Clinical and experimental observations on the inflammatory response following a myocardial infarction

Citation for published version (APA):

ter Horst, E. N. (2018). Clinical and experimental observations on the inflammatory response following a myocardial infarction

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Summary, Future directions & Concluding remarks
Summary of the thesis

An increasing number of preclinical studies have emphasized the necessity of a well-orchestrated phased post-MI immune response. Different inflammatory cells are recruited to the infarcted heart to primarily clear cell debris and subsequently propagate scar tissue formation. Disturbance in either one of these phases has been shown to detrimentally affect cardiac healing and could be a prerequisite for the development of chronic heart failure. The current thesis therefore aimed to clarify important aspects of the post-MI immune response in both clinical and experimental settings to elucidate therapeutic opportunities that could beneficially influence post-MI cardiac healing.

In chapter 2, several novel aspects regarding the post-MI CD14+ monocyte response in patients are uncovered. In this chapter, we showed in human post-mortem tissue specimen that the pro-inflammatory CD14+CD16– monocyte subsets infiltrate the border zone of the myocardial infarct at 12 hours – 5 days post-MI whereas the anti-inflammatory CD14+CD16+ monocyte subsets infiltrate the infarct core at 5–14 days post-MI. This emphasized a unique post-MI spatio-temporal accumulation of monocyte subsets into the human infarcted heart. Furthermore, we were the first to demonstrate a monocyte depletion from the human spleen that coincided with monocyte accumulation in the infarcted myocardium. This indicates that the human spleen contains an important reservoir function for monocytes, that can be recruited upon substantial tissue damage such as MI.

Mesenchymal stem cells (MSC) are a potential therapy to regenerate cardiac tissue and thereby diminish scar tissue formation post-MI. However, animal and patient studies showed a beneficial effect of MSC on post-MI cardiac function with limited engraftment of MSC into the infarcted heart. In chapter 3 we reviewed studies that analysed the role of MSC in post-MI cardiac functioning. In these studies it was suggested that MSC improve post-MI cardiac functioning through paracrine mechanisms that induce a phenotype switch of monocytes and/or macrophages towards a more anti-inflammatory state rather than the differentiation of MSC into cardiomyocytes. Production of IL-10, indoleamine-pyrole 2,3-dioxygenase and prostaglandin E2 is suggested to be one of the main mechanisms by which MSC polarizes macrophages towards the inflammatory resolution subtype, however, the exact mechanism of action still remains to be elucidated.

As the polarisation towards particular monocyte subsets could influence post-MI cardiac healing, we aimed in chapter 4 to further identify proteins that could play a role in the monocyte subset switch. It is known that the so-called NOX proteins are involved in the migration and differentiation capacity of monocytes. However, the role of NOX expression in different monocyte subsets remains to be elucidated. In chapter 4, we uniquely showed that the NOX2 isotype, in comparison to NOX1 and NOX4, is the predominant NOX isoform in all three human monocyte subsets. Subsequently, we showed that in all monocyte subsets, NOX1 expression was located at the plasma membrane whereas NOX2 was located in the cytoplasm. NOX4 uniquely showed a different subcellular expression pattern in the different monocyte subsets. In the pro-inflammatory classical and intermediate monocytes we found
plasma membrane expression of NOX4 whereas in the anti-inflammatory non-classical monocytes, we showed cytoplasmic expression which was proposed to be located in the mitochondria. This could suggest that the difference in subcellular expression of NOX4 might influence functional differences of monocyte subsets.

To further specify monocyte characteristics that are related to post-MI cardiac functioning, we investigated and associated gene transcripts of monocytes to adverse left-ventricle (LV) remodelling in MI patients in chapter 5 and subsequently evaluated intervention with these characteristics in a rat MI model. In patients, we found that a monocyte specific upregulation of interferon (IFN) stimulating genes that are involved in type I IFN signalling coincided with beneficial cardiac healing at 4 months follow up. However, in a rat MI model we found that systemic administration of the type I IFN-α exerted an opposite response. IFN-α namely increased the infarct area and deteriorated ventricular dilatation at day 28 after MI. Additionally, we showed that in comparison to placebo MI rats, IFN-α changed the peripheral monocyte subset distribution towards the pro-inflammatory classical monocyte subset whereas in the myocardium, presence of the anti-inflammatory macrophage subset was increased at day 3 post-MI. Moreover, IFN-α administration resulted in an increase of necrosis and a decrease in granulation tissue in the infarct area at day 3 in comparison to placebo MI. This could implicate that administration of IFN-α delayed infarct healing, ensuing from a lack of cell debris clearance and replacement with granulation tissue. Overall, these results implicate that elevated type I IFN signalling in circulating monocytes does not reflect systemic elevated type I IFN signalling but above all, underscore the importance of the type I IFN response in cardiac healing post-MI.

In chapter 6, we investigated the effect of auto-reactive T-cells from the adaptive immune response on post-MI cardiac healing in mice and extrapolated it to humans. In mice, it has been shown earlier that lymphocytic CD4+ T-cells substantially influence the post-MI cardiac healing response. In chapter 6 we showed that after MI, CD4+ T-cells in the infarct area and in mediastinal lymph nodes (med-LN) beneficially promote cardiac healing and are specifically activated and recruited by the class-II-restricted cardiac myosin peptide antigen (Myhca), which is released by dying cardiomyocytes. We observed this by using different approaches:

1) We primarily transferred cardiac-myosin-specific CD4+ T cells (TCR-M cells) into mice prior to induction of MI. At 7 days post-MI, we showed that these TCR-M cells homed selectively in the infarct area and med-LN. These activated TCR-M cells acquired a Foxp3+ T-regulatory (Treg) phenotype and exhibited a specific gene expression profile that could promote myocardial healing.

2) To elucidate if endogenous CD4+ T-cells are also autoantigen dependent post-MI, we analysed CD4+ T-cells from the heart and med-LN of wild type mice at day 7 after MI. We determined their T cell receptor (TCR) β chains by sequencing analysis, as the diversity in the TCR chains on the plasma membrane fundamentally determines the functioning of T cells. The endogenous CD4+ T cells from the infarcted heart showed a clonally expansion of TCRβV19, a TCR β chain that is mainly associated with antigen-specific responses. To reveal the identity of this antigen, we additionally analysed the TCR repertoire of CD4+ T-cells
purified from axial LN of mice that were immunized with cardiac antigens. There, we found a similar expansion of the TCRβV19 clone, implicating that infiltrated endogenous CD4+ T-cells in the heart post-MI are indeed dependent on the activation by cardiac antigens.

3) The presence of this post-MI T-cell response was confirmed in humans by showing accumulated CD4+ and Foxp3+ cells in infarcted human myocardial autopsy tissue. Additional non-invasive thoracic PET/CT imaging revealed that MI patients display enlarged med-LNs. Furthermore, a radio-ligand for CXCR4, a receptor that is expressed highly in cardiac endogenous CD4+ T-cells and TCR-M cells in mice, revealed increased T-cellularity in the med-LN of patients. Moreover, we showed that an increase in CXCR4 in med-LN could suggest for a positive prognosis with a smaller infarct area as we observed a negative correlation of CXCR4 in med-LN with infarct volume at 4 months follow up. Thus, taken together, in chapter 6 we provide strong evidence that MI induces a temporary protective T cell auto-immunity in mice, and point to the existence of an analogous physiological heart/med-LN/T cell axis in MI patients.

Cardiac ischaemia induces increased reactive oxygen species (ROS) in the heart. This can cause additional damage and thus could increase the infarcted area. To elucidate one of the pathophysiological mechanisms of the ROS induced cell damage we studied in chapter 7 the role of the forkhead box O (FOXO) transcription factor 1. FOXO1 is a redox sensitive transcription factor critically involved in stress induced cell-fate decisions. The nuclear translocation of FOXO is essential for its activity in response to external stimuli. In chapter 7, we elucidated in cultured rat cardiomyoblasts that ROS produced by p47phox, one of the essential activating subunits of NOX2, are critically involved in the nuclear translocation and thereby activation of FOXO1 during metabolic inhibition, a method to mimic ischaemia in vitro.

To study the inflammatory response following MI, animal MI models are indispensable. However, induction of MI in rodents is generally associated with high mortality due to ventricular fibrillation (VF) during ischaemia. The anaesthetic agent used during the procedure can greatly affect the incidence of VF. In Chapter 8 we therefore evaluated the incidence of ventricular arrhythmias during ligation of the coronary artery to induce MI and its putative effect on infarct size comparing two anaesthetic regimens, sufentanil-medetomidine and fentanyl/fluanisone-midazolam in a rat MI model. This revealed that when using sufentanil-medetomidine, loss of animals is avoided by preventing VF related complications and acute death during coronary artery ligation. The usage of sufentanil-medetomidine is therefore recommended for the induction of experimental cardiac ischemia/reperfusion in rats.
Future directions

In this thesis, we determined the post-MI regulation patterns of the different components of both the innate and the adaptive immune response, including the role of reactive oxygen species herein. Overall, from animal studies it is known that therapeutic agents can decrease the inflammatory monocyte or macrophage subsets early post-MI and thereby diminish an exaggerated acute inflammatory response post-MI and as such ameliorate the functional cardiac output.1-3 In other animal studies where macrophage polarization towards the inflammatory resolution phenotype was stimulated at a later stage post-MI, infarct healing post-MI was improved.4, 5 Thus, modulation of the inflammatory response during different stages post-MI is an appealing concept to ameliorate healing and improve cardiac outcome post-MI. In chapter 2, we show that the biphasic post-MI monocyte infiltration pattern in the heart as observed in animal studies also occurs in humans. For this, it could be proposed that monocyte/macrophage modulating agents that improved post-MI myocardial healing in mice, might also form interesting targets for clinical studies. The most potential targets are discussed below.

One method to control the infiltration pattern of monocyte subsets into the infarcted heart is to intervene with the sources of the monocytes.6 Monocytes are namely produced in haematopoietic tissues and have been described to originate from macrophage and dendritic cell (DC) progenitor cells, although the existence of a monocyte specific progenitor cell has also been proposed.7 Several preclinical studies have focussed on targeting exit of monocytes from the bone marrow towards the circulation, which critically depends on the CC chemokine receptor 2 (CCR2) and its ligand monocyte chemoattractant protein-1 (MCP-1).8, 9 CCR2 also regulates monocyte recruitment into atherosclerotic plaques10, 11 and the ischaemic cardiac tissue12, 13 Targeting CCR2 in MI mice has been shown to result in reducing the migration capacity of bone marrow derived monocytes coinciding with a smaller infarcted area of the heart.2 Monocytes of patients with familiar hypercholesterolemia have been shown to contain an increased expression of CCR2 which coincided with an improved migration capacity in comparison to monocytes of healthy controls.14 Moreover, one study that inhibited the binding of MCP-1 to CCR2 in atherosclerotic risk patients showed a reduction of circulating monocytes15, whereas another study demonstrated an increase of circulating monocyte numbers in healthy volunteers after MCP-1 infusion.16 These results implicate that CCR2 and MCP-1 also regulate the entrance of human monocytes into the circulation and neutralizing CCR2 could potentially positively diminish the circulating monocyte count in inflammatory diseases. The development of CCR2-neutralizing drugs are currently in the pipeline and these would be of interest for further implementation in MI patients.15

Next to recruitment from the bone marrow, we showed in chapter 2 that human monocytes are significantly depleted from the spleen after MI. It has been demonstrated in MI mice that the majority of circulating monocytes that infiltrate the myocardium post-MI are derived from the spleen, as splenectomised MI mice showed no increase in blood monocyte numbers after MI coinciding with a 75% decrease of infiltrated monocytes in the ischaemic myocardium.17 Release of splenic monocytes has been shown to be mediated by angiotensin
(Ang) II as Ang II-receptor knock-out mice showed less monocytes egress from the spleen and reduced monocyte accumulation in the infarcted myocardium after MI compared to controls. However, the exact contribution of this rapid release of splenic monocytes in relation to cardiac functioning remains to be determined. It would therefore be necessary to further elucidate the mechanisms that regulate storage, production and release of splenic and bone marrow monocytes together with their accumulation pattern after MI in patients and their ultimate effect on cardiac functioning.

Another method to beneficially modulate the monocyte/macrophage subsets after MI could be through the application of mesenchymal stem cells (MSC). Once engrafted, MSC were thought to differentiate into new functioning cardiomyocytes, however, several clinical trials have shown beneficial effects of MSC on cardiac function post-MI with minimal engraftment of MSC in the infarcted myocardium. Interestingly, as reviewed in chapter 3, an increasing number of studies emphasize that the beneficial effects of MSC therapy on post-MI cardiac function are designated to their immunomodulatory effects through paracrine mechanisms. MSC can namely inhibit active inflammatory substances such as MCP-1 and thereby reduce the acute inflammatory response after MI, initiating the resolution of inflammation that will favour post-MI cardiac functioning. Next to the switch of macrophages towards the more anti-inflammatory subsets, MSC have also been shown to regulate other immune cells and for instance can inhibit the proliferation of T-cells or the maturation of dendritic cells. MSC can even promote angiogenesis through the release of vascular endothelial growth factor. In MI, this caused a beneficial decrease of the inflammatory cell infiltrate in the infarct area that coincided with an increased neovascularisation, improving post-MI cardiac healing. Thus, MSC therapy appears to be an interesting strategy to beneficially modulate the post-MI immune response through both the reduction of acute inflammation and the promotion of cardiac healing processes. It would then be important for clinical trials to also focus on these paracrine mechanisms of MSC and its subsequent effect on cardiac healing. In this way MSC could be applied more effectively to beneficially promote post-MI cardiac healing.

Direct targeting of specific monocyte subset after MI could also be an interesting strategy to influence the activity of a specific monocyte subset. We propose that NOX4 protein would be a promising target herein. We namely show in chapter 4 that expression of the NOX4 protein isoform is localised differently in monocyte subsets of healthy volunteers. We found NOX4 expression on the plasma membrane in the pro-inflammatory classical and intermediate monocytes whereas the anti-inflammatory non-classical monocytes showed cytoplasmic NOX4 expression. Thus, affecting the localisation of NOX4 could theoretically contribute to inducing a switch in the monocyte subsets, which could also affect the functioning of monocytes. Indeed, plasma membrane NOX4 expression in monocytes has been suggested to critically mediate the monocyte migration capacity and a reduction in the migration of the pro-inflammatory classical monocytes into the infarcted heart early after MI have been suggested to improve post-MI cardiac functioning. However, additional studies are needed to further determine the functional role of NOX4 in the different monocyte subsets and the possibilities of targeting NOX4 in monocyte subsets after MI.

In the current thesis, we are one of the first to demonstrate the influential role of type I
interferon (IFN) signalling during post-MI cardiac healing. In chapter 5, we showed that induction of type I IFN signalling in human monocytes coincides with a decrease in adverse LV remodelling. In rats however, administration of the type I IFN-α deteriorated post-MI cardiac healing resulting in an increased infarct size together with ventricular dilatation, although we could not determine the exact mechanism by which type I IFN affects post-MI cardiac healing. Our results could suggest that elevated type I IFN signalling in monocytes does not reflect systemic elevated type I IFN signalling. Interestingly, a recent study in mice suggests that after MI, cardiac macrophages that phagocytosed cellular debris detrimentally induce gene expression of type I IFN related genes and thereby induces an exaggerated type I IFN response which contributes to increased ventricular dilatation and mortality of the mice after MI.25 Exaggerated IFN responses are increasingly associated with human autoimmune diseases and the concentration and duration of IFN production together with specific timing of action can be crucial for accurate IFN functioning.26, 27 Based on the preclinical results, it would be important to further focus on the role of systemic type I IFN during cardiac healing after MI and to evaluate the effects from different systemic levels of type I IFN on cardiac functioning in MI patients. Subsequent investigation on the timely control of type I IFN signalling following MI is certainly required to further elucidate if and how type I IFN should be influenced in MI patients to promote post-MI tissue repair.

Many studies, including those in the current thesis, have showed the important contribution of the innate immune system during cardiac healing after MI. Recently, the beneficial contribution of T-lymphocytes from the adaptive immune response during the processes of cardiac healing is also starting to be recognized.5, 28 In chapter 6, we showed in mice that after MI, cardiac antigens activate CD4+ T-cells in mediastinal lymph nodes (med-LN) which infiltrate into the infarcted heart and polarize towards a non-classical CD4+T cells phenotype and promote the resolution of inflammation and benefit cardiac post-MI healing. Also, we addressed the clinical translational perspective and reported for the first time that this heart/med-LN T cell axis also exists in MI patients and that an increased CD4+ T-cellularity in med-LN could be indicative for a positive prognosis for post-MI cardiac healing. Thus, imaging the heart-draining LNs and its T-cellularity component could provide additional valuable clinical information after MI and might even be a mechanistic tool to modify T-cell directed therapeutic interventions in the future. However, it has also been reported in mice that CD4+ T cell activation in the myocardium can contribute to the development of adverse remodelling after ischaemia29 or stress overload induced heart failure.30 Currently, the antigen specificities of the CD4+ T cells that contribute to dysfunctional myocardial remodelling later on and which factors control this shift remains unclear.29 It would be interesting to estimate these differences to elucidate how particularly the post-MI CD4+ T-cell response should be modified to favour post-MI cardiac functioning.

Next to the involvement of the immune system, we focussed in chapter 7 on oxidative stress induced cellular damage of cardiomyocytes following ischaemia. The redox transcription factor FOXO1 has been suggested to be critically involved in the induction of apoptosis through nuclear transportation of FOXO1. In chapter 7, we showed that reactive oxygen species (ROS) produced by NOX2 in particular, are critically involved in the nuclear translation and thereby activation of FOXO1. Thus, as both NOX2 and FOXO1 appeared to
be crucial in the oxidative stress response of cardiomyocytes, it would be interesting for future studies to elucidate the exact function of FOXO1 during post-MI cardiac healing and how to beneficially modulate its activation after MI. Hypothetically, targeting FOXO1 specific or the components involved in its nuclear translocation might critically influence oxidative stress related apoptosis of cardiomyocytes after ischaemia.

To avoid unnecessary loss of animals in studies that analyse the inflammatory response after MI, we recommended in chapter 8 to use a mixture of sufentanil-medetomidine for the induction of MI. Sufentanil-medetomidine significantly repressed the incidence of ventricular fibrillation (VF) during ischaemia and reduced the mortality during ischaemia. The mechanisms of action of sufentanil-medetomidine on VF during ischaemia still remain to be elucidated. It would also be of interest to study its potential for MI induction in other animal species.

Concluding remarks

Overall, this thesis revealed the important involvement of cells from both the innate and the adaptive immune response during post-MI cardiac healing. We elucidated several promising aspects by which monocytes, macrophages, CD4+ T cells but also oxidative stress challenged cardiomyocytes could be modulated to beneficially improve post-MI cardiac healing to diminish putative cardiac failure. Accurate intervention is highly dependent on timely modulation of the immune cells. This intervention namely should not interfere with the immune response required for proper healing but at the same time should prevent detrimental aspects of the immune response that deteriorate post-MI cardiac healing. Future studies performed in MI patients should reveal which strategies, that ameliorated cardiac healing in experimental studies, are useful to translate into clinical settings. Hopefully, the studies described in the current thesis will contribute to the development of new studies that can establish promising therapeutic targets which eventually could diminish the development of cardiac failure after MI.

References


