**Inflammation and the induction of acute myocardial infarction (AMI)**

It is well known that the inflammatory protein type IIA secretory Phospholipase A$_2$ (sPLA$_2$-IIA) is involved in the pathogenesis of atherosclerosis. sPLA$_2$-IIA releases fatty acids from membrane phospholipids that form precursors for inflammation inducing eicosanoids. Membrane phospholipids from which sPLA$_2$-IIA has freed a fatty acid are lysophospholipids. These lysophospholipids act as binding sites for another inflammatory protein C-reactive protein (CRP), which in turn can activate complement. Furthermore, sPLA$_2$-IIA can also kill cells by itself. In chapter 2 we show that sPLA$_2$-IIA is significantly more expressed in culprit coronary atherosclerotic plaques that caused AMI than in coronary plaques that caused angina pectoris. This sPLA$_2$-IIA may be involved in the death of smooth muscle cells in the plaque, which contributes to plaque instability. This implies that sPLA$_2$-IIA is involved in the induction of plaque complications and therefore also AMI.

Branched off from the coronary arteries that lie on the heart (epicardial) are the arteries that lie embedded in the heart muscle; the so-called intramyocardial arteries. In chapter 3 we investigated the accumulation of advanced glycation end products (AGEs), in this case ε-N-carboxymethyllysine (CML), in these intramyocardial arteries. AGEs are the product of non-enzymatic glycation of proteins which disrupts the functioning of these proteins. AGEs accumulate during aging and at an accelerated rate in diabetes. We found significantly more accumulation of CML in the intramyocardial arteries of the left ventricle in AMI patients than in control patients. These patients did not have diabetes. CML was found in intramyocardial arteries throughout the left ventricle, not just the infarction area, and was found shortly after the onset of infarction. Furthermore, we found in vitro and in a rat AMI model that CML was likely not formed as a result of AMI, but accumulated prior to the onset of AMI. These two studies, sPLA$_2$-IIA in the epicardial coronary arteries and CML in the intramyocardial arteries endorse the role of inflammation in aberrations of cardiac blood vessels that may lead to infarction.

**Inflammation after acute myocardial infarction**

*Death of cardiomyocytes*

As a result of AMI cardiomyocytes die. Cell death can be executed roughly via two processes: apoptosis and necrosis. Apoptose is the regulated form of cell death; self-termination occurs in a regulated manner via different signaling routes that ultimately lead to the activation of caspases. A cell needs energy to complete apoptosis. With necrosis the self-termination is less well regulated (through lack of energy), which leads to the disintegration of the cell. In the infarcted heart both processes take place. In areas where intense ischemia leads to complete depletion of cellular energy, cardiomyocytes will become necrotic. In areas where (sufficient) energy remains, supposedly in the border zones of infarction or after reperfusion, cardiomyocytes will become apoptotic.

It has been shown before that reactive oxygen species (ROS) play a role in apoptosis. In chapter 7 we show for the first time that the ROS-producing protein Nox2 is expressed in human cardiomyocytes and that this expression is upregulated in the infarction area in AMI patients. In chapter 8 we show in cardiomyocytes in vitro that as a result of ischemia this Nox2 translocated to the nucleus and produced ROS there. The Nox2 produced ROS in the cardiomyocyte nucleus play an important role in the process of apoptosis, as these cells did not become apoptotic when Nox2 related ROS were inhibited.

*Membrane flip-flop and reversibly damaged cells*

In the border zones of infarction also reversibly damaged cardiomyocytes exist. Reperfusion of the heart after AMI can cause additional damage in which the sPLA$_2$-IIA – CRP – complement inflammatory cascade plays a key role. The binding of sPLA$_2$-IIA to reversibly damaged cardiomyocytes is an important initial step herein. sPLA$_2$-IIA does bind to (reversibly) damaged cells but not to healthy cells and can thus discriminate between the two. We have shown earlier that cardiomyocytes that bind
sPLA$_2$-IIA had membrane alterations. These cells exposed the membrane phospholipid phosphatidylserine (PS) in the outer layer of the plasma membrane, which in healthy cells is kept actively within the inner plasma membrane leaflet by a trans-membranous protein called flippase. This process is also called membrane flip-flop. Membrane flip-flop is a hallmark of apoptotic and thus dying cells. However, membrane flip-flap also occurs in reversibly damaged cells, as we show in cardiomyocytes in chapter 13. Membrane flip-flop in reversibly damaged cells therefore is an important characteristic that is utilized by inflammation to cause extra damage in the infarcted heart. In chapter 6 we analyzed whether RhoA – ROCK signaling is involved in the process of membrane flip-flop. We found that inhibition of RhoA – ROCK activity led to membrane flip-flop in cardiomyocytes. This coincided with decreased flippase activity, which can explain why these cells exposed PS. These data show that in cardiomyocytes RhoA – ROCK signaling is necessary to prevent membrane flip-flop. Whether this plays a role in the heart after infarction is subject of further study.

Thus cardiomyocytes die after AMI, both through apoptosis, with a probable role herein for Nox2 produced ROS, and necrosis. Also reversibly damaged cardiomyocytes exist. Membrane flip-flop occurs in all these cells. sPLA$_2$-IIA binds to and disposes of these cells, either directly or through CRP – complement activation. This results in the disposal of already dead cells, but also in additional damage through the disposal of reversibly damaged cells.

**Complement activation in the infarcted heart**

After reperfusion of the infarcted heart an inflammatory reaction is induced, wherein sPLA$_2$-IIA, CRP and complement thus play an important role. Complement induces cell lysis and the influx of neutrophilic granulocytes. In chapter 10 we show that in AMI patients that received reperfusion therapy, either the thrombolytic streptokinase or coronary artery bypass grafting (CABG), had significantly more depositions of CRP and complement in the heart than AMI patients who did not receive reperfusion therapy. This was however not the case in AMI patients who received percutaneous transluminal coronary angioplasty (PTCA). Also patients who suffered from reinfarction within two weeks of the first infarction had more extensive CRP and complement depositions. More extensive depositions of CRP and complement implicate more damage in the heart after AMI. These data show that the method of reperfusion can influence the ultimate damage in the infarcted heart. Furthermore, a second infarction relatively soon after a first infarction leads to an increased inflammatory response, leads to more extensive damage in the infarcted heart.

It is known that CRP can activate complement on cells. We have shown before that this occurs in the infarcted heart. Another known complement activator is the immunoglobuline IgM, which for instance can activate complement on bacteria. In chapter 11 we show colocalization of IgM with CRP and complement in the infarcted heart. These data imply that within the infarcted heart complement can also be activated by IgM.

**Acute myocardial infarction and therapy**

The activation of the sPLA$_2$-IIA – CRP – complement inflammatory cascade contributes to the additional damage in the heart after AMI. Inhibition of this cascade therefore may limit this damage. It is however of crucial importance that the clearance of dead tissue is not inhibited, because this leads to a disturbed wound healing. This may lead to a weakened ventricle wall which may lead to rupture and resulting death. Therefore, intervening in the post-AMI inflammation should never be done at the expense of successful wound healing (see also chapter 9).

In chapter 12 we show that an inhibitor of sPLA$_2$-IIA, named PX18, can indeed reduce infarction size in a rat AMI model. Furthermore, we show that PX18, in addition to sPLA$_2$-IIA, can inhibit the extent of membrane flip-flop in cardiomyocytes. In this way PX18 can have a double effect in the infarcted heart: it inhibits sPLA$_2$-IIA and inhibits binding sites for sPLA$_2$-IIA on cardiomyocytes. PX18 did not disrupt normal wound healing, probably because inhibition of sPLA$_2$-IIA does not prevent binding of CRP and complement to dead cells; these cells are still cleared. This study shows that specific inhibition of inflammatory mediators can limit reperfusion damage after AMI, leading to less damage in the heart, lessening the chance of developing heart failure.
In chapter 13 we studied clusterin in myocardial infarction. Clusterin as a multi-potent protein that is widespread expressed in the body and is involved in many different processes. It was found before that clusterin can be cytoprotective through the inhibition of complement. It was also shown that clusterin colocalized with complement in the infarcted heart. This shows that also anti-inflammatory mediators deposit in the infarcted heart, presumably to control the inflammatory response. We have now shown that clusterin is also found in cardiomyocytes of the border zones of infarction, without complement. Furthermore, we show in vitro that clusterin protects cardiomyocytes from ischemia induced cell death, independent of complement. Elevating clusterin levels after AMI might therefore be useful to limit reperfusion damage. Meanwhile we have analyzed the effect of elevated clusterin levels in the blood on infarct size in the AMI rat model. Elevated levels of clusterin indeed led to decreased infarction size, without disrupting normal wound healing.

Conclusions
The studies in this thesis support the notion that inflammation is important in the pathophysiology of acute myocardial infarction. We show that inflammation in blood vessels in and on the heart can play a role in the development of AMI. In addition, we show the existence of reversibly damaged cardiomyocytes and that certain inflammatory mediators can bind to and kill these cells, because they signal their state of stress through membrane flip-flop. We also show that inhibition of these inflammatory mediators in vivo can lead to lessened myocardial damage after AMI and a better prognosis.