Modulation of the inflammatory response following myocardial infarction

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General introduction and outline of the thesis

GENERAL INTRODUCTION

Cardiovascular disease

Cardiovascular disease (CVD) remains the number one cause of death worldwide responsible for an estimated 17.8 million people annually representing 31% of global deaths[1]. Only in Europe it accounts for more than 4.1 million deaths a year representing 45% of total mortality and new cases of CVD are estimated around 19.9 million on a yearly basis. Of these deaths, an estimated 1.7 million are due to ischemic heart or coronary artery disease[2]. Last decades, treatment options for acute myocardial infarction have been drastically improved[3] with the advent of percutaneous coronary intervention (PCI)[4,5], coronary artery bypass grafting (CABG)[6,7], and the *golden five* medical treatment[8-13]. More recently, anti-inflammatory therapies showed interesting therapeutic effects in atherosclerotic disease[14-16]. All together, this resulted in a steady decline of mortality in The Netherlands since 1980 with a fourfold lower chance to die from acute myocardial infarction[17]. Nevertheless, worldwide current incidence rates and increasing morbidity emphasize the importance of ongoing research to improve prevention and treatment.

Atherosclerosis is the main cause of ischemic heart disease and has been shown to be a complex chronic inflammatory disease, encompassing both innate and adaptive immunity[18,19]. Immune mechanisms interact with metabolic risk factors to initiate, disseminate, and activate atherosclerotic lesions in the arterial wall during years or even decades, a process called atherogenesis[18]. Despite this chronicity, acute thrombosis, the most feared complication with clinical consequences, occurs suddenly. Progressive atherosclerosis narrows the lumen of a coronary artery resulting in a vulnerable plaque, which finally could cause a complete occlusion after plaque rupture of the culprit artery leading to a ST-segment elevation myocardial infarction, or non ST-segment elevation myocardial infarction in case of an incomplete or transient obstruction[20].

Atherogenesis

Over the last quarter century inflammation and immunity has shown to play a key role in the pathogenesis of atherosclerosis[21-24] (Figure 1). The development of atheromatous plaques is initiated by endothelial dysfunction caused by irritative stimuli such as dyslipidemia, hypertension or pro-inflammatory mediators[25,26]. This increases the adhesiveness and permeability of the endothelium with respect to inflammatory cells, by expression of adhesion molecules, chemoattractants, and growth factors, provoking an inflammatory process and procoagulant properties. The formation of a fatty streak, a most premature type of lesion with lipoproteins, consisting of mainly monocyte-derived macrophages and T lymphocytes is a fact[27], and already appears in adolescents[28]. Incessant inflammation, mediated by monocyte-derived macrophages and specific subtypes of T lymphocytes, stimulates progression to an intermediate lesion with a core region of foam cells and extracellular lipid accumulation in the intima surrounded by a cap of smooth-muscle cells and a collagen-rich matrix[29]. Finally due to efferocytosis, an advanced, complicated lesion originates by the formation of a necrotic core with a fibrous cap covering a

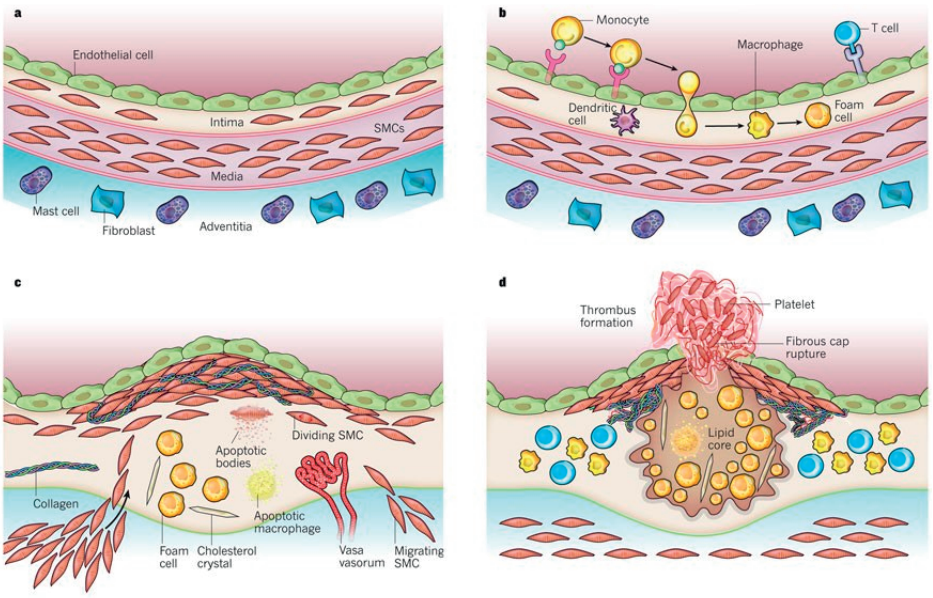

Figure 1: Pathogenesis and development of atherosclerotic lesions. Normal coronary artery with intima, media and adventitia layers (a). During atherosclerosis, blood leukocytes adhere to the activated endothelial layer and directly migrate into the intima, followed by maturation of monocytes into macrophages yielding foam cells upon lipid uptake (b). Migration and proliferation of smooth muscle cells and synthesis of extracellular matrix macromolecules result in lesion progression. A necrotic core is formed from lipid-derived dead and dying cells in the central region of a plaque (c). The ultimate complication of atherosclerosis, known as thrombosis and impeding blood flow, follows a physical disruption of the atherosclerotic plaque (d). Adapted from Libby et al[23] *Nature* 2011;473:317-25.

mixture of leukocytes, extracellular lipids, and cellular debris[30]. As a result of progressive disease and production of inflammatory cytokines, advanced lesions may become unstable and eventually plaque rupture or endothelial erosion induces acute thrombosis[31].

Acute coronary syndrome

A sudden and sustained atherothrombotic coronary artery occlusion causes an acute coronary syndrome (ACS) or type 1 myocardial infarction inducing myocardial cell death due to prolonged ischemia after disruption of myocardial blood flow[32]. Therefore, current medical therapy is based on timely reperfusion or revascularization. The preferred strategy in the setting of an acute myocardial infarction with ST-segment elevation is primary PCI as soon as possible in all patients with symptoms of ischemia of ≤12 hours and persistent ST-segment elevation, under the cloak of 'time is muscle'[33]. Primary PCI has been shown for decades to result in reduced infarct size and better clinical outcome in order of death and reinfarction[34]. Reduced infarct size in its turn, appears to be correlated with a better preserved left ventricular (LV) ejection fraction and

smaller end systolic volume index associated with less major adverse cardiac events and lower mortality[35,36].

In case of ischemia, early reperfusion salvages myocardium from irreversible damage, which may end up mostly functional. However, reperfusion itself has been shown to cause a pathophysiological process with detrimental effects known as lethal reperfusion injury. It may be responsible for up to 50% of the final infarct size. Following ischemia-reperfusion injury many different mechanisms, such as cell death, inflammation, fibroblast proliferation and degradation, and *de novo* synthesis of extracellular matrix take place as a result of numerous local and systemic signals, which ultimately may provoke heart failure due to loss of cardiomyocyte contractile function[37].

Taken together, there is a complex inflammatory interplay between atherosclerosis and myocardial infarction, and vice versa. In most cases, a complex atherosclerotic inflammatory process finally provokes myocardial infarction, its clinical consequence[21]. This initiates an inflammatory reaction activated by the innate immune system[38]. Subsequent reperfusion induces pathophysiologic reperfusion injury, and thereby an additional inflammatory process[37]. To complete this circle, myocardial infarction in its turn has been shown to accelerate atherosclerosis[39].

Current research clarified the importance of both addressing the culprit lesion and also tackle the atherosclerotic process by aiming for rapid stabilization of other plaques to prevent recurrent events[40]. Furthermore, opportunities to reduce the extent of cardiomyocyte necrosis and apoptosis are studied by investigation of immunomodulatory therapies, cell therapy, and other approaches. All this in order to ameliorate post-ischemic inflammation, stimulate cardiac regeneration, and repair large fibrotic scars to ultimately banish ischemic heart disease from the top ranking of diseases with the highest mortality and morbidity. Future targeted therapies should therefore be guided by a more precise pathophysiologic classification of ACS and based on greater mechanistic understanding of its diverse underlying causes[41]. In the following paragraphs, first the atherosclerotic inflammatory process and second, myocardial ischemia and pathophysiological ischemia-reperfusion injury (extensively reviewed in chapter 2) will be described into more detail.

Unraveling the atherosclerotic inflammatory process

Accelerated atherosclerosis development concerns a lipid-driven inflammatory process in which immune responses of the innate and adaptive immune system against circulating and local immunogenic antigens in the arterial wall play a crucial role (Figure 2). Clinical outcome is determined by the balance of pro-inflammatory and inflammation-resolving mechanisms. In experimental atherosclerosis, increased amounts of circulating neutrophils enable monocyte adhesion and transmigration, and contribute to oxidative stress, a major determinant of endothelial cell dysfunction, lesion growth and plaque instability. The monocyte/macrophage subtype is the main cellular contributor to atherosclerotic lesion formation. Generation of oxidation-specific

epitopes (OSEs) as a result of oxidative stress represents a major pathogenic burden that resulted in the conservation of a variety of innate immune responses. OSEs can act as endogenous danger-associated molecular patterns (DAMPs), which are recognized by pattern recognition receptors (PRRs) and the proteins of the innate immune system. Examples of primitive innate immune PRRs include various Toll-like (TLRs), nucleotide-binding oligomerization domain (NOD)-like (NLRs) and scavenger receptors (SRs), natural antibodies, and the complement system[42]. When the innate immune system is dysfunctional or overwhelmed, activation elicits a chronic inflammatory process as atherosclerosis[43] appealing the adaptive immune system[44].

Adaptive immunity is predominant in the chronic inflammatory atherosclerotic process and driven by dendritic cell-mediated antigen capture and presentation to naïve T cells. During a steady state, dendritic cells reside in the aortic wall and affect progression to atherosclerosis[45], possibly by interacting with local T cells resulting in maintained chronic inflammation and induced foam cell formation[46]. T cells, predominantly $CD4^+$, account for approximately 10% of all cells in human plaques, and are divided into proatherogenic T helper (T_H)1 cells, atheroprotective (currently debated) T_H2 cells, regulatory T (T_{reg}) cells, and T_H17 cells and natural killer T cells with both proatherogenic as atheroprotective properties. For example, T_H1-generated interferon (IFN)-γ and tumor necrosis factor (TNF) activate macrophages and propagate inflammation, whereas T_{reg}-generated interleukin (IL)-10 and transforming growth factor (TGF)-β restrict inflammation[47,48]. The role of B cells in atherosclerosis remains controversial, but current literature predominantly indicated atheroprotective effects. For instance, the immunoglobulin (Ig)M responses to OSEs from B1a and B1b cells have been shown protective[49]. However, proatherogenic effects of B2 cells have also been described[48]. In addition, active and passive immunization generally have atheroprotective effects in animals[50,51].

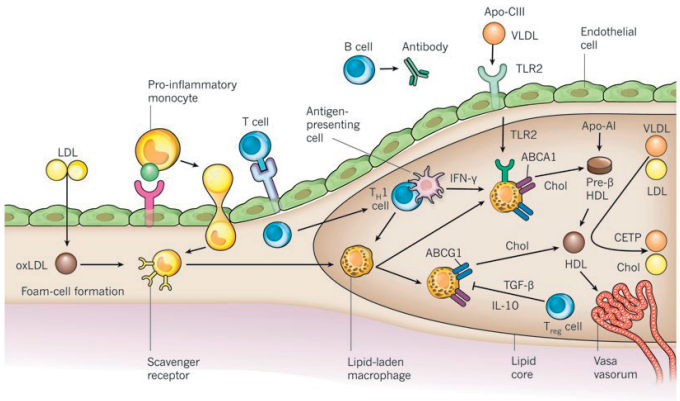

Figure 2: Modulation of atherosclerosis by inflammation and lipid metabolism. Atherogenesis starts with the recruitment of inflammatory cells to the intima. The pro-inflammatory subset of Ly-$6C^{hi}$ monocytes are predominantly captured by activated endothelial cells. Uptake of oxLDL is allowed following inflammatory activation. As a result, foam cells and mature lipid-laden macrophages produce pro-inflammatory mediators, ROS, and tissue factor pro-coagulants. Although fewer in number, T cells, T_{reg} cells, and B cells affect the inflammatory process as well. Adapted from Libby et al[23] *Nature* 2011;473:317-25.

Post-ischemic myocardial inflammatory response

Ischemic myocardial injury causes cellular degradation and loss of oxidative phosphorylation resulting in loss of membrane integrity[52]. The dominant mechanism of cardiomyocyte death is coagulation necrosis, peaking after 12 hours up to 4 days[53]. In addition, apoptosis, peaking after 6 to 8 hours, concerns programmed cell death, in particular induced by reperfusion and also affecting non-infarcted areas[54]. The entire post-ischemic myocardial inflammatory response, especially following reperfusion, is extensively described in the next chapter 2, a state-of-the-art review article. All three overlapping stages in myocardial infarct repair, the inflammatory, reparative, and maturation phases, are thoroughly discussed, including the roles of innate immunity, chemokines, cytokines and inflammatory cells (Figure 3). Since monocytes are key players involved in both the etiology of cardiovascular disease as the disease itself, they are subsequently described into more detail below. In addition, the current status of immunomodulatory therapies in clinical practice is discussed at the end of chapter 2.

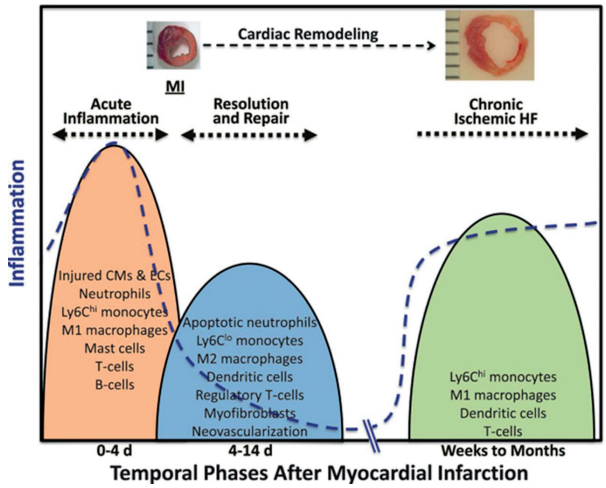

Figure 3: Post-ischemic myocardial inflammatory response. Myocardial ischemia induces an acute pro-inflammatory response through production of DAMPs, ROS, and complement cascade, which mediates accumulation of inflammatory cells through the release of chemokines and cytokines. Subsequently, the anti-inflammatory reparative phase mediates the resolution of the inflammatory response and precedes the final maturation phase. Adapted from Prabhu et al[55] *Circ Res* 2016;119:91-112.

Monocytes - an indispensable immunological link

Besides their contribution to the post-ischemic inflammatory process, monocytes also contribute to the originating process of atherosclerosis, the main contributor of acute MI. Monocytes have been shown to play a role in both the initiation and formation of an atherosclerotic plaque, the acute inflammatory phase in ACS following plaque destabilization, rupture, and acute thrombus formation, as well as the reparative process, either as pro- or antagonist, when they migrate towards the ischemic myocardium in response to an acute ischemic event[56]. Where atherosclerosis

is considered a chronic inflammatory disease with mobilization of leukocytes to the vessel wall due to a chronic low-grade stimulation by native and oxidized lipoproteins, MI triggers an acute inflammatory response following plaque rupture and elicits different triggers that nevertheless recruit similar (sub)types of leukocytes[57]. Despite their frequent concurrence, the interconnection between the chronic and acute inflammatory conditions is mostly neglected.

Atherosclerosis is accompanied by hypercholesterolemia-associated monocytosis dominated by pro-inflammatory Ly-6C^{hi} monocytes in mice. This is explained by increased survival of Ly-6C^{hi} monocytes, continued cell proliferation, and impaired Ly-6C^{hi} to Ly-6C^{lo} conversion[58]. Ly-6C^{hi} monocytes express TNF-α, IL-1β, myeloperoxidase, matrix metalloproteinases (MMPs), cathepsins, and plasminogen activator urokinase and are therefore potentially inflammatory and precursors of M1 macrophages. Whereas Ly-6C^{lo} monocytes express IL-10, TGF-β, and the pro-angiogenic vascular endothelial growth factor (VEGF) and therefore exhibit a reparative phenotype and are precursors of M2 macrophages[59]. Eventually, effects of hypercholesterolemia-associated monocytosis may be subsided upon statin-induced cholesterol reduction[58].

In human, peripheral monocytosis two to three days following reperfused acute MI was associated with LV dysfunction and LV aneurysm. Patients with LV failure or aneurysm had higher peak monocyte counts. More specific, peak monocyte counts were positively correlated with LV end-diastolic volume (EDV) and negatively correlated with ejection fraction[60]. In addition, low monocyte counts were related to markers of effective reperfusion and they were both independently associated with LV functional recovery at 6 months after primary PCI in patients following acute MI[61]. Sequential mobilization of CD14^{+}CD16^{-} (similar to Ly-6C^{hi} in mice), and CD14^{+}CD16^{+} (similar to Ly-6C^{lo} in mice) monocytes was shown after acute MI in patients treated with PCI. Only peak levels of CD14^{+}CD16^{-} monocytes were significantly negatively associated with the extent of myocardial salvage after seven days and recovery of LV ejection fraction after six months[62].

Unreperfused MI in hypercholesterolemic mice resulted in sequential and active recruitment of Ly-6C^{hi} (via CCR2) and Ly-6C^{lo} (via CX3CR1) monocytes. Monocyte/macrophage recruitment is not only restricted to the infarcted area but also substantially affects the non-infarcted remote myocardium, a finding mirrored in patients with acute MI[63]. Pro-inflammatory Ly-6C^{hi} monocytes dominate the early inflammatory phase (day 1 to 4) and exhibit phagocytic, proteolytic, and inflammatory functions and thus digest damaged tissue. Reparative Ly-6C^{lo} monocytes dominate the later reparative phase (day 4 to 8) with attenuated inflammatory properties, wound healing, angiogenesis, and collagen deposition[64]. Moreover, unreperfused MI in hypercholesterolemic mice showed a more pronounced post-ischemic inflammatory gene expression profile associated with the activity of Ly-6C^{hi} monocytes. This consequently resulted in impaired infarct healing and accelerated deterioration of ejection fraction after three weeks[65].

Inflammatory monocytes trigger an autoimmune T cell response following acute MI. Selective inhibition of monocyte recruitment to the injured myocardium limited the autoimmune response, which was associated with improved tissue repair and cardiac function[66]. Furthermore,

the positive correlation between enzyme activity and LV dilation suggests that a prolonged inflammatory phase or compromised reparative phase predisposes to heart failure[64,65]. Altogether, experimental data regarding both unreperfused as reperfused MI reported predominantly negative correlations[64,67-70] between monocyte/macrophage numbers versus LV remodeling and function but positive correlations[71,72] have been reported as well. To conciliate these apparently conflicting results, wound healing of ischemic myocardium presumably requires a monocyte/macrophage response that balances and coordinates inflammatory and reparative functions (Figure 4). Either unrestrained inflammatory activity or extensive suppression of inflammation may counteract or disable the reparative capacity of monocytes.

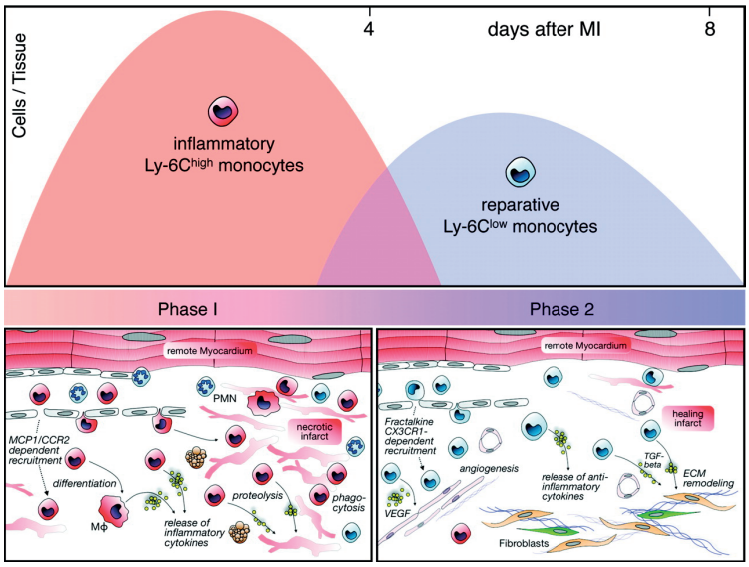

Figure 4: Recruitment of monocytes following myocardial ischemia. Time course of monocyte recruitment following murine MI concerning inflammatory Ly-6C^{hi} and reparative Ly-6C^{lo} subsets. Adapted from Nahrendorf et al[57] *Circulation* 2010;121:2437-45.

LV remodeling

The heart itself has negligible regenerative capacity[73] and therefore cardiomyocyte death triggers a reparative response that ultimately results in scar tissue formation and is associated with adverse dilative ventricular remodeling (Figure 5). Moreover, the uninjured myocardium consists of a 3D myocardial structure of which the specific architectural characteristics enable efficient electrical conduction and mechanical contraction[74] compromised following myocardial ischemia. Myocardial wound healing and scar tissue formation occurs under continuous rhythmic contraction of the non-infarcted myocardium resulting in continuous cyclic stretch on the healing wound. Myocardial scar formation and remodeling impair the functional syncytium, in which

myocardial or LV remodeling affects even both the infarcted and non-infarcted residual viable myocardium[75]. Remodeling regards an adaptive process in which the LV is reshaped by structural changes of the myocardium induced by the increased loading conditions of the heart after MI, due to loss of myocardium. Besides mechanical stress, also cytokines, neurohormonal factors, growth factors and enzymes are involved in the myocardial remodeling process[76,77], ultimately resulting in infarct expansion with concomitant increase of LV wall stress and resultant global LV dilation and wall thinning[78]. Following acute MI, reperfusion therapy cannot be applied soon enough in many cases to reverse pathological processes and prevent extensive myocardial damage. Absence of reperfusion undoubtedly results in adverse myocardial remodeling and progression to heart failure with a poor prognosis[79]. Eventually myocardial remodeling becomes maladaptive as it can no longer compensate for the increased work load, resulting in ventricular arrhythmias, aneurysm formation, global LV dysfunction, and sudden cardiac death[80]. Since the extent of LV remodeling is a major predictor of prognosis in patients with MI, therapeutic approaches to attenuate LV remodeling are critically important, where the modulation of non-infarcted residual viable myocardium provides therapeutic potential.

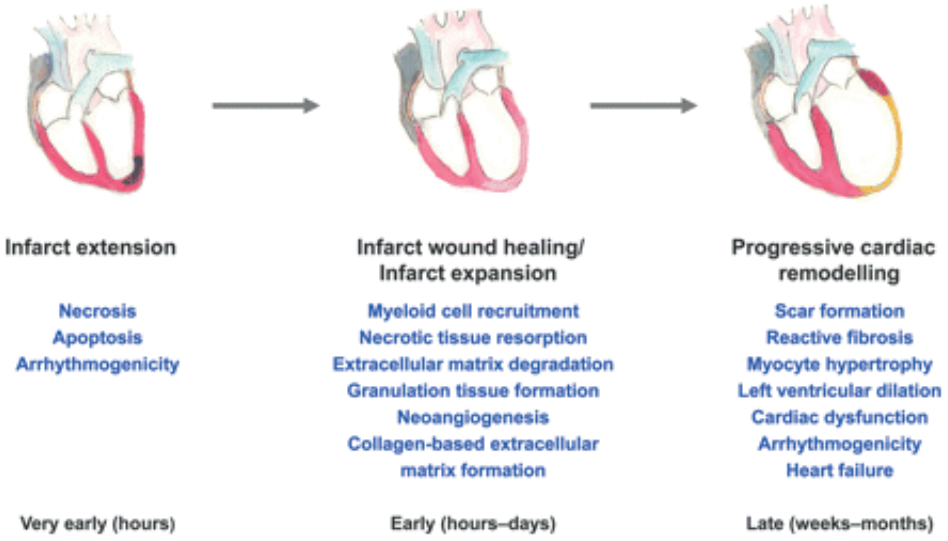

Figure 5: Post-ischemic LV remodeling process. Post-ischemic phases of infarct extension (after hours), wound healing or expansion (hours-days), and final progressive cardiac remodeling (weeks-months). Adapted from Fraccarollo et al[81] *Cardiovasc Res* 2012;94:293-303.

Animal models in translational research

Until now, animal models are indispensable to investigate the etiology of MI and possibilities to treat the consequences of myocardial ischemia. At the same time however, relevancy of animal derived data is questioned whether and to which extent these studies resemble the clinical setting with their human counterparts. The selection of an appropriate animal model to ensure optimal

translation of novel cardioprotective therapies from bench to bedside is of the utmost importance, which has been generally endorsed before[82-85]. Yet until recently, most data regarding MI have been generated in animal models lacking human comorbidity, spared of atherosclerosis and associated heightened inflammatory phenotype[86]. Briefly, discussions are related to two major issues to overcome. First, patients suffering a MI are in the vast majority exposed to atherosclerosis, a long-lasting chronic inflammatory process, finally experiencing rupture of an unstable atherosclerotic plaque with sudden occlusion of a coronary artery as a consequence. However, most experimental data regarding MI and heart failure have been generated in animals lacking this chronic atherosclerotic inflammatory phenotype and associated chronic monocytosis, a problem that has been denounced for several years[57]. Second, current translational research underestimates effects of clinically applied mechanical reperfusion, since studies concerning MI with long-lasting follow-up periods of weeks are mainly performed in unreperfused MI models. However, reperfused MI has been shown to attenuate adverse remodeling accompanied by higher numbers of involved inflammatory cells and enhanced neovascularization[87], and moreover induces reperfusion injury by itself[37]. Other points of interest are periods of ischemia, in patients mostly between 2 and 12 hours, infarct size, in patients between 13% to 16%, which may limit the scope for cardioprotection regarding larger infarct sizes in experimental animal studies, and timing of intervention relative to the period of ischemia and onset of reperfusion[37].

Ideally, translational animal models resemble the clinical setting exhibiting an atherosclerotic phenotype before they are exposed to temporarily MI-R injury. In addition, parameters should be studied after weeks instead of hours or days to draw correct conclusions regarding delayed necrosis or apoptosis in order of therapeutic effects. Although murine atherosclerosis is not associated with occlusive coronary heart disease or MI, transgenic APOE*3-Leiden mice however do develop advanced aortic atherosclerotic lesions resembling their human counterparts when exposed to cholesterol feeding[88]. Therefore, APOE*3-Leiden mice are suitable to study therapeutic effects of MI in case of experimental MI-R injury, providing a clinically more relevant experimental MI-R model.

Immunomodulatory therapies

Following myocardial ischemia, several immunomodulatory therapies showed promising results in preclinical research but failed successful translation towards clinical practice for many reasons. Besides selection of appropriate experimental models, mechanisms of action and timing of interventions regarding modulation of the post-ischemic inflammatory response promoting benign wound healing are of the utmost importance. In this thesis the role of annexin A5 (AnxA5) and phosphorylcholine monoclonal IgG antibodies (PC-mAb) in post-ischemic myocardial immunomodulation are profoundly studied.

Annexin A5

AnxA5 affects apoptosis[89], and has anti-inflammatory[90] and anti-thrombotic effects[91]. As a result of MI-R injury, phosphatidylserine (PS) for example is externalized during early apoptosis and inflammatory cell activation where it functions as an "eat me" signal te ensure early recognition and phagocytosis[92]. PS-expressing cells are targets for annexins, a family of phospholipid-binding proteins, and in particular AnxA5, which binds reversibly, specifically, and with high affinity to PS-expressing cells[93]. Both endogenous AnxA5 plasma levels[94] as wells as uptake in the infarct area[95] are increased following myocardial ischemia. Therefore, a therapeutic post-ischemic effect of AnxA5 seems likely.

Phosphorylcholine antibodies

Phosphorylcholine (PC) is an example of an OSE, which is expressed by oxidized LDL (ox-LDL), an important lipoprotein in the development of atherosclerosis, remained hidden until oxidation[42]. It is the polar headgroup of a major membrane component, phosphatidylcholine, and expressed on the outer membrane of apoptotic cells, and known for its immunogenic and pro-inflammatory properties[96]. For instance, natural antibodies against phosphorylcholine are capable to inhibit apoptotic cell uptake by macrophages *in vitro*[97] and *in vivo*[96] and block the pro-inflammatory effects of PC expressing oxidation-damaged molecules[98]. Moreover, low concentrations of PC IgM antibodies are associated with increased risk for cardiovascular diseases[99], and acute coronary syndrome patients with low PC IgM antibody levels experience a worsened prognosis[100]. Therefore PC seems to be an interesting target for novel immunomodulatory therapeutic interventions as PC-mAb.

Aim and outline of the thesis

Following MI timely reperfusion by primary PCI is the ultimate goal to limit myocardial damage and reduce infarct size resulting in a better clinical outcome, as endorsed by the guidelines. Paradoxically, restoration of myocardial blood flow comes at a price, as it initiates myocardial reperfusion injury by a series of events, which apparently affect post-ischemic infarct healing. Evidenced by the numerous amount of studies, modulation of the post-ischemic inflammatory response has been shown to attenuate infarct healing and LV remodeling preserving cardiac function in translational animal research. Though, promising pre-clinical results are received with some skepticism nowadays, since most of them have failed successful translation into clinical trials for several reasons. This research enabled the evaluation of the effectiveness of specific immunomodulatory therapies and tested their effects in a murine MI model resembling the clinical setting more accurately, taking into account both a hypercholesterolemic phenotype as well as MI-R injury.

As an introduction, the post-ischemic myocardial inflammatory response with the inflammatory, reparative, and maturation phases, including the roles of innate immunity, chemokines,

cytokines and inflammatory cells, is described in detail in **chapter 2** as a state-of-the-art review article. Also the current status of immunomodulatory therapies in clinical practice is discussed.

In **chapter 3**, to validate the murine MI model, we studied the long-term effects of diet-induced hypercholesterolemia on MI-R injury in APOE*3-Leiden mice up to 8 weeks following MI-R. We focused on cardiac function, infarct size, and the post-ischemic inflammatory response regarding monocytes in particular. It has become clear that monocytes play a key role in the etiology of MI since atherosclerosis is associated with chronic monocytosis. Furthermore, MI itself causes an inflammatory response in which a pro-inflammatory monocyte subtype initially dominates, which is replaced by a reparative monocyte subtype. This makes monocytes main contributors of the post-ischemic inflammatory response and an ultimate target for therapeutic immunomodulation.

In the following chapters different anti-inflammatory and immunomodulatory strategies are considered as possible therapeutic applications to modulate post-ischemic infarct healing and preserve cardiac function following MI-R injury. In **chapter 4** we investigated the effects of post-ischemic human recombinant annexin A5 (AnxA5) therapy. By binding phosphatidylserine, which becomes externalized on the outer cell surface following early apoptosis and inflammatory cell activation, AnxA5 earlier has been shown to exhibit anti-apoptotic and anti-inflammatory effects. Focus of **chapter 5** is on the therapeutic use of antibodies directed against phosphorylcholine. So far, low levels of anti-PC have been associated with development of atherosclerosis, cardiovascular disease in humans, and worsening of the prognosis following ACS. By binding of natural IgM anti-PC, uptake of apoptotic cells by macrophages is prevented. We studied the effects of a clinically applicable new developed human monoclonal IgG_1 antibody against phosphorylcholine (PC-mAb) following MI-R injury regarding post-ischemic inflammation and adverse LV remodeling to ultimately preserve cardiac function. In addition, **chapter 6** addresses therapeutic administration of PC-mAb following unreperfused MI to clarify the influence between different mechanisms of myocardial ischemic injury.

In **chapter 7** we questioned the current use of translational animal models to test potential novel therapeutic strategies regarding acute MI in a pre-clinical setting. By using a MI-R injury model in hypercholesterolemic APOE*3-Leiden mice we provided a more clinically relevant translational MI model. By comparing it with an unreperfused MI model we investigated whether this is a validated and suitable murine MI model to better predict hypothesized beneficial effects of newly derived clinical treatment strategies in a pre-clinical setting.

Finally, **chapter 8** provides the summary and conclusions, as well as future perspectives regarding post-ischemic immunomodulatory therapies.

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