Cardiovascular diseases (CVD) are still number one cause of death globally, despite improved prevention and therapy. The major reason for most of the CVD diseases is atherosclerosis. Atherosclerosis is a long term inflammation of a blood vessel. This inflammation is started by a damage of a vessel wall due to a lot of risk factors common in our lifestyle, such as smoking, lots of lipids (also called lipoprotein particles) in blood, obesity, high blood pressure, and stress. Inflammation is a process fueled by our immune system and a lot of immune cells are involved in starting and progression of atherosclerosis. Once the vessel wall is damaged by e.g. lipids in blood, these lipoprotein particles can pass through the vessel wall more easily into the tissue below where they accumulate and cause further injury of the blood vessel. That triggers arrival of even more immune cells, mainly T cells, monocytes/macrophages, dendritic cells, neutrophils, and to less extent also B cells. Monocytes recognize vessel wall injury and start to migrate to the site of damage where they mature into macrophages and together with already present resident macrophages try to clear lipids that are accumulating in the vessel wall. Macrophages can “see” or recognize lipoprotein particles, also if they are modified by e.g. oxidation (then they are called oxLDL), through proteins called receptors on their surface. These receptors help macrophages not only to recognize but also take up oxLDL particles decreasing their amount in the vessel wall tissue. However, if the capacity of a macrophage to take up and process oxLDL is exceeded, it becomes filled with lipids and gains a foamy appearance. Foamy macrophages are releasing high amount of substances such as chemokines and cytokines into the surrounding tissue attracting more immune system cells and further driving inflammation. Another process worsening inflammation of the vessel wall is death of the foam cells when they open up and release all the lipids to the tissue around. That causes accumulation of lipids in the vessel wall and formation of so called fatty streak or in later phases atherosclerotic lesion. Released lipids together with cytokines and chemokines are attracting smooth muscle cells that start to migrate to the top of the formed atherosclerotic lesion. Smooth cells create a fibrous cap and the major function of the fibrous cap is to protect the content of the lesion from being freed to circulation. However, as the inflammation of the vessel wall proceeds, pro-inflammatory substances released from foam cells make the fibrous cap vulnerable for rupture. Once the lesion ruptures, the whole content is released to blood circulation and that activates platelets. Platelets start to aggregate in a similar way as during bleeding and they create a clot that can completely close the vessel. That in fact, is the most dangerous complication of atherosclerosis as the closed vessel cannot provide oxygen to tissue. If this closure happens in the vessel of heart or brain the consequence is myocardial infarction or stroke. In this thesis we looked at different immune cells and how they influence inflammation in atherosclerosis.

Receptors that allow macrophages to recognize not only oxLDL but also bacteria or fungi are named pattern recognition receptors (PRRs). PRRs are very important during infection but they also help to find other potentially harmful antigens. One of the PRRs is dectin-1 that is present in high amount on monocytes and macrophages. Dectin-1 recognizes beta-glucan structure present on fungi and thanks to that a
healthy immune system can fight infection with e.g. candida. Recently, vimentin was identified as an endogenous ligand for dectin-1. Moreover, macrophages can become activated by vimentin which leads to production of reactive oxygen species (ROS). ROS are important in oxidation of lipoprotein particles into oxLDL. Vimentin has been reported to be released by activated monocytes and present in high amount in human atherosclerotic lesions. In Chapter 2 we aimed to investigate if dectin-1 is indeed important also in development of atherosclerosis in vivo (meaning in living organism and not isolated cells). For that we used a well-known mouse model of atherosclerosis where bone marrow of a mouse is replaced by either normal blood cells (wild type) or by cell which miss a certain protein (knock-out). We therefore replaced blood of mice with wild type cells having dectin-1 or by dectin-1 knock-out cells. Mice were for 9 weeks fed high fat diet to get atherosclerosis. After that we looked how big and severe the lesions in their hearts were. We observed that both wild type mice as well as dectin-1 knock-out mice developed lesions of the same size and severity. In fact, no differences were found between these two groups of mice. This finding suggests that dectin-1 is not important in development of atherosclerosis.

Many processes in cells are controlled through phosphorylation and dephosphorylation of proteins by enzymes called kinases and phosphatases. In Chapter 3 we hypothesized that changes in the phosphorylation status of proteins happen also during foam cell formation. We aimed to identify which proteins are phosphorylated or dephosphorylated after monocytes/macrophages encounter oxLDL. Those findings would later help us to develop therapeutic intervention in order to stop the process of foam cell formation and possibly also change inflammation in atherosclerosis. In order to study processes of phosphorylation and dephosphorylation in human monocytes we used a method called phosphoproteomics. During this experiment proteins isolated from monocytes that were exposed to oxLDL for increasing time were analyzed using mass spectrometry. We have noticed that a large amount of proteins gets very quickly phosphorylated after the cells were in contact with oxLDL, already after a few minutes. We focused on one of the prominently phosphorylated kinase called protein kinase C delta (PKCδ) and tested if blocking its function with a drug called rottlerin would decrease uptake of oxLDL by macrophages. We could indeed see decreased uptake of oxLDL by macrophage when we used pharmacological inhibitor rottlerin. However, when we tried to confirm this observation by using macrophages with decreased levels of PKCδ we could not repeat the same result as with inhibitor rottlerin. Therefore, we also used macrophages from PKCδ knock out mice as well as macrophages obtained from patients with a rare mutation in PRKCD gene causing complete lack or very pronounced decrease in PKCδ protein levels. As both mouse and human macrophages lacking PKCδ showed again normal uptake and foam cell formation we concluded that PKCδ is not involved in regulation of foam cell formation. That was in contrast to previously published data by another group where they claim that PKCδ is important in regulation of oxLDL uptake and foam cell formation.
On the other hand, when we looked at functions of neutrophils isolated from patients with none or very low level of PKCδ we noticed that they cannot kill bacteria and fungi as good as healthy neutrophils. Neutrophils use two major ways how to kill bacteria and fungi, namely production of ROS and release of very toxic enzymes called proteases from their granules. We tested if neutrophils from PKCδ deficient patients have normal production of ROS and release of proteases and that was indeed the case. It means that PKCδ is important in a different way in the process of bacterial and fungal killing. These findings are described in Chapter 4.

Other important cells of the immune system involved in modulation of the inflammation in atherosclerosis are B cells. A subset of B cells called B1 cells are capable of producing proteins called antibodies that help to prevent atherosclerosis as they bind to oxLDL. When oxLDL is bound to these antibodies they cannot be taken up by macrophages in the vessel wall. Instead the complexes of oxLDL and antibody are cleared in e.g. spleen, away from sites prone to develop atherosclerosis. These antibodies are produced by B1 cells already from birth on and that gave them name natural antibodies. They can also recognize oxidized proteins on surface of dying cells which helps their clearance from circulation and prevent autoimmune diseases. B1 cells are a minor subset of B cells and we still know very little about their function, especially how production of natural antibodies is controlled. In Chapter 5 we present Signal Regulatory Protein alpha (SIRPα) to be a novel inhibitory receptor on B1 cells. For the first time we described presence of SIRPα on B1 cells in mouse and also on a subset of B cells in humans. We also saw that mice without functional SIRPα loose “a break” and start to produce more natural antibodies already without any stimulation. If cells isolated from mice without SIRPα signaling are used in above explained model of atherosclerosis (blood of a mouse is replaced by either wild type or knock out cells) they are protected from this diseases. We saw higher levels of natural antibodies against oxLDL in mice without functional SIRPα which is most likely the reason why they are protected from atherosclerosis. We also looked carefully at macrophages of these mice as SIRPα has many functions on macrophages. However, we did not find any explanation yet how SIRPα could regulate macrophages in atherosclerosis. Therefore, we concluded that SIRPα is involved in regulation of atherosclerosis because it blocks production of natural antibodies by B1 cells.

Taken together, in this thesis we described different ways of how immune system, namely macrophages and B cells, can control process of inflammation in atherosclerosis. We hope that especially findings about how blocking SIRPα can prevent development of atherosclerosis in Chapter 5 will help us to develop new strategies in the treatment of cardiovascular diseases.