-3, MIA-3 and PSRC-1 [57]. These are just a few examples, as various other genes involved in inflammation, lipid homeostasis as well as matrix production are linked to cardiovascular disease.

GENETIC SUSCEPTIBILITY AND RESTENOSIS

Recently, various genetic mutations have been described which are protective against or augment the occurrence of in-stent restenosis following percutaneous coronary intervention. Restenosis may lead to re-occlusion of a stented (coronary) artery which has serious clinical consequences. It is a multi-factorial process in which vessel recoil, VMSC proliferation and thrombus formation play a major role [60]. Furthermore, studies by Monraats et al. showing a strong association between SNPs in the tumor necrosis factor- α (TNF-α), CCL11 and colony

stimulating factor 2 (CSF2) genes and an increased risk for restenosis, clearly illustrate the impact of inflammation in this process [61, 62]. This notion is corroborated by in vivo studies in which TNF-α deficient mice on a hyperlipidemic background showed a marked decrease in restenosis after non-constricting femoral cuff placement [61].

Particularly attractive targets for the treatment of restenosis are transcription factors, although no direct patient study data are available to support this hypothesis. They are feasible targets, as they are involved in injury-response mechanisms, control a wide variety of downstream effector genes with often overlapping and associated functions. For instance, activation of the nuclear receptor Nur77 with 6-mercapto-purine in a murine restenosis mouse model was seen to protect against abundant VSMC proliferation [63]. Furthermore, the nuclear activator of T-cells (NFAT) inhibitor VIVIT has been proposed as a therapeutic agent for restenosis, as it inhibits the calcineurin/NFAT interaction [64]. These findings are promising but need to be replicated in other experimental models to assess if these transcription factors are effective and safe drug targets. Furthermore, current mouse models cannot be directly extrapolated to the human situation, and the associations of enhanced or decreased Nur77 and NFAT activity with clinical restenosis and hard clinical end-points still needs to be confirmed. This thesis describes one of the first population studies to link SNPs in the promoter and coding region of a transcription factor, notably the mRNA binding protein Quaking, with restenosis.

STUDY AIMS AND THESIS OUTLINE

The research described in this thesis consists of two parts. The first part not only focuses on chemokine profiling in patients with acute coronary syndromes, but also attempts to elucidate the some of the mechanisms by which these chemokines act in atherosclerosis. In **chapter 2**, a broad overview is given on chemokines which have been linked to atherosclerosis and plaque destabilisation. The therapeutic potential which chemokines and chemokine receptors might have for future therapeutic intervention is also discussed.

Two cohorts of patients with acute coronary syndromes (ACS) were used for a multiplex profiling strategy to identify chemokines which are associated with ACS and/or future cardiovascular risk. **Chapter 3** describes the studies in which plasma from patients with unstable angina pectoris from the APRAIS cohort was screened with a multiplex chemokine analysis. We identified two chemokines, CCL5 and CCL18, which were seen to be transiently raised during ischemic symptoms after ELISA follow-up analysis. Furthermore, these chemokines also showed strong prognostic capacities for the development of future cardiovascular events. **Chapter 4** further elucidates the role of CCL18 in atherosclerosis via two strategies. First, we systemically administered synthetic CCL18 to hyperlipidemic atherosclerotic mice to analyse plaque progression. Second, we have focally overexpressed CCL18 in pre-existing plaques and addressed effects on plaque progression and stability at 2 weeks follow-up. Systemic CCL18 exposure led to enhanced atherogenesis and when overexpressed in the plaque attracted T-cells to the

lesion, however plaque stability was not affected. Furthermore, CCL18 carotid atherosclerotic plaque protein distribution was demonstrated. **Chapter 5** more specifically explored the chemokine profile of a selection of patients with myocardial infarction from the MISSION! cohort. Levels of a number of chemokines were altered in myocardial infarction patients compared to control. We were able to confirm the increased levels of CCL3 in the previously mentioned APRAIS cohort with ELISA analysis, linking CCL3 to cardiac ischemia. This was corroborated in an experimental *in vivo* murine model of myocardial infarction, as elevated CCL3 levels were observed upon infarction. To further determine the function of CCL3 in atherosclerosis, we applied in **chapter 6** a bone marrow transplantation model to study the role of CCL3 in circulating white blood cells on atherosclerosis. Deletion of CCL3 causes a decreased atherosclerotic plaque size, which is associated with a decreased neutrophil response in CCL3 deficiency. In **chapter 7** a potential alternative explanation is given for the recent failure of CETP inhibitors in prevention of morbidity in patients with cardiovascular disease and elevated cholesterol levels. We propose that leukocyte CETP expression is repressed during ACS, as judged from APRAIS cohort analysis and experimental studies in a mouse coronary ligation model. An enhanced inflammatory status may induce leukocyte CETP repression, which might be relevant in the pathogenesis of ACS and shed new light on recent negative study results on CETP inhibitors.

The second part of this thesis discusses experiments which focus on specific targets in atherogenesis and plaque stability. Targets were selected on the basis of 1) an established risk factor (aging) and ensuing in house genomic analysis of atherosclerotic tissue from the aging study and 2) a previously published genetic linkage analysis in patients with myocardial infarction. **Chapter 8** describes the studies on the influence of aging on atherogenesis. Surprisingly aging was accompanied by a decreased atherogenic as well as a decreased vascular remodelling response in LDLr-/- mice. Furthermore, we identified genes and genetic pathways which were associated with vascular aging. Specifically, one of the dysregulated vascular genes upon aging was Quaking, a gene with great potential regarding diagnosis and therapeutics in cardiovascular homeostasis and disease. The role and impact of Quaking in cardiovascular disease is further explored in **chapter 9**, where we identified polymorphisms in the Quaking gene as both risk and protective predictors for the occurrence of in-stent restenosis in patients who underwent coronary revascularisation. Furthermore, in concert with this finding, Quaking mutant mice displayed a weakened restenotic response in a dedicated mouse model of intimal hyperplasia which could be attributed to disturbed VSMC homeostasis. **Chapter 10** provides, by experimental in vivo studies in a murine adenoviral overexpression model, mechanistic ground for the recent finding that a mutation in the MEF2A gene associates with increased susceptibility for developing myocardial infarction. Abrogated MEF2 signalling was seen to cause disturbed endothelial cell homeostasis which led to an increased plaque size as well as an enhanced occurrence of intraplaque haemorrhages. Furthermore, via microarray analysis, we unveiled partly overlapping signalling pathways of endothelial MEF2 silencing and VEGF activation.

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