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General introduction
and outline of the thesis
General introduction

Clinical background. Cardiovascular disease (CVD), including coronary heart disease (CHD), accounts for over 17 million deaths each year worldwide (1). Although the mortality rate of CHD steadily declined over the last decade due to improved treatment options, CVD and more specifically CHD, remain the leading cause of death in Europe (2) and the US (3). Also, during this timeframe, the incidence of heart failure has remained unaltered (3). In addition, the aging population and the increased incidence of cardiac risk factors, e.g. smoking, obesity, hypertension, hypercholesterolemia and diabetes mellitus, contributed to increased CHD-related morbidity. Therefore, the expectation is that both the incidence of CVD and the economic costs of CVD will keep rising in the future above the expenditure of $320.1 billion in 2011 in the US and €196 billion in the EU each year (2, 3).

Ischemic heart disease: atherosclerosis. The principle underlying cause of CVD is atherosclerosis, an inflammatory disease associated with increased blood levels of apolipoprotein B-containing lipoproteins such as low-density lipoprotein (LDL) (4, 5). This LDL increase, combined with other cardiac risk factors, may ultimately lead to activation of the inflammatory response through the release of phospholipids by modification of LDL. Platelets are recruited to the site of activation resulting in expression of adhesion molecules by endothelial cells and homing of monocytes and lymphocytes to and subsequently activation of different chemokine and cytokine pathways in the intima. Differentiated macrophages and dendritic cells become lipid-laden foam cells that accumulate in the proteoglycan layer of the intima and eventually form a fatty streak (Figure 1). Progression of a fatty streak towards an atherosclerotic plaque starts with pathological intimal thickening through accumulation of small lipid pools underneath the foam cell layers. These small lipid pools may flow together, forming an atherosclerotic plaque. Connective tissue is formed on the surface of the atherosclerotic lesion by type I collagen deposition by smooth muscle cells, establishing stable fibrous cap. The plaque may develop a necrotic core due to apoptosis and necrosis of foam cells, which ultimately may lead to disruption of the fibrous cap covering the atheroma.

During the progression towards an atherosclerotic plaque, neoangiogenesis from the vasa vasorum to the base of atherosclerotic lesion results in additional entrance sites for inflammatory cells, increasing the local inflammatory response and the risk of intraplaque bleedings (Figure 1).

Ischemic heart disease: myocardial infarction (MI). Prolonged myocardial ischemia results in cell death MI, initiating an inflammatory response ultimately leading to scar formation (6-11). During the first inflammatory phase of myocardial ischemic damage, platelets are activated to prevent bleeding and release growth factors to aid the repair process. Neutrophils are the first immune cells entering the myocardium, clearing the
infarct site from dead cells and debris, followed by monocytes (Figure 2). In addition, neutrophils contribute to the production of reactive oxygen species, which amongst others activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor that regulates genes involving pro-inflammatory cytokines, chemokines, matrix metalloproteinases, growth factors and genes involved in cell survival and proliferation. Besides reactive oxygen species, the Toll-like receptor-mediated pathway and the complement cascade contribute to NF-κB activation. After the clearance of the infarct site from debris and the activation of pro-survival and reparative signaling cascades, the inflammatory phase progresses into the proliferative phase in which the initial inflammation reaction is directed towards a healing process (Figure 2).

Figure 1. Lesion types of atherosclerosis and a proposed sequence of their development. A: Adaptive intimal thickening characterized by smooth muscle cell accumulation within the intima. B: Intimal xanthoma corresponding to the accumulation of foam cell macrophages within the intima. Pathological thickening in C denotes the accumulation of extracellular lipid pools in the absence of apparent necrosis. D: Fibroatheroma indicating the presence of a necrotic core. The necrotic core and surrounding tissue may eventually be calcified, which forms fibrocalcific plaque shown in E. Because some of the advanced lesion types (fibroatheromas and fibrocalcific plaques) evolve simultaneously in life, their interrelationships are difficult to resolve in autopsy studies. Movat pentachrome stain. Reproduced with permission from (4), Copyright by American Heart Association.
The recruitment of both neutrophils and monocytes is reduced and lymphocyte antigen 6C (Ly-6C)low macrophages clear the apoptotic neutrophils from the infarct site. In addition, the presence of anti-inflammatory factors such as interleukin-10 and transforming growth factor β is increased, further stimulating the removal of inflammatory leukocytes. Also, these factors are important for the recruitment of myofibroblasts and the production of extracellular matrix proteins for scar tissue formation.
During the proliferation phase the expression of the angiogenic factor hypoxia-inducible factor 1 results in neoangiogenesis, facilitating oxygen delivery to the injured tissue and maintaining cell metabolism. These processes lead to the resolution of the pro-inflammatory environment and the formation of highly vascularized granulation tissue, initiating the transition towards the maturation phase in which the final collagen-rich scar is formed.

Endothelial cells proliferate to form an extensive microvascular network, stabilizing the scar by providing oxygen and nutrients. When the highly vascularized granulation tissue is replaced by a collagen-rich scar to replace the damaged heart muscle, the process of infarct healing is complete (6-11).

Potential of cell-based therapy for the treatment of ischemic heart disease. The number of therapeutic strategies to treat MI has increased substantially in the past decades (12-15). Current treatment options for MI to ameliorate the effects of myocardial ischemia are diverse (16). In ST-elevation MI (STEMI), primary percutaneous coronary intervention (PCI) is indicated and the delay between the patients’ symptoms and reperfusion therapy should be kept at a minimum. When a primary PCI center is not available or when a PCI is not possible, thrombolysis should be initiated immediately. In case of multivessel disease or when the culprit coronary artery is unsuitable for PCI, emergency coronary artery bypass graft surgery (CABG) may be indicated. In Non-STEMI (NSTEMI) patients, angiography is performed to evaluate whether there is a culprit lesion for which PCI or CABG may be indicated. Additionally, all (N)STEMI patients are treated with antiplatelet and fibrinolytic therapy, beta-blockers, statins and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Also, both lifestyle interventions and risk factor management are provided to optimize cardiac rehabilitation and reduce the risk for a recurrent event.

Patients with significant left ventricular dysfunction and ventricular arrhythmias or at risk of ventricular arrhythmias should be evaluated for implantable cardioverter defibrillator therapy with or without resynchronization therapy (16).

Despite these treatment options, MI patients are still prone to develop heart failure since there are no therapeutic options available to reverse the loss of functional myocardium (17). So far, the only curative option for advanced heart failure is heart transplantation. However, the number of transplant procedures is restricted by limited donor availability (18). Therefore, there is an unmet clinical need for new therapies to treat irreversibly damaged myocardium.

In recent years cell-based regenerative therapy has emerged as a potential therapeutic option for ischemic heart disease (19). The ideal cell-based cardiac regenerative therapy utilizes a cell type that is easily accessible, is able to engraft into the infarcted site and survive, can differentiate into cardiomyocytes and endothelial cells, couple electromechanically to host myocardium, contribute in a meaningful manner to cardiac function and this cell type should be delivered via a safe and minimally invasive
procedure. Additionally this cell type should have immunomodulatory properties in light of the extensive detrimental inflammatory processes that occur after MI, and must be autologous to avoid the need for pharmacological immune suppression. In search of this ideal form of cell-based therapy, a variety of cell types has been studied.

In 2001, Orlic et al reported that intramyocardial transplantation of bone marrow cells after MI in mice resulted in differentiation of these cells into cardiomyocytes and regeneration of the infarcted myocardium (20). However, these results remain controversial, as other labs were not able to reproduce them (21, 22). Since then, a large number of experimental studies has been performed indicating that cell-based therapy may improve cardiac function after MI (23, 24). The skeletal myoblast was one of the first cell types injected after experimental MI, which resulted in improvement of cardiac function (25). In addition, different stem cell populations derived from the bone marrow were studied.

Mesenchymal stromal cells (MSCs) (26, 27), bone-marrow derived mononuclear cells (28, 29), hematopoietic stem cells (22) and endothelial progenitor cells (30, 31) all showed the potential to improve cardiac function when injected after MI. In particular MSCs have been evaluated as source for cell-based therapy after MI, since they are easily accessible and expendable, present limited risks of tumorigenicity, allow allogeneic use because of their immunomodulatory capacities and produce a combination of paracrine factors that diminish the deleterious effects of the post-infarction inflammatory response. (32).

Although all of the above mentioned cell types engraft into the infarcted myocardium, they are not able to quantitatively differentiate towards cardiomyocytes, couple with the host cardiomyocytes or provide meaningful neomyocardiogenesis. So far, the only stem cell types known to have the capacity to differentiate into cardiomyocytes are the embryonic stem cell (ESC) and induced pluripotent stem cells (iPSCs) (33, 34). Transplantation of ESC-derived cardiomyocytes has shown a beneficial effect on cardiac function (35), although this appears to be temporary (36). Despite of their huge potential for cell-based therapy in patients, the use of ESCs remains controversial because of ethical issues, observed teratoma formation and immune rejection (37). However, recently the first in man application of ESC has been performed (38).

The discovery of iPSCs by Takahashi et al. addresses the ethical and immune rejection issues since the patients’ own skin fibroblasts can be used for iPSC generation (34). iPSCs are able to differentiate towards cardiomyocytes and other cells of the cardiovascular lineages (39-41). Injection of these cells after MI did result in improvement of cardiac function (42-44).

Besides ESCs and iPSCs, it has become clear that the heart itself also contains different populations of cardiac progenitor cells (CPCs) which appear to be capable of cardiac regeneration (45). Beltrami et al. questioned the traditional view of the heart as a terminally differentiated organ by proposing that cardiomyocytes may re-enter the cell cycle and undergo mitotic division (46). Recent studies have confirmed this
finding (47-51), but it remains unclear what the exact number and turnover rate of newly formed cardiomyocytes is (52). Hsieh et al. suggested that progenitor cells may play a role in the process of cardiomyocyte renewal (53). However, the contribution of progenitor cells appears to be limited to injury-related cardiomyocyte replacement, as during normal ageing cardiac regeneration mainly occurs through pre-existing cardiomyocytes (53, 54). The different CPCs populations residing in the heart that have been identified over the last few years are characterized by expression of several surface marker proteins, including c-Kit (55, 56), islet1 (57) and stem cell antigen-1 (cardiomyocyte progenitor cells, or CMPCs) (58, 59). Additionally, cell populations have been characterized by expressing a combination of stem cell markers and the potential to grow as self-adherent clusters (cardiospheres) (60) or by their ability to exclude Hoechst dye (61, 62). Transplantation of these different cell populations (55, 63, 64) and cardiospheres (65-67) after MI all resulted in beneficial effects on cardiac function.

Besides CPCs, other progenitor cells which are important for the development of the heart, e.g. epicardium-derived cells (EPDCs) have been investigated. Both injection of EPDCs alone (68) and injection of a combination of EPDCs and CMPCs (69), contributed to functional improvement of heart function after MI.

**Targeting hypercholesterolemia as treatment for ischemic heart disease.**

Hypercholesterolemia, either familiar or diet-induced, is an important risk factor for the development of ischemic heart disease due to the initiated inflammatory response by increased blood levels of lipoproteins, e.g. LDL, leading to the development of atherosclerosis (4, 70). The APOE3*Leiden mouse model provides an opportunity to study the pathophysiology of diet-induced hypercholesterolemia and the development of atherosclerotic plaques. While on a high-cholesterol diet, these transgenic mice developed hypercholesterolemia and hyperlipidemia (71). A subsequent study by van Vlijmen et al. showed that after 14 weeks of high-cholesterol feeding, APOE*3 Leiden mice developed atherosclerotic plaques similar to humans (72). However, in these mice atherosclerotic plaques were mainly present in the aortic arch and carotid arteries, whereas no lesions were found in the coronary arteries as is the case in the clinical situation of CHD.

So, despite the fact that APOE*3 Leiden mice develop atherosclerosis in a similar way as humans, this mouse model cannot be used to study the origin and development of a myocardial infarction. However, this model still provides us more insight in the development and pathophysiology of hypercholesterolemia and atherosclerosis, which may eventually lead to additional treatment options for or (secondary) prevention of CVD.
Outline of the thesis

This thesis focuses on the potential of cell-based therapy in ischemic heart disease and the role of the inflammatory response after MI. Chapter 2 reviews the specific myocardial inflammatory events that occur following MI and explores the potential role of cell therapy, in specific of the MSC, to positively influence this process. In chapter 3 we studied the usefulness of a clinically relevant transient ischemia MI model in immunodeficient mice to investigate the potential of human stem cell therapy and compared this to the commonly used animal MI model via permanent ischemia. Besides a comparison of cardiac function between both MI models, infarct size and inflammatory response were also evaluated. Next, in chapter 4 we aimed to extend our previous research regarding the positive therapeutic effects of MSC therapy after MI by injecting MSCs stimulated with the pro-inflammatory cytokine interferon-γ, since pro-inflammatory priming has shown additional beneficial effects in several experimental disease models. In addition to determining cardiac function, infarct size, cell engraftment and inflammatory cell influx were also evaluated.

Chapter 5 evaluates the short-term effect of human CMPC (hCMPC) infusion on cardiac function in an animal MI model. Cells were isolated from human fetal heart tissue and infused into infarcted hearts. Afterwards, engraftment and differentiation capacities were evaluated by histology.

Chapter 6 discusses the effect of diet-induced hypercholesterolemia, one of the important risk factors for developing ischemic heart diseases, on both cardiac function and inflammation after myocardial ischemia-reperfusion injury.

Finally, chapter 7 provides an overview of the results described in this thesis, and discusses future perspectives.
Reference List


cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. Dev Biol 2004;265:262-75.


