Atherosclerosis & inflammation: Macrophage heterogeneity in focus

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Summary

Atherosclerosis is a chronic inflammatory condition of the arterial vasculature that is characterised by imbalanced lipid metabolism and maladaptive immune responses leading to the accumulation of lipoproteins, inflammatory cells and fibrous material in the intimal layer of the vessel wall. Over time, plaque progression may lead to rupture and subsequent thrombus formation, which is the most important cause of acute cardiovascular events such as myocardial infarction and stroke.

Macrophages form the main immune constituent of atherosclerotic lesions and are central to plaque development and progression. Their morphology in the vessel wall varies from small inflammatory cells to large, lipid-laden foam cells. Moreover, these cells are characterised by a great degree of functional diversity. Based on their respective characteristics, M1 macrophages are hypothesised to aggravate atherosclerotic plaque development, whereas their M2 counterparts may fulfil a more favourable supportive role in the vessel wall.

In Chapter 1, we describe the pioneering efforts of key figures in the field of atherosclerosis research. Combined, these early lines of evidence provide valuable insight into how our view on the etiology of cardiovascular disease has evolved through the centuries. Thereby, we provide some historical context for the issues addressed in this thesis.

In Chapter 2 we draw on available evidence for the involvement of polarised macrophages in diseases associated with a chronic inflammatory response to consider the functional contribution of these macrophage subsets in atherosclerosis development. Polarised macrophage subsets differentially participate in the pathogenesis of for instance obesity, where a phenotypic switch from M2 to pro-inflammatory M1 cells outlines the onset of surplus weight. Conversely, cancer is often associated with a profound alternative activation profile that dampens anti-tumour responses and facilitates disease progression. In spite of reports recognising the presence of M1 and M2 macrophages in plaques, insight into the temporal and spatial dynamics of these populations in the atherosclerotic vessel wall is limited at best. The effects these changes exert on plaque composition and disease course are of key interest as well.

As such, there is a definitive need to characterise and assess polarised macrophage distribution in atherosclerotic plaques. To address these issues, we call for additional, more specific markers to aid in demarcating macrophage subtypes and their purpose. Furthermore, we advocate the need for direct functional studies in mice lacking appropriate macrophage-specific cytokine receptors to clarify their in vivo relevance.

Chapter 3 expands on Chapter 2 by focusing on new areas of interest with regard to the regulation of macrophage heterogeneity. We address evidence illustrating that macrophages
do not merely display phenotypical differences in response to various environmental triggers, but employ distinct signalling pathways, transcription factors and epigenetic modulators to translate these stimuli into a functional gene program. Continued investigation of these mechanisms will not only greatly augment our general understanding of macrophage heterogeneity, but also direct our efforts to find a reliable intervention that can skew macrophage function and ultimately manage cardiovascular disease risk.

In Chapter 4, we examine the presence and spatial distribution of several macrophage polarization markers in a series of human atherosclerotic lesions. We recognise that gene expression signatures for both M1 and M2 macrophages increase incrementally as plaques develop more advanced morphology. In addition, our results indicate that markers associated with either M1 or M2 polarization allocate to different morphological compartments of the vessel wall. Most importantly however, by identifying M1 macrophages as the dominant phenotype in the plaque shoulder – a section particularly vulnerable to complication – we are the first to offer basic evidence in support of a deleterious role for M1 polarization in atherogenesis.

In Chapter 5, we investigate the hypothesis that the pleiotropic anti-inflammatory cytokine IL-10 exerts its protective effects on atherogenesis through myeloid cells. In support of this theory, loss of IL-10 receptor signalling in primary murine macrophages elicits an exaggerated LPS-induced inflammatory response. However, by transplanting myeloid IL-10R1 deficient bone marrow to atherogenic LDLR⁻/⁻ mice, we are able to demonstrate that deletion of the IL-10R1 gene in myeloid cells grants a strong protection from plaque development. This phenotype involves markedly reduced plasma cholesterol and plaque neutral lipid accumulation, decreased myeloid cell numbers and enhanced apoptotic cell counts relative to lesion area. Concluding, we provide new insights into IL-10 function by establishing that this cytokine provokes conflicting effects on its target (immune) cells in atherosclerosis.

Chapter 6 outlines our pursuit of the mechanisms involved in reduced plasma VLDL and LDL cholesterol in myeloid IL-10R1-deficient LDLR⁻/⁻ mice. We assess systemic cholesterol balance to reveal that loss of myeloid IL-10R signalling impairs intestinal absorption of cholesterol, while amplifying faecal sterol loss through bile-independent pathways. Notably, strong compensatory upregulation of de novo cholesterol synthesis is unable to replenish the resulting deficit. As the ratio of esterified and free cholesterol is disturbed in IL-10R1⁻/⁻ transplanted LDLR⁻/⁻ mice, we subsequently postulate that decreased ACAT2 function in these animals changes the composition of VLDL and LDL cholesterol to direct these lipoproteins towards transintestinal excretion. We conclude that myeloid IL-10R1-deficiency provides resistance from diet-induced hypercholesterolaemia by altering intestinal cholesterol fluxes.
In **Chapter 7**, we explore whether *Schistosoma mansoni*-derived soluble egg antigens (SEA) can be used to prevent atherosclerosis development in mice. Although parasitic helminths and their eggs are strong immunomodulatory agents that can protect from hyperlipidaemia and plaque formation, they also cause harm to their host. Interestingly, subcutaneous SEA-treatment of hyperlipidaemic LDLR⁻/⁻ mice does not produce negative side effects, but indeed strongly attenuates plaque size and severity. In treated mice, SEA prompts an anti-inflammatory phenotype in myeloid cells and reduces plaque inflammation. Thus, our data support SEA as a potential therapeutic agent in cardiovascular disease.

In **Chapter 8**, we hypothesise that Glatiramer acetate (Copaxone®), an immunomodulatory therapy for multiple sclerosis (MS), skews macrophages to an anti-inflammatory phenotype and is thereby able to reduce atherosclerotic plaque formation in LDLR⁻/⁻ mice. We show that incubating bone marrow-derived macrophages with GA augments LPS-induced cytokine production. Most notably, IL-10 gene expression and secretion is strongly increased, whereas pro-inflammatory cytokines TNF and IL-6 are also significantly upregulated. In vivo, fours weeks of subcutaneous GA treatment significantly reduces plasma cholesterol in LDLR⁻/⁻ mice, but does not affect plaque burden or systemic inflammation. Moreover, assessment of *ex vivo* cytokine production by peritoneal macrophages obtained from GA-treated mice reveals similar concentrations to PBS-injected mice. We conclude that GA modifies macrophage TLR-induced inflammation *in vitro*, but is not effective as an immunomodulatory approach under hyperlipidaemic conditions.

Finally, **Chapter 9** discusses the main findings presented in this thesis within the context of the current state of affairs within the respective fields and offers some perspective for the future.