

Summary

Chapter 1: Introduction

Worldwide cardiovascular disease is the number one cause of death. Both myocardial infarction (MI) and infectious inflammation of the heart muscle, i.e. lymphocytic myocarditis (LM), are important contributors to cardiovascular mortality. Inflammation plays a pivotal role in the pathogenesis of viral myocarditis and in the induction and the outcome of MI. This thesis describes inflammation both in viral myocarditis and MI aiming at better understanding of its mechanisms in search of improving and therapy.

In general myocarditis is defined as inflammation (*itis*) of the heart muscle (*myocardium*). The cause of myocarditis can be infectious agents such as viruses, bacteria, protozoans and fungi, but also an autoimmune reaction. In Europe most cases of myocarditis are caused by a viral infection. Due to the large varieties in clinical presentation, viral myocarditis is a complicated clinical entity and is difficult to diagnose. Moreover, to date there is a lack of specific therapies to treat viral myocarditis. Therefore, there is a need for a better understanding of the pathogenesis of viral myocarditis to enable the development of improved diagnostic tools and potential new therapies.

A MI is usually an acute event as a result of an obstruction within the coronary arteries that supply the heart with oxygenized blood. Such obstructions are generally the result of erosion, inflammation and/or rupture of atherosclerotic plaques in the coronary artery leading to blood clot formation. The clinical presentation of MI is more clear than that of myocarditis. The diagnosis of MI is based on specific changes in electrocardiogram, biomarkers, echocardiography and angiography. Although treatment of MI has improved significantly in recent years there still is a demand for improved therapies to prevent loss of cardiomyocytes and/or to regenerate myocardial tissue.

Chapter 2: For the diagnosis of lymphocytic myocarditis CD45 is more sensitive than CD3

The gold standard for diagnosing LM is the pathological examination of endomyocardial biopsies (EMB). In these heart biopsies various inflammatory cells can be detected via immunohistochemistry. To diagnose LM with immunohistochemical stainings clear guidelines have been suggested: in the EMB ≥ 14 leukocytes per mm^2 need to be present, composed of ≥ 7 (CD3-positive) T-lymphocytes and maximum 4 macrophages per mm^2 . We hypothesized that a more common leukocyte marker, CD45, instead of CD3 could increase the diagnostic sensitivity. In hearts of mice with acute viral myocarditis we found that, using the guidelines of ≥ 14 leukocytes per mm^2 , only 33% of the mice classified for the diagnosis of LM with the CD3 T-lymphocyte marker, while 89% classified with the CD45 lymphocyte marker. Also in human autopsy material of deceased patients with proven LM we found that the diagnosis of LM could be increased from 17% to 50% with the use of CD45 instead of CD3 in the EMB sampling area using the guideline of ≥ 14 leukocytes per mm^2 . Thus, the use of the common leukocyte marker CD45 increases the sensitivity of the diagnosis of LM. Moreover, we also found more inflammatory cells within the EMB area compared with the rest of the ventricular wall, indicating that the endomyocardial sampling area constitutes the highest chance for positive histological diagnosis of LM.

Chapter 3: Lymphocytes infiltrate the quadriceps muscle in lymphocytic myocarditis patients: A potentially new diagnostic tool

As described above, the gold standard to diagnose LM is via endomyocardial biopsies (EMB). Since EMBs are small there is a chance that the inflammation is not present within the EMB (sampling error). Moreover, with the collection of EMBs there is a small risk of potentially serious complications, such as cardiac perforation and valvular damage. Taken together new diagnostic methods are necessary to improve the diagnosis of LM. We know that in other diseases if the heart changes in the heart muscle may sometimes be reflected in other muscles also. In this chapter we examined whether the inflammatory cells in a biopsy of the quadriceps skeletal muscle can serve as a potential new diagnostic tool for LM. In autopsy material of patients with proven LM and of healthy controls various inflammatory cells were quantified within a biopsy of the quadriceps muscle. Compared to the controls, LM patients had significant more lymphocytes within the quadriceps muscle. Using the number of lymphocytes it was possible to diagnose LM with a specificity of 100% and a sensitivity of 71%. This study clearly shows that immunohistochemistry on a biopsy of the upper leg thigh muscle is a potential new method of diagnosing LM.

Chapter 4: Colchicine aggravates coxsackievirus B3 infection in mice

Currently there is no specific therapy for patients with LM. For LM patients that have reduced cardiac function, treatment usually consists of supportive therapy and cardiovascular stabilization therapy. Although the standard heart failure therapy is useful in symptom repression and preventing on-going decline of cardiac function, the underlying disease is not treated (the LM). Therefore, there is a clinical need to find effective treatments for LM. In this chapter we investigated the effect of the immunosuppressive drug colchicine, that is often used to treat patients with pericarditis i.e. inflammation of the pericardium, as treatment option for LM. We treated mice with colchicine that had acute coxsackievirus B3 induced myocarditis. We found that colchicine treatment rapidly resulted in severe disease symptoms, such as weight loss, inactivity and in some cases even led to death. Tissue investigation revealed that colchicine treatment induced massive degeneration of the pancreatic acini, which was most likely the cause of the severe disease symptoms. Moreover, we found in the heart that colchicine treatment induced an increased the number of neutrophils and decreased the number of macrophages. It was also found that in mice with coxsackievirus B3-induced myocarditis, that colchicine treatment, in contrast to placebo treatment increased the level of coxsackievirus B3 in both the heart and the pancreas.

Chapter 5: Lymphocytic myocarditis coincides with myocardial infarction and concurs with increased inflammation, hemorrhage and instability in coronary artery atherosclerotic plaques

Patients with LM can present with clinical symptoms similar to MI. In clinical practice, LM is only considered as a potential underlying cause of infarct-like complaints when MI is ruled out, based on the absence of coronary artery narrowing or obstruction. The general consensus is that although LM and MI can be similar in clinical presentation they are distinct clinical entities. In this chapter we have observed in clinical autopsies a high prevalence (32%) of very recent MI coinciding with LM in a cohort of patients diagnosed post mortem with LM. To investigate whether LM affects coronary atherosclerotic plaques, we analyzed the inflammatory infiltrate and stability in coronary atherosclerotic lesions in autopsied

patients with LM and/or MI. Compared to controls, patients with LM or MI showed increased numbers of macrophages and neutrophils in the tissue sections of the coronary segments, while patients with both LM and MI or MI alone showed an increase in lymphocytes and mast cells. Moreover, in patients with both LM and MI or MI alone, this coincided with an increase of unstable plaques and thrombi. Finally, LM patients showed more intraplaque hemorrhage than controls, which was even more pronounced in patients with MI, with and without LM. This study demonstrates prevalent co-occurrence of LM with a very recent MI at autopsy, and inducibility of inflammation and remodeling of the atherosclerotic plaques by LM.

Chapter 6: Infectious myocarditis: the role of the cardiac vasculature

In this chapter we reviewed the existing literature regarding the role of infectious myocarditis on the vessels of the heart. First we found that the blood vessels of the heart, both the intramyocardial microvasculature and the large epicardial coronary arteries, play an important role in the pathogenesis of infectious myocarditis. Moreover, endothelial cells, which form the inner lining of the heart vessels, are direct targets for infection. The blood vessels assist in shaping the cellular immune response in infectious myocarditis through the expression of adhesion molecules and antigen presenting Major Histocompatibility Complex molecules. In addition, damage and dysfunction of the cardiac (micro)vasculature are associated with thrombus formation as well as aberrant regulation of vascular tone including coronary vasospasm. These in turn can cause cardiac perfusion abnormalities and even MI.

Chapter 7: Development of a new therapeutic technique to direct stem cells to the infarcted heart using targeted microbubbles: StemBells

Stem cell therapy has been proposed as a promising therapy for regenerative tissue repair and to prevent heart failure development after MI. One of the major problems of stem cell therapy is lack of engraftment of sufficient stem cells at the site of injury. To overcome this problem we designed a novel targeting technique by assembling adipose-derived stem cell-microbubble complexes, named 'StemBells'. Microbubbles are small gas-filled bubbles originally developed as contrast agents for echocardiography. In this research the microbubbles were labeled with an antibody against CD90 to bind them to the CD90 marker of the adipose-derived stem cells. Additionally, the microbubbles were labelled with an ICAM-1 antibody. ICAM-1 is expressed on activated endothelium of blood vessels within the infarct area, allowing the StemBells to theoretically target the infarct area. To investigate the therapeutic effect of StemBells rats were injected with StemBells 7 days after MI. We proved that it was possible to inject StemBells safely. Moreover, StemBells were found, albeit in small numbers, within the infarct area. Using echocardiography we also found that StemBell therapy induced improved cardiac function on the long term (5 weeks after therapy). This functional improvement neither coincided with a reduction in infarct size nor with a change in anti- and pro-inflammatory macrophages within the infarct area. This study shows that StemBell technology is a novel method to improve cardiac function after MI.

Chapter 8: A comparison in therapeutic efficacy at several time points of intravenous StemBell administration in a rat model of acute myocardial infarction

StemBell therapy is a promising new therapeutic option to improve cardiac function after MI (see *chapter 7*). MI leads also cell death and inflammation in the heart. This inflammatory

response is beneficial, since inflammatory cells remove the dead cells from the infarcted area allowing the heart to recover. However, this response may be unfavorable for the survival rate of stem cells entering the infarct area. Therefore, we initially chose to administrate StemBell therapy 7 days after MI. However, since 7 days after MI a lot of damage is already initiated there may be more therapeutic effect when StemBell therapy is given earlier. In this study we compared the efficacy of StemBell therapy in rats 1 and 7 days after MI. Using echocardiography, we observed that StemBell therapy given 1 day after MI improved cardiac function already at one week after MI. At 6 weeks after MI StemBells administrated either 1 or 7 days after MI resulted in a similar improvement of cardiac function. Furthermore, there was no difference in infarct size or the number of pro- and anti-inflammatory macrophages in the infarct area between the different times of StemBell administration. Thus, StemBell therapy leads independently from the time of administration to prolonged improvement in cardiac function and can be administrated either 1 or 7 days after MI.

Chapter 9: StemBell therapy stabilizes atherosclerotic plaques after myocardial infarction

After MI the development of atherosclerotic plaques is accelerated and the amount of inflammation (mainly consisting of macrophages) is increased within the plaque, leading to destabilization of the atherosclerotic plaque. In theory, stem cell therapy is a therapeutic option for atherosclerosis. In this chapter we investigated the use of StemBell therapy on the atherosclerotic plaque after MI. Mice with atherosclerosis received StemBell or vehicle treatment 6 days after MI. Compared to the vehicle group, StemBell therapy resulted in a thicker (more stable) fibrous cap of the aortic plaque and a decreased number of macrophages within the plaque 4 weeks after MI. Additionally, StemBell treatment induced an increased percentage of anti-inflammatory macrophages both in the atherosclerotic plaques and in the infarct area of the heart, coinciding with a trend to increased percentage of anti-inflammatory monocytes within the blood. Moreover, we found that the effect of StemBells on atherosclerosis was independent of cholesterol and triglyceride levels, since these were similar in the blood after both treatments. Furthermore, StemBell treatment did not affect cardiac function nor infarct size 4 weeks after MI. Since StemBell therapy did result in more anti-inflammatory macrophages in the tissue and a trend towards more anti-inflammatory monocytes in the blood this point to a systemic effect. Thus, in atherosclerotic mice after MI, StemBell therapy not only has a positive result on the development of the atherosclerotic plaque, but also on the inflammation within the infarct area.

Chapter 10: Conclusion

In this thesis, we studied the role of inflammation in LM and MI in search of improving both diagnostic and treatment options.

In LM we found that increased inflammation was not limited to the heart, but was also found in the atherosclerotic plaques of the coronary arteries and even within the skeletal quadriceps muscle. The increased inflammation in the skeletal quadriceps muscle may be a viable alternative for EMB to diagnose myocarditis. Moreover, the immunohistological diagnosis of LM can be improved by staining CD45 lymphocytes within the heart, since we found that the inflammatory infiltrate consists predominately of CD45 compared to CD3 positive lymphocytes. Importantly, we have provided evidence that LM can coincide with MI

and that LM, via destabilization effects on atherosclerotic plaques, may facilitate MI development.

After MI an inflammatory response develops within the heart and the atherosclerotic plaques of the coronary arteries. We have developed a so-called StemBells technique that not only improves inflammation in the atherosclerotic plaque and the infarct area, but also improves cardiac function. Therefore, StemBell therapy may be a promising therapy after MI.