The inflammatory response in myocarditis and acute myocardial infarction
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Chapter 10

General Discussion
DISCUSSION

Myocarditis and acute myocardial infarction (AMI) are important contributors to cardiovascular disease-related morbidity and mortality worldwide. The research chapters included in this thesis have provided new information on the pathogenesis of myocarditis and AMI and explored novel options in diagnosis and therapy. Note that the pathogenesis of both acute viral myocarditis and AMI presented in this general discussion chapter has been simplified for the sake of overview. The purpose of this discussion section is to provide the context of the individual chapters within the 'whole picture'.

ACUTE VIRAL MYOCARDITIS

Myocarditis is primarily caused by viral infection, but can also be attributed to other infectious causes (bacteria, fungi and eukaryotic parasites) and non-infectious causes (for instance drug-induced hypersensitivity, autoimmune disorders and exposure to heavy metals). Of these aetiologies, coxsackievirus B3 (CVB3) is by far investigated best (figure 1). CVB3 enters the circulation through the venous portal system [1] after faecal-oral transmission (natural transmission route) or intraperitoneal injection (in animal experiments). After reaching the systemic circulation, CVB3 infiltrates the myocardium and infects cardiomyocytes [2].

In chapter 2 and chapter 4, we found that CVB3 does not only infect the heart, but also the quadriceps muscle and the pancreas respectively [3]. Indeed, coxsackieviruses (as well as other cardiotropic viruses) have been previously demonstrated to infect skeletal muscle tissue. Coxsackieviral infection is in addition a well-known cause of pancreatitis in mice and men alike. CVB3 replication in the heart results in direct injury to the myocardium, and with activation of the host immune response. The viral infection induces maturation of dendritic cells [4]. Mature dendritic cells migrate to a nearby lymph node [5], inducing activation and proliferation of antigen-specific T-lymphocytes [6]. In addition, pro-inflammatory cytokines are produced by cardiomyocytes, cardiac macrophages and dendritic cells [7], which enhance inflammatory cell responses during acute viral myocarditis. Pro-inflammatory cytokines can also be found in the systemic circulation, although to what extent these cytokines affect other organs during acute viral myocarditis is not known.

In chapter 4, we investigated the spleens of CVB3-infected mice that received the anti-inflammatory drug colchicine. Besides massive apoptosis in the white pulp and a reduction in megakaryocyte numbers, the nucleated cells in the red pulp also appeared to be present at a lower cell density. In recent years, monocyte recruitment from the spleen to the heart has been described in response to AMI, and may be orchestrated by cytokines produced in the
heart. Possibly, in the colchicine-treated CVB3-infected mice, a similar response has occurred, where splenic monocytes were recruited to the heart and/or pancreas as a systemic response to the high viral load [12]. If and how this monocyte recruitment also occurs during acute myocarditis is not yet known however. Alternatively, although we were not able to determine this ourselves, CVB3 has been described before to infect the mouse spleen,19, 20 indicating that alterations in the spleen could have been induced locally as well.

In chapter 3, we found that both lymphocytic myocarditis and catecholamine-induced myocarditis in patients results in infiltration of inflammatory cells in the atria [13].21 Myocarditis is a known underlying cause for the development of atrial fibrillation (AF),22 although the involved mechanism of this relation is not clear. Our observation that myocarditis patients as a group have increased inflammatory cells in the atria is similar to a previous study.
carried out of atrial tissue of AF patients. This similarity suggests that atrial inflammation is a promising research target to include in the mapping of the events that lead from myocarditis to AF. Lymphocytic myocarditis is generally associated with viral infection. Therefore, the most obvious explanation for lymphocytic infiltration of the atria is that cardiotropic viruses do not only infect the ventricles but also the atria. This has been demonstrated before for CVB3 in mice. For patients with lymphocytic (viral) myocarditis however, it is not known if viruses infect the atria as well.

In chapter 2, we found that lymphocytes infiltrate the quadriceps skeletal muscle tissue of both lymphocytic myocarditis patients and mice with acute CVB3-induced myocarditis [14]. Again, this is likely a direct response to viral infection of skeletal muscle tissue. In the lymphocytic myocarditis patients, even though lymphocyte numbers were significantly higher compared to control patients without any form of cardiac disease, the numbers were quite low (3 cells per mm² tissue on average). Possibly, viral infection/inflammation of the cardiac muscle either precedes or succeeds viral infection/inflammation of the skeletal muscle (just as CVB3-induced inflammation in the mouse heart succeeds CVB3-induced inflammation of the pancreas). This can explain why only a small increase in lymphocytes was found in the skeletal muscle tissue at the time the patients succumbed to lymphocytic myocarditis. At this point however, we cannot exclude that an autoimmune response may also have been involved in these patients. Skeletal-muscle specific auto-antibodies have been described before in myocarditis patients.

The reliance on endomyocardial biopsies (EMB) is an important limitation of the diagnostic process of patients with suspected myocarditis. With the observed lymphocytic infiltration of quadriceps muscle tissue, we have provided a potential alternative that may in time reduce the number of suspected myocarditis patients that will undergo an EMB.

Finally, in chapter 4, we observed that inflammatory cells also infiltrate the pancreas following CVB3 infection [15]. CVB3-induced pancreatitis has been described to precede myocarditis in mice. In addition, coincidence of viral myocarditis and pancreatitis has been described in several case reports, not only for enteroviruses, but also for Epstein-Barr virus.

**Colchicine therapy**

In chapter 4, we studied the effects of colchicine on the pathogenesis of acute CVB3-induced myocarditis in mice, and found that it unexpectedly affects the pathogenesis in several ways. First of all, colchicine increased the viral load in both heart and pancreas. We are unsure how colchicine affects viral infiltration and/or replication. Colchicine is known to disrupt the endothelial cell barrier. Possibly, this has made it easier for CVB3 to cross the endothelial barrier into the tissues beyond. Remarkably, opposite effects of colchicine were observed on the number of macrophages (which were decreased) and
neutrophils (which were increased) in both the heart and the pancreas. Colchicine has been described to reduce inflammatory cell infiltration by reducing endothelial cell expression of intercellular adhesion molecules. However, an increased viral presence and disrupted endothelial barrier function may have provided a positive effect of inflammatory cell infiltration which outweighed the effects on adhesion molecule expression. In turn, colchicine may have had local toxic effects on (virus-activated) macrophages and as such reduced their numbers. The fact that colchicine treatment of CVB3-infected mice reduces macrophage numbers even below the level of the control mice indeed suggests a local macrophage-specific toxic effect.

Finally, the most important finding presented in chapter 4 was that colchicine treatment caused damage to the exocrine mouse pancreas, especially in the presence of CVB3. We can speculate that similarly, colchicine treatment may also result in damage of the pancreas of patients, especially in the presence of an active viral infection. This indicates that colchicine may not be safe for patients with viral myocarditis, but may also have implications for pericarditis patients. Also, the most common side-effects of colchicine treatment in these trials namely are related to the digestive system (diarrhoea and nausea). As the pancreatic acini produce enzymes that greatly affect the digestive system, it may be worthwhile to keep an eye on the pancreas in patients that receive colchicine.

ACUTE MYOCARDIAL INFARCTION

The pathophysiology of AMI is schematically depicted in figure 2. AMI is induced by decreased perfusion of one or more of the coronary arteries, resulting in ischemia of the downstream myocardium [1]. Ischemia induces cardiomyocyte necrosis, which in turn activates the innate immune response locally in the infarcted myocardium. As part of this response, pro-inflammatory cytokines are produced, which enter the systemic circulation. This has several systemic effects. First of all, these cytokines trigger the production acute phase proteins (APPs) in the liver, including components of the complement cascade, but also activators and inhibitors of complement such as C-reactive protein (CRP, activator) and C1-inhibitor (C1-Inh). At the same time, the sympathetic nervous system and the spleen are activated, resulting in monocyte proliferation and migration towards the infarcted myocardium.

Restoration of the blood flow coincides with the infiltration of pro-inflammatory neutrophils, lymphocytes and macrophages into the infarcted myocardium, triggered by the presence of necrotic cardiomyocytes accompanied by complement activation. Next to components of the complement cascade, both activators and inhibitors of complement, such as IgM, CRP and clusterin, can be found in the infarcted heart at the same time.
Figure 2. Schematic overview of pathways involved in acute myocardial infarction (AMI). Numbers in brackets correspond with the main text.


In chapter 8, we observed in AMI patients and in a rat AMI model that, in addition to the infarct area, lymphocytes and monocytes/macrophages also infiltrate the atria and non-infarcted right ventricle [8]. Thus far, it is not yet
known what triggers infiltration of these leukocytes in these areas despite the absence of necrotic cells. AMI is also a known precursor of AF. Therefore, similar to myocarditis, atrial inflammation is also a promising research target for uncovering how AMI causes AF.

Despite the fact that the influx of pro-inflammatory cells enlarges the infarct area, the process is necessary to remove necrotic cell debris. Four to five days after the inflammation starts, the necrotic cardiomyocytes are cleared, and the inflammatory response is down-regulated by anti-inflammatory M2 macrophages and regulatory T-lymphocytes [9]. As inflammation subsides, cardiac repair takes over. TGF-β is known to be produced locally in the heart as result of AMI, and stimulates the transformation of resident cardiac myoblasts into myofibroblasts. This transformation is hindered by inflammation. As the inflammation subsides, myofibroblasts produce extracellular matrix proteins which results in the formation of a fibrotic scar to replace cardiomyocyte tissue lost to ischemia and inflammation.

C1-inhibitor

In chapter 5, we described that C1-inh therapy has been studied extensively in the context of AMI, both in animal models and clinical trials. In the majority of the pre-clinical studies, a reduction of infarct size was found, albeit only in the first five hours following reperfusion (further time points were not included).

C1-inh has an anti-inflammatory effect by inhibiting complement activation and/or by interfering with leukocyte infiltration. In pigs, an inhibiting effect of C1-inh on complement activation was observed, visualized by decreased levels of C3a and C5a in plasma. Decreased levels of complement activation products were also found in the clinical trials on AMI patients. In several pre-clinical studies, a decreased neutrophil infiltration was observed as a result of C1-inh therapy. One possible mechanism underlying this decrease is a reduced production of anaphylatoxins out of the complement cascade, which are known to be involved in leukocyte recruitment in general. Alternatively, C1-inh has been suggested to directly interfere with leukocyte-endothelial cell interaction by binding to E- and P-selectin, which can also explain this reduction in neutrophil infiltration. Interestingly, it was also described that C1-inh therapy reduced expression of both ICAM-1 and P-selectin in the infarcted myocardium.

In chapter 8, we found in rats that C1-inh therapy decreases the amount of N(epsilon)-(carboxymethyl)lysine (CML) in post-AMI intracardial blood vessels of both the infarcted left ventricle and the non-infarcted areas (right ventricle and both atria). CML is an advanced glycation end-product (AGE), which forms on endothelial cell surfaces in the heart as result of ischemia and reperfusion, and induces oxidative damage and production of pro-inflammatory cytokines, as well as increased expression of adhesion molecules. AGEs in general are known to contribute to infarct expansion and cardiac dysfunction. In the rat
model we used, we observed that the CML formation resulting from ischemia and reperfusion also occurs in the non-infarcted areas of the heart [13], which is consistent with earlier observations in AMI patients, where CML depositions were found to be increased on activated endothelium (E-selectin-positive) in both infarcted and non-infarcted areas of the heart.72 To what extent CML accumulation functionally affects the non-infarcted areas of the heart is not yet known. Also, we only focussed on the effects of C1-inh at 42 days post-AMI. Effects of C1-inh need to be studied in the rat model at earlier time points as well. Finally, it was recently demonstrated that CML depositions are also increased in the atria of AF patients.23 If C1-inh can also decrease atrial inflammation in AMI patients, it may become a useful therapeutic option to prevent AF.

In chapter 5, we concluded that for C1-inh, studies employing either AMI animal models or AMI patients consistently show beneficial effects. Despite that clinical research on C1-inh as therapeutic option for AMI patients appear to have been abandoned we found no reason that speaks against the C1-inh as potential treatment for AMI. We hope that this will rekindle the interest in C1-inh administration as therapeutic strategy, as well as complement inhibition in general.

A practical problem of C1-inh administration in rat models is that repeated intravenous injections in the tail vein causes considerable damage to the tail itself. In chapter 7 we evaluated subcutaneous C1-inh injections as alternative. Despite the fact that subcutaneous C1-inh administration is rapidly developing in clinical practice,73 it was found to be ineffective in rats compared to intravenous administration.74 With this, we hope to prevent unnecessary negative results in future studies on C1-inh treatment in rats.

Finally, in contrast to C1-inh as exogenously administered compound, very little is known on the role of endogenous C1-inh in the context of AMI. In chapter 6, we observed that endogenous C1-inh is also present in the infarcted human myocardium, coinciding in time and location with activated complement. In addition, we observed in rats (in vivo) and in endothelial cell and cardiomyoblasts (in vitro) that ischemia resulted in local production and expression of C1-inh [14].75 This local production, on top of systemic up-regulation by the liver, may result in local complement regulation which is induced faster and more effective. Determining to what extent this local production contributes to complement regulation in the post-AMI heart requires further research.

Adipose-derived stem cell therapy

Adipose-derived stem cell (ASC) administration is an emerging therapeutic strategy which has been investigated extensively in AMI animal models.76 Multiple studies in rat AMI models do report both a reduction in infarct size and an improvement of cardiac function following ASC administration.77-82 A practical difficulty of ASC therapy is the poor engraftment of intravenously
injected cells in the infarcted myocardium. For this, we previously developed a technique where ASCs are coupled to gas-filled microbubbles (StemBells) which enables the ASCs to be 'pushed out' of the bloodstream at the site of the infarction using ultrasound (Woudstra et al., in preparation), thereby improving ASC infiltration of the heart following intravenous administration. Using StemBells, we first demonstrated that we could improve fractional shortening and decrease cardiac hypertrophy when administered 7 days post-AMI. In chapter 9, we demonstrated that StemBells administered at 1 day post-AMI resulted in a faster improvement of fractional shortening compared to StemBells administered 7 days post-AMI.

ASCs are able to differentiate into cardiomyocytes in vitro. However, it is generally assumed that the therapeutic potential of ASCs lies primarily in the production of paracrine factors, as little evidence of ASC differentiation can be found in the heart in vivo. For instance, ASCs secrete IL-10, Galectin-1 and Galectin-3, which stimulate regulatory T-lymphocytes and inhibit pro-inflammatory T-lymphocytes. Indeed, cytokine measurements in rat AMI models also point towards anti-inflammatory effects of ASCs. Additionally, ASCs support cardiac repair by limiting the amount of permanently damaged myocardium through stimulation of neovascularization and inhibition of cardiomyocyte apoptosis. In chapter 9, we also found that StemBell administration reduced cardiac hypertrophy without reducing the infarct size. This indicates that paracrine effects of ASCs also affect the non-infarcted areas of the heart.

For bone marrow-derived stem cells (BMSCs), it is well known that inflammation may be further reduced by stimulating a macrophage phenotype switch towards the anti-inflammatory M2 phenotype through production of IL-10. For ASCs, this effect has never been demonstrated before. As ASCs and BMSCs are both of mesenchymal origin and similar in functionality, it is plausible that ASCs also facilitate the phenotype switch. In our study however, we did not observe this, although the time point of analysis (42 days post-AMI) may have been too late to be able to find evidence of an enhanced phenotype shift.

Finally, the most important implication that can be made from chapter 9 is that it may not be necessary to wait with administration of StemBells until the post-AMI inflammatory response subsides, and that earlier administration may even improve patient outcome. Translation of these findings towards clinical practice may contribute to an ASC-based therapy for AMI patients that is easy, safe and cost-efficient.
CONCLUDING REMARKS

The most important message of this thesis as a whole is that during heart disease, inflammation is more widespread than one would initially consider. Myocarditis and AMI are both diseases in which inflammation is primarily described in the ventricles, yet we found that inflammation is also present in the atria (for both myocarditis and AMI) and the skeletal muscle tissue (for myocarditis). This is a concept which has thus far been underappreciated. As this thesis is written, we are already continuing this line of research, where the clinical significance of extraventricular inflammation will be further investigated. A prospective clinical trial (the INFLAME-trial) is currently being conducted to investigate whether myocarditis can be diagnosed based on skeletal muscle tissue inflammation. Moreover, we are currently aiming to discover a serological marker that can specifically diagnose atrial inflammation. Atrial fibrillation is an important complication of both myocarditis and AMI, and a diagnostic test for atrial inflammation may predict if a patient is predisposed towards atrial fibrillation development.

References

37. Frangogiannis NG. The mechanistic basis of infarct healing. Antioxid Redox Signal 2006;8:1907-1939.


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