ABSTRACT

Atherosclerosis is a complex inflammatory disease localized in medium-sized and large arteries and it is the most important contributor to cardiovascular disease. Complications of atherosclerosis such as myocardial infarction and stroke are leading causes of mortality in many countries. Leukocyte recruitment to the arterial intima is crucial for the development of atherosclerotic lesions. The roles of macrophages and T-lymphocytes in promoting atherosclerotic plaque development and destabilization have been extensively studied. However, the most abundant white blood cell in the circulation, the neutrophil, has until recently rarely been associated with disease pathogenesis. The work of this thesis aimed at investigating the potential presence and roles of neutrophils in atherosclerosis.

Previous in vivo studies of leukocyte recruitment in atherogenesis have not been able to selectively detect individual subpopulations of leukocytes. In order to study neutrophils more specifically, we aimed at introducing a system, by which we could selectively study the roles of monocytes and neutrophils by microscopy. By crossing mice deficient in apolipoprotein E (ApoE⁻/⁻ mice) with mice homozygous for a knock-in mutation for enhanced green fluorescent protein (EGFP) in the lysozyme M (lys-M) locus, we generated lysozyme M-deficient atherosclerosis-prone mice with endogenously fluorescent neutrophils and monocytes (ApoE⁻/⁻/LysEGFP/EGFP mice, Paper I). In order to address whether absence of lys-M and replacement with EGFP influence atherogenesis, we compared the generated mice with their littermate ApoE⁻/⁻ mice and found no differences in white blood cell count, cholesterol profile, plaque composition or lesion area between the two strains. The generated mouse strain enabled us to use intravital microscopy to efficiently detect fluorescent monocytes and neutrophils that were interacting with atherosclerotic endothelium in vivo, and to use confocal microscopy to observe individual cells within lesions.

In order to specifically study neutrophil presence in, and recruitment to, atherosclerotic lesions, we used ApoE⁻/⁻/LysEGFP/EGFP mice in several experiments. By use of intravital microscopy we showed that a vast majority of leukocytes interacting with endothelium on lesion shoulders are neutrophils, suggesting a significant recruitment of these cells to plaque (Paper II). Furthermore, flow cytometry and confocal microscopy showed that neutrophils make up for 1.8% of CD45⁺ leukocytes in the aortic wall of ApoE⁻/⁻/LysEGFP/EGFP mice and that their contribution relative to monocyte/macrophages within lesions is approximately 1:3. Interestingly, we could show that neutrophils accumulate at sites of high density of monocytes and preferentially in shoulder regions of plaques. In some regions of plaque neutrophils actually outnumber monocytes/macrophages.

Atherosclerosis is known to aggravate during systemic inflammatory diseases, and common infections can trigger acute cardiovascular events. In Paper III, we investigate the potential for systemic inflammatory stimuli to induce recruitment of leukocytes to the walls of large arteries in normal and atherosclerotic mice. ApoE⁻/⁻ and control C57Bl/6 mice were challenged with cytokines (TNF-α and IL-1β), LPS or infection with Influenza A in order to induce a systemic inflammatory response. The stimulation triggered a rapid systemic cytokine release and an increase in the relative number of peripheral neutrophils. Interestingly, there was a significant increase in the number of leukocytes adherent to atherosclerotic endothelium as detected with scanning electron microscopy on aortic endothelium. Furthermore, flow cytometry on aortic cells revealed a marked recruitment of neutrophils following inflammatory challenge.

Altogether, this thesis demonstrates that neutrophils are recruited to atherosclerotic lesions and that neutrophils represent the principal subset of leukocytes that interact with atherosclerotic endothelium. Furthermore, neutrophils invade lesions in significant numbers under baseline conditions and are found especially in shoulder regions and at sites of high inflammatory activity. Neutrophil invasion is significantly increased during systemic inflammation. These findings establish neutrophils as potentially important players in the pathogenesis of atherosclerosis.