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

ter Horst, E.N.

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.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
1

General introduction & Outline of the thesis

Text partly adapted from:

*Modulators of Macrophage Polarization Influence Healing of the Infarcted Myocardium*

Ellis ter Horst, Nazanin Hakimzadeh, Anja M van der Laan, Paul AJ Krijnen, Hans WM Niessen, Jan J Piek

International Journal of Molecular Sciences

(2015)16; 29583-29591
CHAPTER 1

Introduction

In the Netherlands, currently over 300,000 people suffer from the consequences of an acute myocardial infarction (MI) and eventually develop severe chronic heart failure (source: the Dutch Heart Foundation). Worldwide, cardiovascular disease remains the leading cause of death over the past 15 years.1 These numbers reflect the continuous necessity for cardiovascular research to improve treatment options, which was the primary aim to start the current thesis.

MI is most often caused by the rupture or erosion of an atherosclerotic plaque in one of the coronary arteries. This can lead to total artery occlusion and subsequent ischaemia of the myocardial tissue distal to the occluded area. The damaged myocardial area then culminates in apoptotic or necrotic cell death of cardiomyocytes which triggers the immune system to eliminate and replace the damaged cells with scar tissue. Accumulating evidence emphasize that an exaggerated post-MI immune response directly negatively affects the prognosis after MI.2, 3 These studies emphasize the important and influential role of inflammatory cells following MI and also suggest that increased understanding of the immune response following MI could reveal potential therapeutic targets that ameliorate cardiac healing and functional outcome.

The studies described in the current thesis therefore aimed to clarify important aspects of the immune response following MI in both clinical and experimental settings to elucidate therapeutic opportunities that may beneficially influence post-MI cardiac healing.

Inflammation during post-MI cardiac healing

The most important in hospital therapeutic intervention following MI is to re-open the occluded coronary artery and thereby initiate reperfusion of the ischaemic myocardium. On the one hand, reperfusion leads to restoration of blood supply towards the injured myocardium, but on the other hand easy access is provided for a wide variety of cells of the immune system can then also infiltrate the myocardium which can cause additional injury referred to as reperfusion injury.4

The immune system is subdivided into the innate immune system, readily present to directly mediate blunt protection, and the more specific adaptive immune system, which is more slow but also more specialized and effective. Following MI, the cardiac healing response can also be divided into subsequent, partly overlapping phases, all characterized by a more or less typical infiltrate of inflammatory cells.5, 6 In Figure 1, macroscopic and microscopic images of infarcted human myocardial tissue specimens in the different phases are shown. At autopsy, infarcted areas of the heart are primarily macroscopically identified using nitro blue tetrazolium (NBT) decolouration, indicative for jeopardized cardiomyocytes that show loss of lactate dehydrogenase (LDH) (Figure 1A). Subsequently, microscopic criteria are used to estimate the age of infarction.7

Early after MI, from around 2 hours - 6 hours following MI, macroscopic LDH loss can be detected but no microscopic changes of the myocardium are observed (Figure 1C). Subsequently, from 6 hours – 5 days following MI, (Figure 1D), the innate immune system
including neutrophilic granulocytes is immediately triggered in response to ischemic injury. In this phase, irreversibly damaged cardiomyocytes are eliminated and extracellular matrix degradation is promoted and simultaneously, inflammation induced cell damage of cardiomyocytes occurs (41). Thereafter, from 5 days - approximately 2 weeks after MI, (Figure 1E), the adaptive immune system dominates, consisting of mainly lymphocytes and macrophages. Cytokines and growth factors are produced that particularly repress inflammatory signals and regulate granulation tissue formation. This phase is mainly associated with processes involved in ventricular remodelling, such as matrix deposition and generation of a microvascular network. Finally, after more than 2 weeks following MI, vascular cells and fibroblasts undergo apoptosis resulting in the maturation of a collagen-based scar.

During the different phases of healing post-MI, a wide range of inflammatory cells are involved that each maintain specific processes that are all needed to provide sufficient healing. Amongst these cells, it has been shown that monocytes, macrophages, CD4+ T-lymphocytes and the coinciding production of reactive oxygen species and lytic enzymes in particular essentially influence the progression of post-MI cardiac healing. Hence, negative disturbance and/or exaggerating of their activities can greatly influence the development of subsequent cardiac failure. The current thesis therefore focussed on unravelling the regulation patterns of these inflammatory processes post-MI in particular and how to potentially beneficially manipulate their processes.

Figure 1. Examples of post-MI infarcted areas in time
A) Infarcted area is visualised macroscopic using NBT decouloration to reveal LDH loss (white dotted area).
B) Example of an H&E stain of normal myocardium. (C) The early phase shows macroscopic NBT decolourisation but no microscopical changes. (D) The inflammatory phase, 12 h – 5 days after MI, is mainly characterized by infiltration of neutrophilic granulocytes in the infarcted area (blue). E) In the proliferation phase, 5–14 days after MI, the formation of granulation tissue is mainly visible. Nuclei are stained blue. Black scale bare indicates 200 µm. NBT=nitro blue tetrazolium; LDH=lactate dehydrogenase, H&E=hematoxyline and eosin; MI=myocardial infarction.
CHAPTER 1

Monocyte and macrophage subsets

Monocytes and their descendant macrophages have the ability to both stimulate and repress inflammation using different phenotypes. Monocytes originate from progenitors cells in the bone marrow and are released into the peripheral blood where they circulate before being recruited into inflamed tissues. The circulating population of human monocytes are a heterogeneous pool of cells that display different receptor repertoires allowing them to be mobilized selectively. Discrimination of the different subsets is mostly based on the expression of cell surface receptor markers CD14 and CD16 (Figure 2). The classical monocytes are defined as CD14++CD16-, intermediate monocytes are identified as CD14++CD16+ and non-classical monocytes as CD14-CD16+ as presented in Figure 2.

Tissue macrophages are generally subdivided into two well-established polarized phenotypes, namely the classically activated M1 macrophages and alternative activated M2 macrophages. The M2 macrophage is a collective term to define multiple forms of macrophage activation (M2a, M2b and M2c) which are alternatively activated as compared to M1 macrophages and are all involved in the adaptive immune responses.

It remains disputable how monocyte subsets relate to the differentiation of macrophage subsets once they enter the extravascular space. It has been described in mice that following accumulation of classical monocytes in inflammatory sites, this subset often differentiates into M1-like macrophages. However, it has also been demonstrated in mice that following recruitment of classical monocytes into tissue, these classical monocytes can switch their phenotype to obtain an anti-inflammatory profile and eventually differentiate into M2-like macrophages or even retain their monocyte phenotype.

Figure 2. Distinction between circulating human monocyte subsets

Post-MI cardiac infiltration of monocytes and macrophages

During the first few days post-MI, it has been established by preclinical studies that classical monocytes and M1 macrophages dominate the cellular infiltrate together with neutrophilic granulocytes, to clear cellular debris. The monocytes and macrophages subsequently secrete cytokines, chemokines and growth factors that influence the consequent phases of healing and initiate tissue regeneration coordinated by non-classical monocytes and M2 macrophages that promote healing and attenuate inflammatory processes.

Although preclinical MI models are also essential to elucidate the post-MI cardiac infiltration pattern of inflammatory cells, substantial differences exist between experimental MI and the circumstances of MI patients. Most importantly, patients primarily develop atherosclerosis preceding a spontaneous infarct which already activates several inflammatory cells, whereas experimental MI is generally induced by mechanic coronary artery ligation in relatively young and healthy animals. Not to mention other co-morbidities that exist in individual patients which are too complex to incorporate in experimental settings. It is therefore indispensable to additionally study the infiltration patterns of the key inflammatory cells in human MI material before evaluating new therapeutic opportunities.

Altered monocyte/macrophage levels and post-MI cardiac healing

Experimental MI studies have demonstrated that a prolonged presence of M1 macrophages extends the pro-inflammatory environment and causes an expansion of the infarcted area post-MI. This then hindered the formation of scar tissue by a delayed transition to M2 macrophages, predisposing heart failure development due to alterations in the size, shape, physiology and volume of the left ventricular (LV), often referred to as adverse LV remodelling (Figure 3). Moreover, high levels of circulating classical monocytes has also been shown to greatly correlate with impaired cardiac healing and subsequent heart failure development in MI patients.
Experimental studies that target the inflammatory classical monocytes or the M1 macrophages post-MI and thereby diminish the duration of the initial highly inflammatory phase, have shown to improve cardiac output post-MI.\textsuperscript{14, 16, 17} Additionally, animal studies wherein macrophage polarization towards the M2 phenotype was stimulated have shown to promote resolution of inflammation and improve infarct healing post-MI.\textsuperscript{18, 19} Therefore, the amelioration of post-MI healing by modulating the polarization of monocyte/macrophage subtype is an appealing concept to beneficially influence healing following MI.\textsuperscript{17, 18, 20} However, effective therapeutic targets that beneficially influence the post-MI monocyte response in clinical settings has not yet been developed. It is therefore of interest to perform different approaches to unravel specific monocytes characteristic involved in subset differentiation or that are related to poor cardiac outcome following MI. This could reveal interesting targets that could be addressed to beneficially affect post-MI cardiac healing.\textsuperscript{21}

**Lymphocytic CD4$^+$ T-cells**

Next to monocytes and macrophages, the lymphocytic CD4$^+$ T-cells from the adaptive immune response have been shown to be activated post-MI and subsequently substantially influence the post-MI cardiac healing response.\textsuperscript{19, 22} A study in mice showed that T-regulatory cells (Tregs) in particular, which are a subset of CD4$^+$ T-cells that express the forkhead box P3 (FoxP3) transcriptional regulators, can be therapeutically activated in mice and thereby ameliorate post-MI cardiac healing.\textsuperscript{23} Tregs are generally involved in the suppression and regulation of innate immune responses in wound healing following injury.\textsuperscript{24, 25} In mice, post-MI expansion of Tregs has been shown to result in increased M2 macrophage associated mRNA levels in the infarcted myocardium at 5 days post-MI and an increased expression of myocardial healing contributors.\textsuperscript{19, 26} This implicates a potential role for Tregs in macrophage polarization and beneficial transition of the inflammatory phase towards the proliferation phase post-MI. However, it has not yet been established how the post-MI T-cell responses are driven and if there are relevant autoantigens that drive this response. Additionally, knowledge about the CD4$^+$ T-cells infiltration pattern in MI patients is still lacking which is crucial to estimate the influence of Tregs on cardiac post-MI healing in patients.\textsuperscript{27}

**Reactive oxygen species**

Reactive oxygen species (ROS) are produced in mammalian cells in response to a multitude of physiological conditions.\textsuperscript{28} It has become recognized that ROS can act as second messengers in cellular signaling through altering the activity in signal transduction proteins trough the reversible oxidation of cysteine residues.\textsuperscript{29, 30} However, after prolonged ischaemia ROS are produced excessively, resulting in oxidative stress and thereby the induction of apoptosis/necrosis and the release of pro-inflammatory cytokines.\textsuperscript{31, 32} Hence, excessive ROS production post-MI can contribute to a deterioration of the myocardial healing process. Additionally, restoration of the blood supply by in-hospital percutaneous coronary intervention therapy post-MI also triggers increased ROS production resulting in additional post-MI cardiomyocyte damage.\textsuperscript{33, 34} Several studies have suggested that the scavenging of ROS following ischaemia/reperfusion either through delivery or overexpression of antioxidants could reduce myocardial
damage, however no successful therapeutic target has yet been developed.\textsuperscript{35-39}

An important source of ROS are NOX proteins, which are shown to be highly upregulated in the myocardium following MI.\textsuperscript{40} NOX are important in the functioning of monocytes, however information about the exact subcellular localization and expression pattern of NOX isoforms in different monocyte subsets are lacking even though this could influence the different monocyte subsets effector functions. In cultured ischaemic cardiomyocytes, nuclear NOX2 derived ROS was implicated in mediating cellular apoptosis.\textsuperscript{41} Moreover, it has been demonstrated that NOX2 is upregulated in the human myocardium following MI \textsuperscript{40} and knock down of NOX2 in mice following MI has been shown to ameliorate cardiac functioning.\textsuperscript{20, 42} However, the exact regulation of the subcellular components that activate the NOX2 protein in ischaemic cardiomyocytes remains largely unknown which is of interest to unravel for the development of therapeutic targets that control the post-MI oxidative stress response.

**Outline of the thesis**

Infiltration of both innate and adaptive inflammatory cells following an acute myocardial infarction (MI) can greatly determine post-MI healing process and functional recovery. However, information regarding their infiltration pattern in infarcted human tissue material was lacking so far. In the current thesis we addressed this issue and evaluated the infiltration pattern of monocytes subsets over time following MI in **chapter 2**, together with their potential sources of recruitment.

Polarization of monocyte/macrophage subsets is an appealing concept to ameliorate post-MI healing. Mesenchymal stem cells (MSC) have initially been suggested to regenerate cardiac tissue and thereby diminish scar tissue formation. However, increasing studies suggest that MSC improve post-MI cardiac functioning through modulation of monocyte/macrophage subsets. Studies that focussed on these paracrine effects of MSC during healing of the infarcted myocardium are therefore reviewed in **chapter 3**. Additionally, alterations in post-MI monocyte subset levels or myocardial recruitment has been shown to highly associate with post-MI cardiac outcome. To target monocyte subsets, specific subset characteristics needs to be further identified. As NOX are important proteins for the functioning of the different monocyte subsets, we elucidated the expression pattern of NOX proteins in the different human monocyte subsets of healthy volunteers in **chapter 4**. Additionally, we investigated and linked gene transcripts of monocytes to adverse LV remodelling in MI patients in **chapter 5** and subsequently evaluated intervention with these characteristics in a rat MI model.

It has also been demonstrated in mice that MI triggers CD4+T-cell activation, which then can modulate post-MI inflammation, healing, and remodelling. To increase insight into the post-MI CD4+ T-cell response, we investigated in **chapter 6** if the post-MI T-cell responses are antigen-driven and which autoantigens are relevant, together with the infiltration pattern of CD4+ T-cell subsets into the myocardium of MI patients.

Post-MI oxidative stress by excessive ROS production highly contributes to increased
cellular damage. In chapter 7, we investigated in cultured cardiomyoblast the intracellular activation pattern of NOX2, the NOX isoform that largely accounts the ROS production during ischaemic conditions.

Additionally, to stimulate future research on the inflammatory response following MI, we evaluated and described the efficiency of two experimental rat MI models in chapter 8.

Finally, the findings and conclusions of the current thesis are summarized and discussed in chapter 9 together with suggestions for future directions in research on the inflammatory response following MI and plausible therapies to diminish the development of post-MI heart failure.

References


