The Inflammatory response in myocarditis and acute myocardial infarction

Emmens, R.W.

Citation for published version (APA):

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: https://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.
Chapter 1

General Introduction
INTRODUCTION

Worldwide cardiovascular disease (CVD) is a major cause of mortality and morbidity, in which acute myocardial infarction (AMI) is playing an important role. Over the years it has become clear that myocarditis, an inflammatory condition of the heart mostly related to a viral infection, is playing a considerable role in CVD morbidity and mortality as well. In both myocarditis and AMI, inflammation is essential in the development of the disease, but also in its outcome. This thesis encompasses eight original studies related to myocarditis and AMI. The aim of this general introduction section is to give a summarized overview of myocarditis and AMI and to provide the rationale for each study.

MYOCARDITIS

The term ‘myocarditis’ literally means ‘inflammation (-itis) of the heart muscle (myocardium)’. Myocarditis as a clinical entity is a cardiomyopathy (disease of the myocardium associated with cardiac dysfunction), and in the context of cardiomyopathies is categorized as ‘inflammatory cardiomyopathy’.1 Myocarditis is primarily recognized by its distinct histopathological appearance in the myocardium, which was defined by Aretz et al. as: ‘Inflammatory infiltrates within the myocardium associated with myocyte degeneration and necrosis of non-ischaemic origin’.2 In Western Europe and North America, myocarditis is most often caused by a viral infection (viral myocarditis), although a wide array of other aetiologies have been described, including bacterial infection, fungal infection, protozoal infection, stress, hypersensitivity reactions, metal poisoning, adverse drug reactions and autoimmune disorders.3,5 This wide array of aetiologies also results in several distinctive patterns of inflammatory cell infiltrations of the heart, by which myocarditis is often subcategorized.

Disease progression of viral myocarditis

Viral myocarditis is not only the most common form of myocarditis, but also the most widely investigated. It is generally accepted that both environmental (culprit virus) and genetic factors influence the way viral myocarditis manifests itself, although the precise underlying mechanisms are not yet fully determined.3,6 Coxsackievirus B3 (CVB3) is one of the more common viruses associated with myocarditis.3 CVB3-induced myocarditis is a widely used mouse model to study disease mechanisms of viral myocarditis. A simplified representation of viral myocarditis, largely based on knowledge from CVB3-induced myocarditis models3, 7, 8, is displayed in figure 1. In the acute phase, viral infiltration and replication occurs in the myocardium, resulting in cell death of cardiomyocytes, although this can be quite limited. This triggers activation of macrophages, followed by infiltration of natural killer cells and lymphocytes (the subacute phase), after which the virus and the necrotic cardiomyocytes are eliminated.
In patients, several clinical scenarios are known to follow after the (sub)acute phase of viral myocarditis.\textsuperscript{5}

In some patients, the inflammation subsides following viral clearance and the myocardium restores to its normal situation (recovery). In other patients, the virus does not get cleared, resulting in a chronic viral infection (viral persistence). Finally, in some patients molecular mimicry occurs. Here, antibodies created to bind viral proteins cross-react with endogenous cardiac proteins such as α-myosin, resulting in a chronic autoimmune response targeting cardiomyocytes (autoimmune myocarditis).\textsuperscript{9} Viral persistence and autoimmune myocarditis phases can result in several different clinical complications (see below).

**Diagnosis of myocarditis**

Myocarditis is a notoriously difficult condition to diagnose. The clinical symptoms vary greatly per patient, ranging from asymptomatic disease to heart
failure symptoms such as chest pain, palpitations, exercise intolerance, fatigue and shortness of breath. Often, symptoms suggestive of viral infection are found, including fever, muscle/joint pain and respiratory symptoms. Myocarditis can also result in alterations of the electrocardiogram (ECG: usually ST-elevation or T-wave inversion\(^\text{19} \text{10}\)) or an increase in serological markers (\text{i.e.} Cardiac Troponins\(^{11} \text{12}\)). However, ECG and serology lack sufficient sensitivity and specificity to provide a clear diagnosis of myocarditis.

Cardiac magnetic resonance imaging (CMR) is becoming a standardized part of the diagnostic process for myocarditis. Combining different CMR techniques (T2-weighted imaging, late gadolinium enhancement and global relative enhancement) has been demonstrated to diagnose myocarditis with 85% accuracy.\(^{13}\) Even though CMR is a rapidly advancing field, there are still some limitations. For instance, borderline myocarditis (an increase of inflammatory cells without cardiomyocyte damage or a limited increase of inflammatory cells) is difficult to detect using CMR.\(^{14}\) Therefore, analysing cardiac tissue samples, obtained via an endomyocardial biopsy (EMB), remains the golden standard to determine the underlying cause of the disease.\(^{14}\) Current guidelines require \(>14\) inflammatory cells per mm\(^2\) tissue to confirm myocarditis.\(^{15}\) However, inflammatory cells are often present in the myocardium as focal pockets and random tissue samples may miss such pockets, resulting in a false negative diagnosis. Furthermore, EMB is an invasive procedure with the risk of complications like ventricular wall perforation, arrhythmia and damage to the heart valves.\(^{16}\) Several case reports have demonstrated that cardiotropic viruses can infect not only cardiac but also skeletal muscle tissue.\(^{17} \text{18}\) In \textbf{chapter 2}, we have investigated quadriceps muscle tissue analysis as alternative for EMB in lymphocytic myocarditis patients.

\textbf{Clinical complications of myocarditis}

Inflammatory damage to the myocardium as result of myocarditis can result in different complications. On the long term it might result in a so-called dilated cardiomyopathy (DCM).\(^{19}\) In DCM, the loss of cardiomyocytes and the coinciding increase in cardiac fibrosis is so extensive that it results in a decreased contractility of the heart and thus heart failure.\(^{20}\) Despite advances in heart failure therapeutics, DCM remains a common cause for cardiac transplantation,\(^{21}\) with a population-wide transplant-free survival rate after 4 years of 88%.\(^{22}\)

A more acute complication of myocarditis is sudden cardiac death (SCD). SCD is defined as a death that occurs within one hour of symptom onset. Especially in young patients (<35 years old), myocarditis is a common cause of SCD.\(^{23}\) SCD is mostly attributed to ventricular arrhythmia,\(^{24}\) although it has also been related to atrial fibrillation (AF).\(^{25}\) It is unknown however what the mechanisms are that underlie the incidence of AF in myocarditis patients. In \textbf{chapter 3}, we have studied atrial inflammation in myocarditis patients as a possible underlying cause of AF.
Treatment of myocarditis

Treatment of myocarditis is dependent on the clinical symptoms of the particular patient. Patients with heart-failure symptoms are treated with a standard heart failure treatment regimen, which usually consist of angiotensin converting enzyme (ACE) inhibitors and beta(β)-blockers.26 ACE inhibitors inhibit the renin-angiotensin system, which results in reduced activity of the sympathetic nervous system, vasoconstriction and a reduction in adverse cardiac remodeling.27 β-blockers block adrenergic stimulation of beta-adrenergic receptors in the heart, resulting in a decreased heart rate.28 Acute myocarditis patients with ventricular arrhythmia or AF are treated according to standardized anti-arrhythmogenic treatment protocols.29 30 Anti-arrhythmogenic drugs generally interfere with ion channels on atrial cardiomyocytes, increasing electrical excitability and prolonging action potential length, thereby reducing the chance of abnormal signal transduction across the tissue.

Even though these treatment regimens serve as a symptom reliever and prevent further decline of cardiac function, they do not target the underlying disease. Therefore, the potential application of anti-inflammatory therapy in myocarditis patients is a widely researched topic. Lymphocyte inhibitors such as prednisone and azathioprine have shown promising results in clinical trials. However, the efficacy of these compounds is largely diminished when myocarditis is accompanied by an ongoing viral infection.31 33 An anti-inflammatory agent that can be applied despite an ongoing viral infection would be highly beneficial for the management of myocarditis. Clinical trials on pericarditis, a disease which is also often caused by viral infection,34 have indicated that colchicine is an effective treatment in pericarditis patients.35 Colchicine prevents polymerisation of microtubules, thereby interfering both with inflammatory cell migration across the endothelial cell barrier, as well as the release of inflammatory cytokines from macrophages.36 In chapter 4, we have studied whether colchicine is also effective in the treatment of acute CVB3-induced myocarditis in mice.

ACUTE MYOCARDIAL INFARCTION

In Europe, acute myocardial infarction (AMI) is responsible for 20% of all deaths.37 In AMI, part of the coronary circulation is obstructed, resulting in cardiac ischemia. This is usually caused by thrombus formation in one or more of the coronary arteries, in majority triggered by the rupture of an unstable atherosclerotic plaque.38 Alternatively, the coronary circulation can also be dysfunctional as result of vasospasm or coronary embolism.39
Post-AMI inflammation and remodelling.

The histopathological alterations in the human myocardium following AMI have been described in detail already in the 1930s. Based on histopathology, AMI can be divided in three different phases (figure 2A). The acute phase (0-12 hours after AMI) is the period of cardiac ischemia immediately following the occlusion of a coronary artery. In the core of the ischemic area of the myocardium, cardiomyocytes become irreversibly damaged, forming a necrotic infarct core. Blood supply to the ischemic areas can be restored with reperfusion therapy (see following section) or it restores naturally due to the outgrowth of pre-existing collateral arteries (arteriogenesis), or by natural thrombolysis. This restoration of blood flow coincides with extravasation of inflammatory cells, which infiltrate the infarcted area. Initially, the inflammatory infiltrate consists of neutrophils. Later on monocytes/macrophages and lymphocytes also appear. The neutrophil infiltration hallmarks the start of the polymorphonuclear leukocyte (PMN) phase (12 hours – 5 days after AMI). The inflammatory response of the PMN phase is needed for clearance of necrotic cell debris and the initiation of reparative pathways. However, simultaneously the inflammatory response also results in enlargement of the necrotic infarct core partly through production of reactive oxygen species and lytic enzymes, and via pro-inflammatory changes of the plasma membrane of cardiomyocytes, which especially occurs in the border zone of the infarction area, and as such increases the area of cell death (figure 2B). In the chronic phase (5-14 days after AMI), the inflammation subsides and cardiac fibroblasts turn into myofibroblasts, producing extracellular matrix proteins that stimulates the formation of a collagen scar in the regions of cardiomyocyte loss.
Treatment of acute myocardial infarction

An important aspect of post-AMI treatment is reperfusion therapy, which limits the ischemic damage to the myocardium. Artificial reperfusion can be achieved either by enzymatically dissolving the blocking thrombus (thrombolytic therapy\(^{45}\)), mechanical clearance of the obstruction (coronary angioplasty\(^{46}\)) or creating a bypass around the obstruction (coronary artery bypass grafting\(^{47}\)). Although developments in reperfusion therapies have decreased AMI-related mortality, heart failure remains a common complication of AMI at present day.\(^{48}\) As post-AMI inflammation results in infarct size expansion and an increased risk of post-AMI complications such as heart failure, a large amount of research has been carried out on inhibiting post-AMI inflammation as therapeutic strategy. Anti-inflammatory therapy is not yet part of common clinical practice, although a number of therapeutic compounds have been studied thus far in clinical trials. These include glucocorticoids (inhibits NF-\(\kappa\)B and MAPK-mediated production of pro-inflammatory cytokines), non-steroidal anti-inflammatory drugs (NSAIDs, inhibit Cyclooxygenase-2-mediated production of pro-inflammatory cytokines), disabling antibodies targeting Interleukin(IL)-1\(\beta\) or Tumour Necrosis Factor(TNF)-\(\alpha\), and inhibitors of the complement system.\(^{49}\)

The complement system

An important component of the post-AMI inflammatory response is the complement system. The complement system is part of the humoral immune response and forms a cascade network consisting of over 30 extracellular and membrane-bound proteins.\(^{50}\) Complement components are found in the necrotic infarct core specifically during the PMN phase (figure 3).\(^{52}\) Complement activation here appears to be tightly regulated. We and others have found C-reactive protein, IgM\(^{52}\) (activate the classical complement pathway), C4b-binding protein\(^{53}\) (induces dissociation and decay of C3b and C4b), clusterin, vitronectin and C8-binding protein\(^{54}\) -\(^{55}\) (prevent assembly of the membrane-attack complex) present in the infarcted human myocardium at the same time and location as complement activation products. Despite the presence of endogenous inhibitors, activation of the complement system results in lysis of damaged cardiomyocytes through the membrane-attack complex, and production of anaphylatoxins. In the context of AMI, anaphylatoxins (C5a in particular) in the infarcted myocardium are considered primarily to function as a chemoattractant for neutrophils, stimulate neutrophil production of reactive oxygen species (ROS) and pro-inflammatory cytokines and stimulate leukocyte extravasation by up regulation of adhesion molecules on vascular endothelial cells (figure 3).\(^{56}\)

In 1971, a landmark paper was published which demonstrated that disabling the complement system significantly decreases AMI-related damage to the myocardium.\(^{57}\) This discovery triggered a great deal of interest in the complement system as possible therapeutic target for AMI patients. While showing promising results in animal models, the effect of complement inhibitors
in clinical trials was limited. In chapter 5, a systematic overview of studies investigating various complement inhibitors as treatment for AMI in both animal models and clinical trials is discussed.

C1-inhibitor
One of the complement inhibitors of interest is C1-inhibitor (C1-inh). C1-inh is an endogenous plasma protein which is primarily synthesized in hepatocytes, but can also be produced by other cell types, including monocytes, fibroblasts and endothelial cells.\textsuperscript{58} Besides inhibiting complement activation, C1-inh is also known to inhibit the coagulation system and has been suggested to interfere with leukocyte binding to endothelial cells.\textsuperscript{59} As mentioned above, a number of
endogenous complement activators and inhibitors have been described to coincide with complement in the post-AMI myocardium, indicating their involvement in post-AMI complement regulation. Even though C1-inh is a well-known complement inhibitor, it has never been investigated whether endogenous C1-inh would also play a role in post-AMI complement regulation. In **chapter 6** we have studied the production and expression of endogenous C1-inh in the heart following AMI.

In clinical practice, plasma-derived human C1-inh is used as therapy for patients with hereditary angioedema (HAE), which is caused by a genetic deficiency in functional C1-inh.\(^6^0\) C1-inh is administered in those patients intravenously. In the past several years the possibility of subcutaneous C1-inh administration was also approached, as this would make it easier for patients to self-administer C1-inh.\(^6^1\)–\(^6^2\) For rodent models, subcutaneous administration would also be beneficial, as repeated intravenous injections causes considerable damage to the tail veins. In **chapter 7** we have therefore compared subcutaneous C1-inh administration with intravenous C1-inh administration in rats.

**Acute myocardial infarction and atrial fibrillation**

Similar to myocarditis (see above section ‘clinical complications of myocarditis’), AMI is a known precursor of AF.\(^6^3\) Unknown is whether AMI coincides with atrial inflammation. In **chapter 8**, we have studied if AMI coincides with atrial inflammation in patients. At the same time we have studied whether C1-inh administration would have an effect hereon in a rat AMI model.

**Adipose-derived stem cells**

Reperfusion therapy and anti-inflammatory drugs can limit post-AMI damage to the myocardium, but they cannot restore lost cardiomyocytes. In recent years studies have been published in which the effect of stem cell therapy was analysed in AMI. Adipose tissue derived stem cells (ASCs) theoretically have the potential to repair lost tissue,\(^6^4\)–\(^6^5\) as ASCS can differentiate into cardiomyocytes.\(^6^6\) In addition, ASC can secrete cytokines that inhibit inflammation, such as IL-10 (stimulates regulatory T-lymphocytes) and Galectin1/3 (deactivates inflammatory monocytes/macrophages and lymphocytes).\(^6^7\) In addition, ASCs can limit the amount of permanently damaged myocardium by production of vascular endothelial growth factor (VEGF, stimulates neovascularization) and mobilization of local cardiomyocyte progenitor cells.\(^6^8\)

An unresolved problem of ASC therapy however is the low amount of ASCs that reach to infarcted myocardium following administration. For this, we have previously developed a technique called ‘StemBells’-technique (figure 2C). For this, ASCs are coupled to gas-filled microbubbles. These microbubbles are used to target the ASC-microbubble complex to the infarct area in the heart by binding an antibody to the microbubble that is directed against intercellular
adhesion molecule 1 (ICAM-1), which is present on activated endothelial cells and cardiomyocytes in the infarcted myocardium.\textsuperscript{69} Theoretically, in this way more ASCs could infiltrate the infarcted area, and as such could improve stem cell therapy of the heart. Even more, using ultrasound, StemBells can be pushed against the vessel wall to facilitate binding (acoustic radiation force), via a direct effect on the microbubbles.\textsuperscript{70} We have previously found that StemBells administered 7 days post-AMI in rats improve post AMI heart function (unpublished results). However, administering StemBells at an earlier time point (1 day post-AMI) theoretically might result in an additional anti-inflammatory effect early after AMI. In chapter 9, we have compared the therapeutic efficacy of both time points of StemBell administration (1 and 7 days post-AMI) in a rat model of AMI.

References

26. McMurray JJ, Adamopoulos S, Anker SD et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J 2012;33:1787-1847.