The role of inflammation in cardiac and vascular remodelling

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Chapter 1

General introduction and outline of thesis

Introduction

Atherosclerosis is a lipid-driven chronic inflammation of the vessel wall, which can lead to formation of an atherosclerotic plaque[1]. This can lead to various clinical manifestations like stable angina, acute coronary syndrome and heart failure[1], but also stroke[2] and peripheral artery occlusive disease[3]. As a consequence is atherosclerosis and its subsequent clinical manifestations one of the leading causes of morbidity and mortality worldwide[4, 5].

Acute coronary syndrome

Acute coronary syndrome can be divided in unstable angina and myocardial infarction (MI). In case of unstable angina the coronary artery is partially blocked, while during a MI the coronary artery is completely blocked, for example due to rupture of an atherosclerotic plaque[6]. In both situations the myocardium downstream of the (partially) occlusion is excluded from oxygen supply and becomes hypoxic, while waste products like carbon dioxide accumulate in that hypoxic area. Subsequently, this leads to death of cardiomyocytes or even worse, cardiac arrest and death[6]. In case of survival of the patient, the loss of viable cardiomyocytes leads to cardiac remodelling, which can ultimately lead to heart failure[7].
Last decades many improvements are realized in revascularization strategies following acute coronary syndrome. Balloon angioplasty, usually in combination with stenting, is commonly used, but coronary artery bypass grafting is also used, especially in patients with multivessel disease[6]. Although these revascularization strategies resulted in a decreased morbidity and mortality rate, a significant part of the patients suffer from complications like restenosis, accelerated atherosclerosis and heart failure. It is of high importance that the ischemia time is kept as short as possible, preferable less than 60 minutes, since ischemia time is shown to correlate with morbidity and mortality[6]. Furthermore, one can imagine that a short ischemic period reduces the amount of adverse cardiac remodelling.

Cardiac remodelling

Cardiac remodelling can be described as a change in shape, size and function of the heart. This can be the result of exercise, in which cardiac function is often increased, or following injury of the heart muscle, for example after a MI, in which cardiac function is decreased. The latter is also termed adverse cardiac remodelling[8]. Following a MI adverse cardiac remodelling is the response to a sudden loss of cardiomyocytes in which the heart tries to compensate for the dead tissue and to maintain its function[9]. This leads to a decreased left ventricle (LV) wall thickness in the infarct zone due to the loss of

cardiomyocytes, which are replaced by a collagen scar[10]. In the non-infarct zone the LV wall thickness is increased due to hypertrophy of cardiomyocytes which is a response to hypertensive stress. This initially results in maintained cardiac function, and thus called a compensatory response, however, on the long run, cardiac hypertrophy is an independent risk factor for cardiovascular pathologies[8]. Furthermore, the LV becomes progressively dilated, and together with the expansion of connective tissue throughout the ventricle wall, which cause ventricular stiffness, this leads to loss of cardiac function and subsequently pathological conditions like heart failure[9].

Adverse cardiac remodelling is a process that can be divided into different phases (Figure 1). First after a MI tissue injury, reactive oxygen species and necrosis initiate the inflammatory phase, in which neutrophils and macrophages remove dead cells and matrix debris. This phase is followed by the reparative and proliferative phase (in some cases these two phase are considered two different phases), in which the inflammatory response shifts to inflammation resolution, myofibroblasts are activated leading to scar formation and wound healing[7].

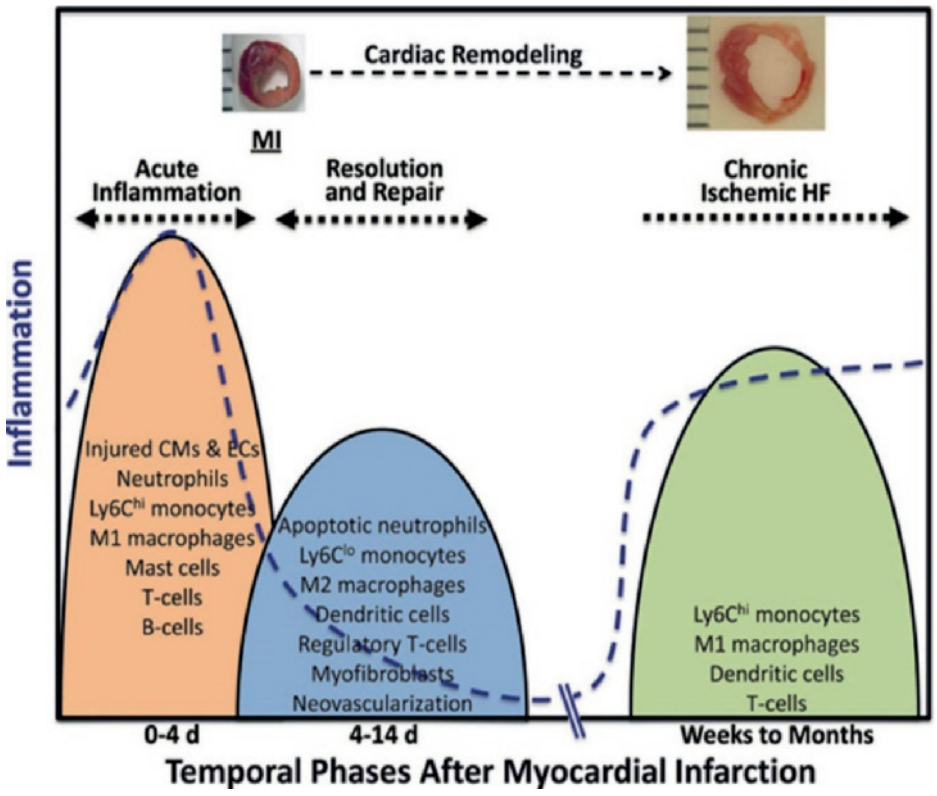

Figure 1. An overview of the different phases in cardiac remodelling following MI. Figure adapted from Prabhu *et al*[7].

Inflammatory phase

Inflammation plays a critical role in the different phases after a MI. The next paragraphs describe in brief the role of different immune cells and different molecules that influence inflammation following a MI. Further details regarding the role of inflammation following MI can be found in different reviews[7, 11, 12].

During the inflammatory phase cardiomyocytes in the infarct area become necrotic and release so-called damage associated molecular patterns (DAMPs), like high mobility group box-1(HMGB1), fibronectin extra domain A, heat shock proteins, singe-stranded RNA, double-stranded RNA and many more[7]. DAMPS are recognized by pattern recognition receptors (PRRs), like toll-like receptors (TLRs), nucleotide-binding oligomerization domain-lie receptors (NLRs) and receptor for advanced glycation end-products (RAGE)[7]. Binding of a DAMP with a PRR leads to downstream signalling and following a MI this signalling may activate several pathways that stimulate leukocyte infiltration and other pro-inflammatory mechanisms.

Binding of DAMP to endothelial cells leads to an increase in the expression of adhesion molecules like E-selectin and P-selectin[13], and subsequently the leukocyte infiltration is increased. Furthermore, due to the ischemic event vessel wall integrity is affected leading to increased leukocyte infiltration in the infarct area[14]. Leukocyte infiltration is also enhanced by the expression of chemokines. Chemokines are cytokines that attract immune cells to injured tissue, examples of chemokines are: chemokine (C-C motif) ligand 2 (CCL2), which is known to attract mainly mononuclear cells, like monocytes, and chemokine (C-X-C motif) ligand 8 (CXCL8; also known as interleukin 8 (IL-8)), which attracts mainly neutrophils[15].

Neutrophils are the first immune cells that arrive in the infarct area and they play an important role in clearing the infarct site from matrix debris and dead cells[11]. Neutrophils help clear the infarct site by phagocytic activity, but also by releasing extra cellular matrix (ECM)-digesting enzymes which helps in the removal of DAMP containing molecules[13]. Furthermore, it is believed that neutrophils stimulate leukocyte infiltration by releasing azurocidin, cathepsin G and a complex of interleukin 6 (IL-6) and its soluble receptor16, and expression of triggering receptor expressed on myeloid cells-1 (TREM-1)[17].

Shortly after the neutrophil infiltration, pro-inflammatory monocytes infiltrate into the injured myocardium. Especially Ly6C^{high} monocytes are abundantly present in the inflammatory phase of cardiac repair[18]. Ly6C^{high} monocytes clear the infarct area from dead cells and matrix debris by their phagocytic actions and expressing of proteolytic mediators, like cathepsins and urokinase-type plasminogen activator[19]. Furthermore, Ly6C^{high} monocytes amplify the inflammatory response by expressing pro-inflammatory cytokines like, tumour necrosis factor α (TNF-α), interleukin 1β (IL-1β) and IL-6[19]. Pro-inflammatory macrophages, also known as M1 macrophages, display comparable

functions as Ly6C^{high} monocytes following a MI in infarct healing. This seems plausible since the main part of the cardiac macrophage pool are derived from infiltrated monocytes[20, 21]. However, not all cardiac macrophages are derived from monocytes, about 8% of all cardiac cells are resident macrophages in healthy myocardium[21]. The exact functions of these resident macrophages in the inflammatory phase of infarct healing are not known yet.
Emerging evidence suggest that both T and B lymphocytes play a role in the early phase of infarct healing. It is believed that cytotoxic (CD8^{+}) T cells infiltrate the infarct area and exhibit undesirable cytotoxic effects on healthy cardiomyocytes[22]. Furthermore, it has been shown that mature B lymphocytes recruit Ly6C^{high} monocytes by expression of chemokine (C-C motif) ligand 7 (CCL7)[23].

Reparative and proliferative phase

The inflammatory phase of infarct healing is followed by the reparative and proliferative phase. This phase is characterized by inhibition and resolution of the inflammatory response, neovascularization and the formation of a scar[7]. The shift from a pro-inflammatory response to an anti-inflammatory response involves a whole range of (immune) cells. Neutrophils, which cleared the infarct area from dead cells and matrix debris, undergo apoptosis and the apoptotic neutrophils are phagocytized by macrophages. Uptake of apoptotic neutrophils by macrophages is followed by a phenotypic shift from pro-inflammatory macrophages to anti-inflammatory macrophages. These anti-inflammatory macrophages express cytokines and growth factors that promote tissue repair and neovascularization, like interleukin 10 (IL-10), transforming growth factor β (TGF-β) and vascular endothelial growth factor (VEGF)[7].
Whereas Ly6C^{high} monocytes where mainly recruited to the infarct site in the inflammatory phase, it are the LyC6^{low} monocytes that are recruited during the reparative and proliferative phase. Ly6C^{low} monocytes express, just like anti-inflammatory macrophages, cytokines and growth factors that promote tissue repair and neovascularization. Furthermore, a part of the anti-inflammatory macrophages are derived from Ly6C^{low} monocytes[20]. It should be noted that macrophages cannot simply categorized to pro- or anti-inflammatory macrophages, but a broad spectrum of different macrophage subsets is involved in infarct healing. Future research will probably discover more macrophage subsets, all with their specific phenotypes and functions.
Dendritic cells (DCs) seem to play an important role during the reparative and proliferative phase of infarct healing. DCs infiltrate into the myocardium following a MI and with a peak seven days post infarction. Furthermore, it has been shown that DC ablation leads to adverse ventricular remodelling and reduced cardiac function, most likely caused by increased Ly6Chigh monocyte infiltration[24].

During the reparative and proliferative phase of infarct healing different T-cells subsets of are involved. For example, Foxp3$^+$ CD4$^+$ regulatory T cells (Tregs) influence inflammation resolution and wound healing, and thereby improve infarct healing[25]. Furthermore, it has been shown that activation of natural killer T cells leads to a reduction in adverse ventricular remodelling and cardiac failure, through increased expression of IL-10[26].

Myocardial ischemia reperfusion injury

As mentioned before many improvements have been made the last decades regarding revascularization following a MI. Undoubtedly, restoring the blood flow (reperfusion) to the ischemic area is the best treatment to save as much cardiomyocytes as possible, and currently it is clinical routine to perform revascularization therapy directly after a MI. However, reperfusion following a MI also causes myocardial ischemia-reperfusion (MI-R) injury. MI-R injury results in vascular leakage, no reflow phenomenon, cell death, transcriptional reprogramming, autoimmunity and increase of the inflammatory response[14].

Reactive oxygen species (ROS), which are produced in response to the sudden re-oxygenation of ischemic tissue, are in part responsible for the increase in inflammatory response[27]. These ROS act on several pathways related to pro-inflammatory cytokine and chemokine production, like the nuclear factor kappa B (NFκB) pathway, mitogen-activated protein kinase (MAPK) pathway and type 1 interferon pathway[27]. Taken together, it seems that the ultimate goal following a MI should be: fast revascularization in combination with treatment against MI-R injury. In the first part of this thesis we focus on anti-inflammatory therapy to treat MI-R injury and MI without reperfusion.

Vascular remodelling

Vascular remodelling is a response to environmental changes, which leads to structural changes in the vessel wall. It involves at least four processes, namely cell migration, cell growth, cell death and production or degradation of ECM[28]. Just like cardiac remodelling, vascular remodelling can be divided in beneficial and adverse vascular remodelling. In beneficial vascular remodelling, the vascular changes result in creation (e.g. vasculogenesis during embryonic development) or restoration of blood flow (e.g. neovascularization following ischemia). In adverse vascular remodelling, on the other hand, the changes in the vessel wall result in reduced blood flow (e.g. atherosclerosis and restenosis), which subsequently can lead to several clinical manifestations.

Restenosis

In case of ischemic problems caused by narrowing or obstruction of a vessel, the preferred

therapy is percutaneous coronary intervention (PCI) like, balloon angioplasty, often in combination with placement of a stent. A major drawback of PCI is restenosis, which is the re-narrowing of the opened vessel leading to new clinical symptoms[29]. Intimal hyperplasia and accelerated atherosclerosis (Figure 2) are the two main contributors of restenosis. In case of intimal hyperplasia the increase in the intimal layer of the vessel consist mainly of vascular smooth muscle cells (VSMCs), while in case of accelerated atherosclerosis the enlarged intima consists of VSMCs, macrophages and foam cells.

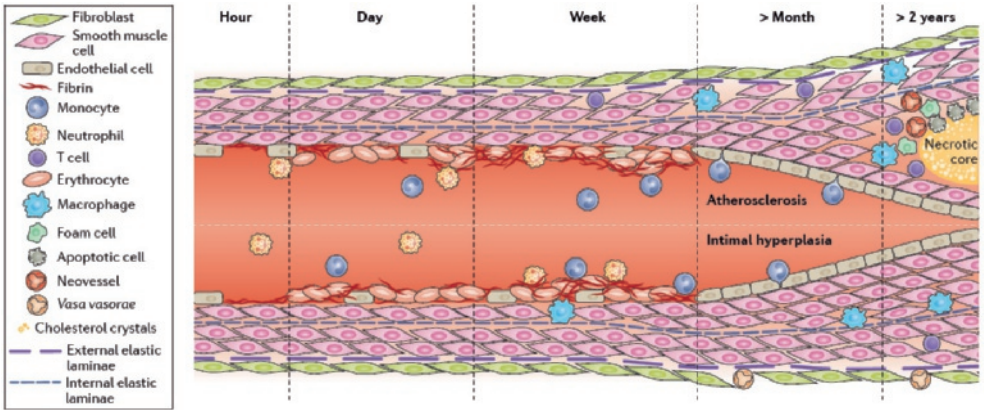

Figure 2. An overview of the development of both atherosclerosis and intimal hyperplasia. Figure adapted from de Vries *et al*[34].

Intimal hyperplasia is caused by local tissue injury during PCI. During the interventional procedure the vessel wall is de-endothelialized or the remaining endothelium is activated. Platelets and fibrinogen bind to the de-endothelialized vessel wall and they express different adhesion molecules like, P-selectin and glycoprotein Ib-α. The damaged endothelial cells also express adhesion molecules and thereby both de-endothelialization and endothelial injury lead to increased leukocyte infiltration of the vessel wall[30]. These leukocytes express cytokine and chemokines, like IL-6, TNF-α and CCL2, that further boost the inflammatory response[31]. Furthermore, leukocytes and activated platelets also release growth factors like, TGF-β[32], and matrix metalloproteinases (MMPs), which lead to ECM remodelling and migration of VSMCs from the media to the intima[31]. As mentioned before, the newly formed neointima consists mainly of VSMCs and they release, together with leukocytes, several growth factors and MMPs, which leads to more proliferation of VSMCs and thus further increase of intimal thickening[31].
Under hypercholesterolemic conditions lipoproteins, like oxidized low density lipoprotein (oxLDL), are formed within the vessel wall. When this occurs at an injured site of the vessel wall, this will lead to a further increase in inflammatory response. Furthermore, macrophages in the vessel wall will recognize and phagocytize oxLDL particles, leading to the formation of foam cells[33]. Therefore, we refer to accelerated atherosclerosis, instead of intimal hyperplasia,

when we study intimal thickening under hypercholesterolemic conditions.

Animal models

To study cardiac remodelling we used two different mouse models, namely a mouse model for MI and MI-R[35]. Basically these models are quite similar to each other. In our MI mouse model (Figure 3) we permanently ligate the left anterior descending (LAD) coronary artery, simulating a permanent MI. In the MI-R mouse model the ligation of the LAD is removed after 45 minutes ischemia, simulating MI-R injury. Both models were used in combination with magnetic resonance imaging (MRI), which is in our belief the most reliable method to study cardiac function in vivo.

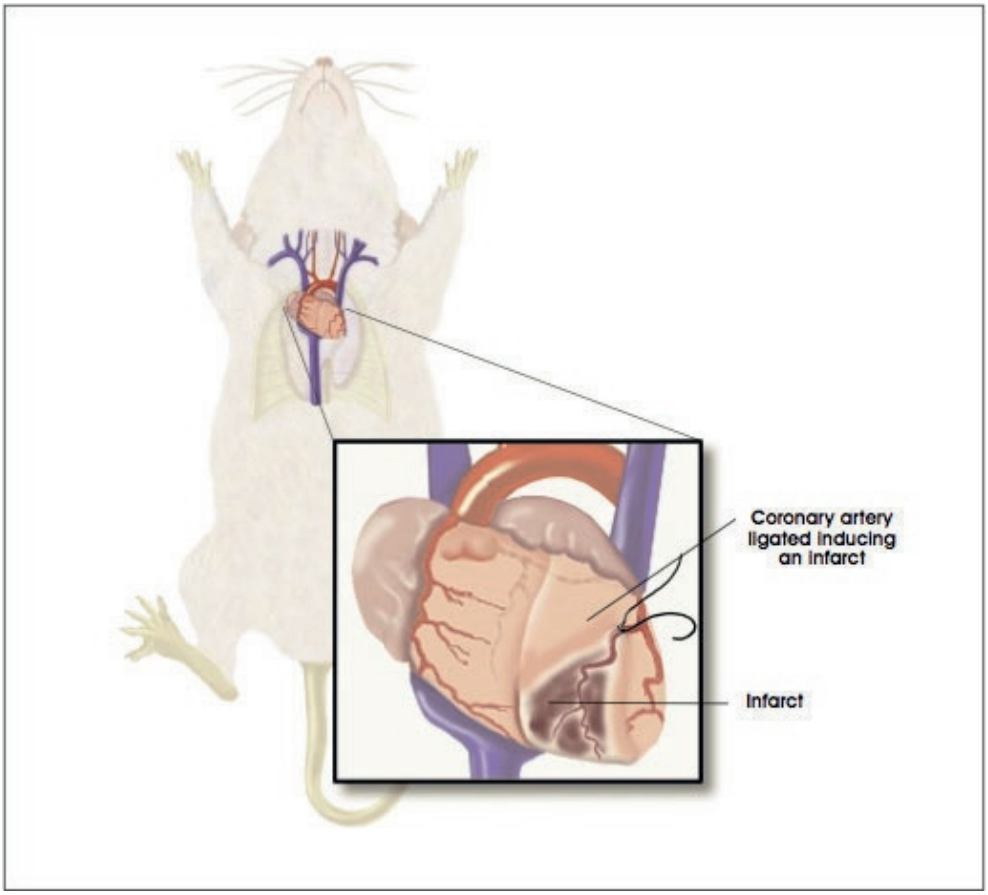

Figure 3. A schematic overview of the MI mouse model.

To mimic the clinical situation often experienced by patients suffering from MI and MI-R injury hypercholesterolemic ApoE*3-Leiden mice were used[36]. These mice experience hypercholesterolemia when fed an high fat diet, but not when fed a chow diet. Other

hypercholesterolemic mouse strains, like ApoE$^{-/-}$ and LDLR$^{-/-}$ are also often used to study cardiac and vascular remodelling. However, ApoE$^{-/-}$ mice already experience hypercholesterolemia on a chow diet and LDLR$^{-/-}$ mice have a cholesterol metabolism which is less comparable to the human situation compared to the cholesterol metabolism of ApoE*3-Leiden mice. Therefore ApoE*3-Leiden mice experience, in our opinion, the best resemblance of the clinical situation of most patients regarding hypercholesterolemia.

To study post-interventional intimal hyperplasia we used the femoral artery cuff mouse model, in which we place a non-constrictive cuff around the femoral artery. The local injury inflicted during this procedure resembles the local injury inflicted during PCI. In response to this injury a VSMC-rich neointima is formed within three weeks if this model is used under normocholesterolemic conditions[37].

Different mouse models were used to study accelerated atherosclerosis, namely the femoral artery cuff model and the carotid collar model (Figure 4), both under hypercholesterolemic conditions. To perform the femoral artery cuff mouse model under hypercholesterolemic conditions, we used ApoE*3-Leiden mice on a high fat diet[37]. In this situation a neointima, which consists of VSMCs, macrophages and other leukocytes, will develop within two weeks. In the carotid collar model we used ApoE$^{-/-}$ mice on a high fat diet. In this model, a semi-constrictive collar is placed around both the left and right carotid artery[38]. Disturbed flow and increased shear stress lead to damage and activation of the endothelial cells. Together with the hypercholesterolemic conditions this leads to development of an atherosclerotic plaque, which consist of a necrotic core, ECM, VSMCs and leukocytes, within four weeks.

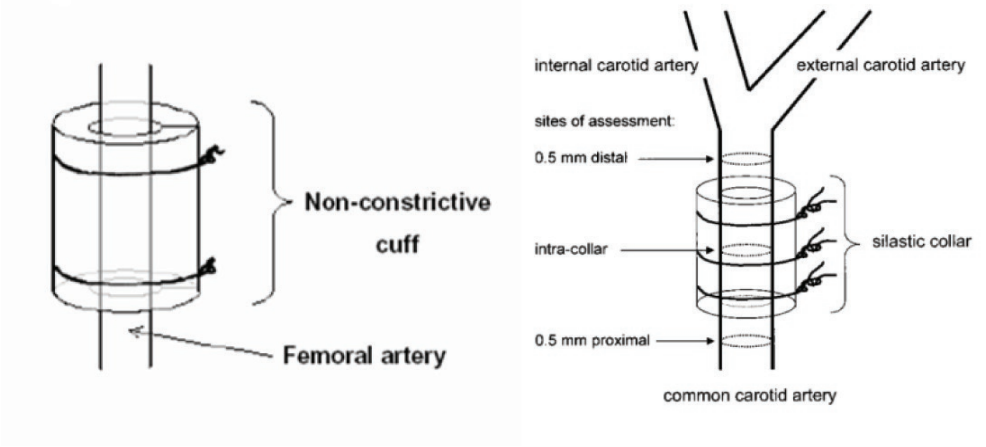

Figure 4. Schematic overview of different mouse models for accelerated atherosclerosis. Left a schematic overview of the femoral artery cuff model and right the carotid collar model.

Non-interventional therapies

Currently, non-interventional therapies focus mainly on prevention of cardiovascular diseases, for example by lowering LDL cholesterol levels using statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Furthermore, several immunomodulatory therapies showed promising results in animal models, but in most clinical trials these therapies were not as successful as expected. One important reason for the failure of current immunomodulatory therapies is that they are focussed on single factors or pathways, which are not able to sufficiently modulate the complex inflammatory process. Therefore, new potential therapies should focus on targets that can modulate multiple processes. The next paragraphs describe several molecules/proteins that are able to modulate multiple processes.

Phosphorylcholine

Phosphorylcholine (PC) is the polar headgroup of a major membrane component, phosphatidylcholine. Phosphatidylcholines are not only vital for all eukaryotic cells, but they are also a membrane component of prokaryote microorganisms, like Streptococcus pneumoniae, and they are also present in lipoproteins, like LDL. PC is a so-called cryptic epitope, which means that eukaryotic cells and lipid molecules have to be modified before they are recognized by the innate immune system[39]. Therefore, viable cells are not recognized in a PC-dependent manner. However, if cells undergo apoptosis, a process in which ROS cause oxidation of molecules, like phospholipids, the PC epitope is modified. This oxidation-induced modification during apoptosis makes the PC epitope accessible for the innate immune system, therefore it is also called an oxidation specific epitope (OSE). In native LDL the PC epitope also remains hidden until it becomes oxidized, which results in the formation of the atherogenic lipid molecule oxLDL[40].
Apoptotic cells and oxLDL, which contain OSEs are immunogenic and pro-inflammatory[39]. Therefore, blocking the PC epitope may be an interesting target for treatment of adverse cardiac and vascular remodelling. Natural antibodies against PC, such as the EO6 or T15 antibodies[41], have anti-inflammatory properties[39, 42] and they inhibit the uptake of apoptotic cells and oxLDL by macrophages in vitro[43, 44]. In vivo it has been shown that B-1a and B-1b cells produce OSE specific antibodies, which display atheroprotective properties[45-47]. Furthermore, sterile inflammation in the spleen triggers splenic B-cells to produce OSE specific antibodies which reduce the development of atherosclerosis[48].
In patients suffering from cardiovascular diseases, low levels of natural IgM antibodies against PC are associated with a worsened prognosis[49]. Furthermore, low levels of natural anti-PC IgM antibodies itself are associated with development of cardiovascular diseases[50-53]. Using different experimental mouse models it has been shown that active and passive immunization with anti-PC IgM antibodies reduces atherosclerosis

development[54, 55] and reduces vein graft disease[56]. Taken together, these data indicate that the use of anti-PC antibodies may be an interesting therapeutic agent against vascular and cardiac remodelling.
A major drawback for using IgM antibodies as a therapeutic agent is that they are relatively expensive and difficult to produce. To overcome this problem our group participated in the development of a fully humanized anti-PC IgG antibody, further referred as PC-mAb, which is compared to IgM antibodies easier to produce and less expensive. Using the femoral artery cuff model in hypercholesterolemic ApoE*3-Leiden mice, we have shown that PC-mAb treatment inhibits accelerated atherosclerosis development and has anti-inflammatory properties. Therefore, PC-mAb treatment may have beneficial effects against adverse cardiac remodelling following MI and MI-R injury , which is described in chapter 2 and 3.

Annexin A5

Annexins are a family of proteins which bind reversibly to negatively charged phospholipids in a calcium dependant manner[57]. One member, Annexin A5 (AnxA5), is known for its ability to bind to phosphatidylserine (PS), which is expressed by activated platelets during the blood coagulation process. PS binds to factor Va, factor Xa and prothrombin leading to the formation of the prothrombinase complex, which ultimately leads to formation of a blood clot[58]. Extracellular AnxA5 binds to PS on activated platelets, thereby it competes with factor Va, factor Xa and prothrombin and preventing formation of a blood clot[58]. In addition to this anti-thrombotic effects[59, 60], AnxA5 is also known to affect apoptosis and inflammation.
In viable cells PS is expressed at the inner plasma membrane leaflet, however during early apoptosis PS is externalized and thus expressed at the outer plasma membrane leaflet[58]. Upon apoptosis several mechanisms are responsible for PS expression on the outer leaflet of the cell membrane which might be cell and signal specific[61]. When PS is expressed at the surface of apoptotic cells it functions as an “eat me” signal which ensures phagocytosis by specialized leukocytes[61]. AnxA5 binding to PS on apoptotic cells has been used diagnostic tool to visualize cell death[60] and even to assess atherosclerotic plaque vulnerability[62].
AnxA5 binds to PS forming two-dimensional crystals, which may shield PS from recognition by leukocytes and subsequently prevent the resulting inflammatory response[58]. Furthermore it has been shown that AnxA5 interacts with the interferon (IFN)-γ receptor modulating the downstream IFN-γ signalling[63]. In addition, it has been shown that treatment with human AnxA5 inhibits the pro-inflammatory response in LPS challenged mice[64]. Previously, our group found that AnxA5 treatment inhibits the post-interventional pro-inflammatory response leading to reduced development of intimal hyperplasia[65] and accelerated atherosclerosis[66]. Taken together these results

indicate that AnxA5 can be used as an anti-inflammatory agent.
In addition to the anti-inflammatory properties of AnxA5 it also affects apoptosis. Upon apoptosis, for example after MI-R injury, cells express PS. It has been found that following an ischemic event cardiomyocytes express PS for at least six hours on their outer membrane. Treatment with AnxA5 resulted in PS internalization restoring the membrane composition regarding PS asymmetry with no PS expression left on the outer membrane, thereby possibly reversing the apoptotic process[67]. Furthermore, it has been reported that following a MI plasma levels of endogenous AnxA5 are increased[68] and that AnxA5 is taken up in the infarct area in patients with an acute MI[69]. Taken together, due to its anti-inflammatory and anti-apoptotic properties, AnxA5 may be an interesting therapeutic agent against MI-R injury. The effects of AnxA5 treatment against MI-R injury are described in chapter 4.

PCAF

Epigenetic factors are proteins that modulate gene expression in response to environmental changes without changing the DNA sequence, for example by regulating histone acetylation and de-acetylation. Chromatin is a complex which consists of proteins, mainly histones, and DNA, and chromatin can be divided in the more loosely packed euchromatin and the more densely packed heterochromatin. The degree of compactness of the chromatin directly influences gene expression, since the DNA is better accessible for transcription factors in the more loosely packed euchromatin[70]. Acetylation of lysine residues, by lysine acetyltransferases (KATs), on histone proteins leads to the formation of euchromatin, and thus activation of genes. On the other hand, de-acetylation, by lysine deacetylases (KDACs), lead to the formation of heterochromatin and subsequently silencing of genes. Therefore, the balance between KAT and KDAC activity are important in gene regulation[71].
P300/CPB-associated factor (PCAF) is such a KAT, which plays an important role in gene activation, and especially inflammatory gene activation[72]. By acetylation of histone proteins at the site of nuclear factor kappa-beta (NFκB), which is an important transcription factor of many inflammatory related genes, PCAF regulates expression of NFκB-regulated genes, like cyclooxygenase-2 (COX-2) and TNF-α[73]. In addition, PCAF is also known for its ability to acetylate non-histone proteins and thereby modulate inflammatory gene expression. NFκB consists of two subunits, namely p50 and p65, and requires a complex of coactivators to induce NFκB-mediated gene expression. It has been shown that PCAF is an important protein of the coactivator complex required for activation of the p65 subunit and subsequent NF-κB-mediated gene expression[74]. Furthermore, it has been shown, in a model for inflammation induced neovascularization, that the absence of PCAF results in 3505 differentially expressed genes, and more importantly, impaired induction of different pro-inflammatory genes[75].

Additionally, in vitro studies have shown that PCAF knockdown downregulates pro-inflammatory gene expression, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and CCL2[76].
Many NFκB-regulated inflammatory genes, like TNF-α and COX-2, are also known to be involved in development of restenosis[77] and atherosclerosis[78]. Furthermore, three large prospective studies have shown an association between the -2481C variant allele in the promotor region of the PCAF gene and reduced risk of vascular morbidity and mortality[79-82]. In a mouse model for restenosis it was shown that Pcaf mRNA levels were elevated upon vascular injury[82]. The role of PCAF in vascular inflammation is investigated in chapter 5.

MicroRNAs

MicroRNAs (Figure 5) are short endogenous RNA molecules, usually about 22 nucleotides long, which are capable to regulate gene expression[83]. MicroRNAs are

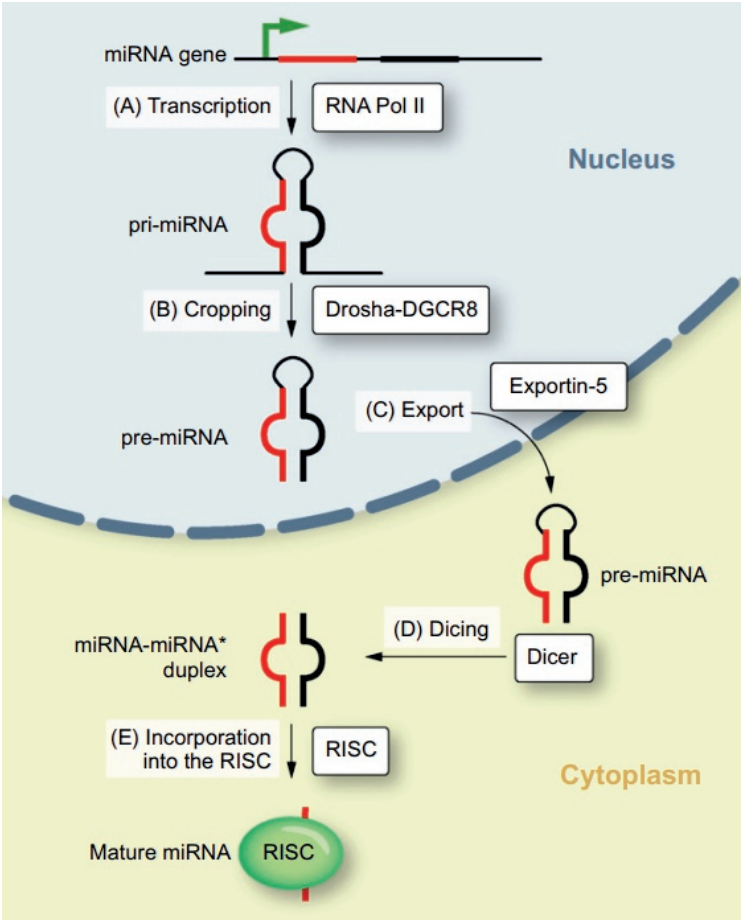

Figure 5. An overview of microRNA biogenesis. Figure adapted from Borel *et al*[87].

transcribed in the nucleus as primary microRNAs, which are subsequently cleaved to form pre-microRNAs by different enzymes, like Drosha and the cofactor DGCR8[84]. These pre-microRNAs are exported from the nucleus to the cytoplasm by the protein Exportin-5. In the cytoplasm pre-microRNAs are further processed by the enzyme Dicer into mature microRNA duplexes[85]. These mature microRNA duplexes are incorporated into the RNA inducing silencing complex (RISC), where they can bind to the 3' untranslated region of their target mRNAs. Perfect complementary binding of the microRNA with its target mRNA will lead to degradation, while imperfect binding will lead to translational repression of the target mRNA[86]. In both situations binding of a microRNA to a target mRNA will lead to downregulation of the target gene.

The fact that microRNAs can target up to several hundred mRNAs means that they can fine-tune a large set of target genes and thereby can have a major impact on multifactorial processes like vascular remodelling. Several microRNAs are found to influence restenosis and atherosclerosis and their specific role is described in several reviews[88, 89].The 14q32 gene cluster, located on chromosome 14 in human and chromosome 12F1 in mice, contains one of the largest microRNAs clusters, which is highly conserved in mammals[90]. Due to the high degree of homology of the microRNA genes in this cluster, it is an relevant cluster to investigate in mouse models. The 14q32 cluster contains 54 human and 59 murine microRNA genes and previously several microRNAs of the 14q32 gene cluster were investigated regarding their effect on vascular remodelling. It has been shown that inhibition of 14q32 microRNA-329, -487b, -494 and -495 results in increased neovascularization in a hind limb ischemia mouse model[91]. Furthermore, inhibition of microRNA-494 leads to decreased accelerated atherosclerosis development and decreased cholesterol levels[92]. Additionally, other microRNAs of the 14q32 cluster (microRNA-431, -668 and -758) are upregulated in atherosclerotic aortas of ApoE$^{-/-}$ mice[93]. Finally, extensive hypomethylation of 14q32 microRNAs was observed in human atherosclerotic plaques, resulting in the upregulation of several 14q32 microRNAs[94]. The effect of 14q31 microRNA-329, -494 and -495 inhibition on restenosis is discussed in chapter 6.

Outline of the thesis

The aim of this thesis was to further unravel the role of inflammation in cardiac and vascular remodelling, as well as to investigate potential therapeutic agents to treat cardiovascular diseases.

The first part of this thesis focuses on potential immunomodulatory therapeutic agents for the treatment of adverse cardiac remodelling following a MI or MI-R injury.

In **chapter 2** we investigate the therapeutic potential of a humanized IgG antibody against PC (PC-mAb). Using a mouse model for MI in combination with MRI assessment

of infarct size (IS) and cardiac function, we found that PC-mAb treatment reduces LV dilatation and IS by inhibiting both the early and late inflammatory response.

Chapter 3 describes the effect of PC-mAb treatment on adverse cardiac remodelling following MI-R injury. In a mouse model with permanent reperfusion following 45 minutes ischemia, we found that PC-mAb treatment leads to preservation of cardiac function. Furthermore, IS was decreased in PC-mAb treated mice compared to vehicle treated mice. Both the early and late inflammatory response was attenuated following PC-mAb treatment.

In **chapter 4** we study the therapeutic potential of the anti-apoptotic and anti-inflammatory protein AnxA5 against MI-R injury. Administration of AnxA5 resulted in reduced LV dilatation, preservation of ejection fraction and decreased IS. This reduction in adverse cardiac remodelling was accompanied by a reduced early and late inflammatory response.

In the second part of this thesis we focus on epigenetic manipulation against adverse vascular remodelling.

In **chapter 5** we investigate the role of the epigenetic factor PCAF, which is known for its ability to acetylate lysine residues on histone proteins, on vascular inflammation and intimal hyperplasia development. Using the femoral artery cuff mouse model, we found that PCAF deficiency lead to a reduction in intimal hyperplasia development. Using hypercholesterolemic ApoE*3-Leiden mice, we found that the pharmacological PCAF inhibitor garcinol reduces injury-induced vascular inflammation. In addition, in vitro experiments showed that both PCAF deficiency and garcinol downregulate protein expression of several pro-inflammatory proteins.

Chapter 6 zooms in on the role of different microRNA members of the 14q32 gene cluster, namely microRNA-329, -494 and-495, on vascular remodelling. Using the femoral artery cuff mouse model we found that inhibition of microRNA-495 reduced intimal hyperplasia development. This reduction in intimal hyperplasia was accompanied with a reduction of macrophage influx and VSMC proliferation. In a mouse model for accelerated atherosclerosis we showed that inhibition of microRNA-495 leads to smaller atherosclerotic plaque size, while plaque stability was increased. Furthermore, microRNA-495 inhibition led to reduced plasma cholesterol levels, via reduction of the VLDL-fraction.

Finally, **chapter 7** summarizes the results of this thesis and future perspectives are discussed.

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