Atherosclerosis & inflammation: Macrophage heterogeneity in focus

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Chapter 1

Introduction - A historic perspective on atherosclerosis research
Given the prevalence of cardiovascular disease (CVD) in man, one could argue that atherosclerosis is a unifying trait of our kind. Together, ischaemic heart disease and cerebrovascular disease (stroke) account for more than a fifth of all deaths worldwide. Recent estimates indicate that 1 in 3 American adults (approximately 80 million people) is afflicted by some kind of CVD. Closer to home, CVD claimed close to 40,000 lives and caused ±380,000 hospital admissions over the course of 2010 alone (data provided by the Dutch Heart Foundation, www.hartstichting.nl). Consequently, our collective battle against CVD is fought on many fronts. As medical science gradually uncovers the inner workings of atherosclerosis development, we find ourselves closer to new and improved ways by which we can prevent, detect and treat myocardial infarction and stroke. Much of the progress we have witnessed over the last decade is founded on the hotly debated premise that high circulating cholesterol levels and inflammation join forces to enhance cardiovascular risk. Since these two topics feature heavily in this thesis, this chapter recounts the early lines of evidence that fuelled the two corresponding theories of atherogenesis: the 'lipid hypothesis' and 'inflammatory hypothesis of atherosclerosis'. This brief historic perspective thereby commemorates some of the groundbreaking work by pioneers of the field and provides a backdrop for upcoming discussions.

The humble beginnings

The term 'atherosclerosis' dates back to 1904, when the German pathologist Felix Merchand used it to describe the key features of arterial lesions; deriving 'athero' from the Greek word for gruel (i.e. the plaque's necrotic core), he used 'sclerosis' to refer to the induration of the vessel wall. The relative novelty of this description would imply atherosclerotic disease is quite a modern phenomenon, where in fact it has troubled mankind for millennia. Ancient texts such as the Hippocratic works (4th century BC) already mention symptoms consistent with angina pectoris and sudden cardiac death. Moreover, detailed examination of Egyptian and North American mummies conducted over the last century revealed not only the existence of atherosclerosis in antiquity, but even comparable histopathology with modern times. Thus, our current understanding of CVD is the culmination of centuries of insightful observation. Claudius Galen (129-200 AD) serves as the single most influential physician in ancient history. He defined the heart as a muscle and recognized the presence and movement of blood in the arteries. Galen also believed blood moved from one side of the heart to the other, but erroneously assumed it went through small pores in the interventricular septum to do so. Although many of his beliefs later turned out to be inaccurate, Galen's teachings held undisputed authority over the medical scientific landscape for centuries after his passing. It was not until the Renaissance that rebellious voices marked the gradual departure...
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from his school of thought. Among the first critics were Paracelsus (1493-1541) and Andreas Vesalius (1514-1564), who at great personal expense openly refuted Galenic medicine, instead promoting observation and experimentation as the foundation of scientific pursuits. Around that time, Leonardo Da Vinci (1452-1519) offered one of the earliest descriptions of atherosclerotic changes. In a display of characteristic brilliance and mechanistic vision he wrote how the nurturing qualities of blood ‘overfed’ the adjacent vessel wall causing it to become thickened, impeding blood flow in the process. Furthermore, he not only illustrated the coronary arteries but also grasped their function. Going forward, the exploits of William Harvey (1578-1657) brought an end to on-going speculation on the architecture of our cardiovascular system; he was the first to correctly describe its two circulatory systems and the flow of blood through the heart. From here, the likes of Heberden (1710-1801), Jenner (1749-1823) and Parry (1755-1822) continued to advance the field by characterising angina pectoris and defining its coronary pathophysiology, while others such as Cruvelheir and Quain continued the steady flow of post-mortem accounts of atherosclerotic changes.

From awareness to understanding - inflammation and cholesterol in the aetiology of atherosclerosis

Over the course of the 19th century, atherosclerosis researchers capitalised on these early developments and started to focus on disease aetiology. In the year 1815, Joseph Hodgson cited inflammation as the cause of atherogenesis, a process he (correctly) believed to occur within the intima. This period in time also saw two celebrated pathologists face-off over their respective views of the pathophysiology involved. Both Rudolph Virchow (1821-1902) and Carl von Rokitansky (1804-1878) observed evidence of cholesterol accumulation and inflammation in atherosclerotic lesions. Specifically, Virchow attributed intimal thickening to retention of soluble components of the blood and noted cellular accumulation in the subendothelial space. Although Virchow lacked the means to assess the origin and significance of these cells, he considered these inflammatory changes to be of primary importance to atherogenesis. Von Rokitansky instead perceived inflammation as something of a secondary nature, but saw himself forced to abandon his views because of the intensity of Virchow’s criticism. Interestingly, this historic dispute was recently settled in favour of Virchow through modern analysis of autopsy specimens from von Rokitansky’s personal collection. Yet, the newly acquired insight regarding a possible inflammatory origin of arterial lesions failed to garner momentum and subsequently fell off the radar altogether. In the first half of the 20th century, the playing field shifted towards Russia, where a selection of papers in short succession kicked off the lipid hypothesis. Following earlier reports of fatty depositions in arterial lesions, Aschoff was the first to define this material as cholesteryl esters in 1907. Along these lines, Windaus in 1910 showed that in comparison to healthy
vessel wall, human arterial plaques contain substantial amounts of cholesteryl esters and free cholesterol. Just 2 years earlier, Ignatovsky had stumbled upon a relation between a cholesterol-rich diet and plaque development in his experimental rabbit model, while pursuing an unrelated hypothesis. It was this study that inspired the landmark experiments by the young experimental pathologist Nikolai Anichkov (for a more personal account of his life I recommend). It was he who demonstrated that pure cholesterol feeding is sufficient to cause atherosclerosis in rabbit arteries. Rather than having to found his conclusions on post-mortem snap-shots of disease, the availability of an inducible model of experimental atherosclerosis allowed Anichkov to track the evolution of atherosclerotic plaques through time. Thus, he gave detailed descriptions of cholesterol crystals and foam cells in arterial fatty streaks, the distribution pattern of plaques in the aorta and the progression of fatty streaks to fibrous atheromas. Furthermore, he learned that the degree of plaque burden was proportional to the extent and duration of hyperlipidaemia and postulated that cholesterol and mononuclear blood cells invaded the arterial wall from the lumen. Although these findings were met with considerable scepticism at the time, they mark the start of the modern era of atherosclerosis research.

Critical support for the lipid hypothesis came when in 1929 Macheboeuf identified lipoproteins as cholesterol’s mode of transport through the circulation. Later, Gofman and his team classified the different lipoproteins according to their separation after ultracentrifugation as very low-, low- and high-density lipoproteins. Over the next decades, these discoveries triggered investigation into human lipoprotein metabolism and its relation to atherosclerosis (beautifully reviewed in), thereby pushing cardiovascular research beyond its roots in pathology to involve more functional biochemical and molecular disciplines. This included work by Müller in 1939, focussing on the familial expression of xanthomatosis, hypercholesterolemia and CVD. He and others were able to establish this syndrome (FH) as a monogenic defect, where heart disease developed as a consequence of elevated plasma cholesterol, thereby ultimately proving Anichkov’s case in a select human population. More than 30 years later, Brown and Goldstein investigated the nature of the gene defect involved and through cell culture techniques found a cellular transport receptor for low-density lipoprotein (LDLR) to be lacking in FH patients. Their discovery would go on to reveal the receptor-mediated endocytosis pathway as a manner of regulating cell behaviour and metabolism. It was also around that time that Russell Ross put forward his response-to-injury theory, focussing on smooth muscle cells, growth factors and matrix deposition as the cause of clinically significant coronary obstruction. This paved the way for the return of inflammation to the forefront of atherosclerotic research. Over the following years, Ross’ theory evolved to increasingly feature lipoprotein modification and inflammation, culminating in publication of his landmark paper in 1999. Thus, together with the likes of Hansson and Libby, Ross revived atherosclerosis as a disease of inflammatory origin.
Macrophages at the interface of inflammation and cholesterol metabolism

Although Virchow already provided thorough reports of lipid-laden foam cells in 19th century human atherosclerosis, these cells had not been immediately apparent as macrophages. As Elie Metchnikoff established the phagocytic nature of mononuclear cells in 1905 \(^{39}\), Anichkov also noted that different leukocytes often infiltrated the aortic wall from the lumen, supporting the view that foam cells represented cells of monocytic origin \(^{17}\). However, it would be decades later, in 1958, before Poole and Florey could provide conclusive evidence that foam cells are derived from circulating monocytes/macrophages \(^{40}\). Still, it remained unclear just how these cells became loaded with cholesterol, as the role of lipoproteins in foam cell formation was incompletely understood. Again, it were Brown and Goldstein who provided a key piece of the puzzle when they realized that although macrophages from FH patients lacked the ability to take up LDL, their plaques consisted of many foam cells. They postulated that modified forms of lipoprotein could be taken up through different receptor pathways, ultimately leading to the discovery macrophage scavenger receptors \(^{41-43}\). Their findings combined macrophages and lipoproteins in the origins of foam cell formation, a view subsequently complemented by the discovery of oxidized LDL (oxLDL) \(^{44}\). Knocking out the scavenger receptors CD36 and SR-A in mice provided the final evidence that oxLDL uptake by macrophages drives foam cell formation and atherogenesis \(^{45,46}\).

Macrophage effector functions are equally influential in homeostasis and disease. Although Metchnikoff was the first to recognise the importance of macrophage phagocytosis in host-pathogen interactions \(^{47}\), it was George Mackaness in the 1960’s who introduced the term ‘macrophage activation’ through his work with the intracellular bacterium Listeria monocytogenes. By analysing a phenomenon he called acquired cellular resistance, he identified that macrophages convey resistance to other unrelated bacterial pathogens after exposure to L. monocytogenes \(^{48}\). Continued efforts revealed how this occurrence depended on a coordinated response between lymphocytes and macrophages, but could not explain how the former influenced the latter \(^{49}\). Following in Mackaness’ wake, Nathan resolved this issue by establishing that antigen-stimulated T-cells activate macrophage microbicidal activities through a soluble factor \(^{50}\) that was ultimately identified as the pro-inflammatory cytokine interferon-γ (IFNy) \(^{51}\). After Siamon Gordon described an alternative type of macrophage activation by interleukin (IL)-4 in 1992 \(^{52}\), the phenotypical disparity in comparison with ‘classical’ IFNy-activated macrophages planted the seed for what would become the M1/M2 paradigm of macrophage heterogeneity \(^{53,54}\).
Aim of this thesis

Macrophages characteristically feature a considerable degree of heterogeneity and plasticity to accommodate for the enormous variety in stimuli and challenges that their microenvironment presents them with. By virtue of their activation status, these cells can be classified into one of several subsets with more or less unique effector profiles. The most frequently used nomenclature in this context is the M1/M2 paradigm, which represents extremes in the spectrum of macrophage subsets and has been steadily expanded over the last decade to allow for other subsets to be incorporated (e.g. M1a-b, M2a-c, Mox, Mhem). M1 macrophages are derived from exposure to IFNγ in the presence or absence of LPS and are uniquely adept at killing intracellular pathogens (as described above) and pro-inflammatory effector functions, characterised by secretion of pro-inflammatory cytokines. M2 macrophages on the other hand include several types of alternative activation that are elicited through IL-4/IL-13, IL-10 and several other mediators. Functionally, these cells are critical to the resolution of (excessive) inflammation, host defense to extracellular parasites and wound healing. With regard to atherosclerosis, due to their pro-inflammatory functions, M1 subsets are hypothesised to aggravate atherosclerosis, while M2 macrophages are expected to attenuate atherosclerotic disease processes. Yet, research addressing the ramifications of polarized macrophage subsets in plaque development is few and far between.

Therefore, the aim of this thesis was to 1) elucidate the functional contribution of macrophage subsets in the development of atherosclerosis and to 2) investigate whether systemic immunomodulation of macrophage subsets can be used to combat atherogenesis.

To this end, Chapter 2 describes some of the lessons learnt in other fields of study involving macrophage heterogeneity and makes an effort to apply this knowledge to atherosclerosis research. Chapter 3 addresses emerging aspects in the regulation of macrophage phenotype and their effector functions and discusses these factors with regard to atherosclerosis development. Chapter 4 deals with the distribution of selected macrophage markers in human atherosclerotic lesions. Here, we were able to provide important insight into the putatively detrimental role for M1 macrophages in atherosclerosis by showing these cells dominate the rupture-prone plaque shoulder. Chapter 5 and Chapter 6 respectively illustrate novel aspects of IL-10 function in atherosclerosis and cholesterol metabolism by revealing that myeloid IL-10R1 deficiency paradoxically attenuates atherosclerosis development, while altering intestinal cholesterol fluxes. Finally, Chapter 7 recognizes the ability of helminth-derived SEA to counter myeloid cell-driven inflammation and atherogenesis in a murine model of atherosclerosis, thereby contributing to the development of novel therapeutic avenues for cardiovascular disease. As another potential therapeutic approach, glatiramer acetate treatment of murine atherosclerosis as described in Chapter 8 sadly failed to affect atherogenesis.
Atherosclerosis research has come of age over the course of the 20th century and now combines lines of evidence from a multitude of disciplines. However, several critical issues that testify to the complex, multifactorial aetiology of cardiovascular disease remain to be resolved. As will be discussed in the chapters to come, the field of macrophage biology holds considerable potential in this regard.
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References


