Macrophage Differentiation and Function in Atherosclerosis: Opportunities for Therapeutic Intervention?

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Abstract

The macrophage is exquisitely sensitive to its microenvironment, as demonstrated primarily through in vitro study. Changes in macrophage phenotype and function within the atherosclerotic plaque have profound consequences for plaque biology, including rupture and arterial thrombosis leading to clinical events such as myocardial infarction. We review the evidence for dynamic changes in macrophage numbers and macrophage differentiation within the atherosclerotic plaque microenvironment and discuss potential approaches to target macrophage differentiation for therapeutic benefit in cardiovascular disease.

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Historical Overview

Atherosclerotic Plaque Composition

Pathologists have long speculated on the mechanisms that underlie the formation of atherosclerotic plaques in patients with myocardial infarction and ischemic stroke. The presence of macrophage foam cells in early focal ath-

erosclerotic lesions (fatty streaks) in the arteries of otherwise healthy young adults identified this disease process as an unusual form of chronic inflammation that begins early in life and that initiates within the tunica intima [1]. In humans, these early ‘fatty streak’ lesions take decades to grow into fibro-fatty plaques, which can develop into either rupture-prone unstable plaques characterized by large areas of necrosis and a higher content of macrophages or stable atherosclerotic plaques containing fewer macrophages and more substantial collagen-containing fibrous cap structures. Postmortem studies can identify ‘culprit’ atherosclerotic lesions that have ruptured and caused fatal arterial thromboembolism [2, 3]. In addition to macrophages, human atherosclerotic plaques contain dendritic cells, T lymphocytes, some B lymphocytes and vascular smooth muscle cells (VSMCs). Clear evidence for adaptive immune responses affecting atherogenesis have been presented from epidemiological studies and experimental models [4]. Current vascular imaging technologies do not allow us to unambiguously distinguish macrophage-rich vulnerable plaques prone to rupture from larger more stable atherosclerotic plaques in high-risk patient groups.

Conceptual Models of Atherogenesis

Competing models to explain the pathological sequence of events that gave rise to atherosclerotic plaques emerged

in the 19th and 20th centuries, including Rudolph Virchow's 'lipid imbibition hypothesis' (ca. 1856), which emphasized the cellular uptake of lipid within atherosclerotic lesions, while the Austrian pathologist Carl von Rokitansky (ca. 1855) emphasized the role of humoral factors in arterial thrombosis associated with atherosclerotic plaques [5]. Both Virchow and von Rokitansky noted features consistent with ongoing inflammation in their atherosclerotic plaque specimens, but a more complete appreciation of the role of inflammation in atherogenesis had to await the development of monoclonal antibodies for immunohistochemistry and mouse models of atherogenesis. In the 20th century, VSMC proliferation and endothelial dysfunction took centre stage. Barrett and Benditt [6] emphasized the role of platelet-derived growth factor in driving clonal proliferation of VSMCs as a key process in atherogenesis, while the work of Russell Ross and colleagues emphasized the role of endothelial damage and subsequent VSMC proliferation in the initiation and progression of atherosclerosis. In a series of influential reviews, Ross [7] expounded the idea that atherosclerosis was the result of a maladaptive 'response to injury' in which changes in activated endothelial cells promoted the recruitment of inflammatory cells, particularly macrophages, within the intima.

In the 1970s, accumulating data from prospective studies such as the Framingham Heart Study and Multiple Risk Factor Intervention Trial clearly implicated dyslipidemia, especially elevated plasma low-density lipoprotein (LDL) cholesterol, as an important risk factor for the development of coronary heart disease. In the 1980s and 1990s, Steinberg et al. [8] championed the role of LDL oxidation as a key driver of atherosclerosis on the basis of experiments showing that modification of LDL by cultured endothelial cells made LDL more atherogenic in a range of *in vitro* assays. In 1995, Williams and Tabas' [9] 'response to retention' model of atherogenesis combined aspects of Ross' and Steinberg's thinking in a model which emphasized the role of trapped apolipoprotein B-containing lipoproteins, such as LDL, within the tunica intima as the key driver of intimal hyperplasia. More recently, the role of local and systemic inflammation as drivers of atherosclerotic plaque progression and especially plaque rupture has gained prominence [10], but our current best medical treatments remain lipid-lowering drugs such as statins and antiplatelet drugs such as aspirin and clopidogrel. Though some of the effects of these and other drugs are thought to have an anti-inflammatory component, so far drugs that specifically target vascular inflammation or macrophage differentiation have not been tested in the treatment of cardiovascular disease.

Experimental Models of Atherosclerosis

Animal models have played an important role in framing our ideas about atherogenesis. All animal models of atherosclerosis rely on dramatic remodeling of endogenous lipoprotein profiles to initiate atherosclerotic lesion development within a realistic time frame [11]. The very earliest experimental evidence for the central role of elevated plasma cholesterol in the development of atherosclerosis came from the studies of Anitschow and Chaltow, who showed that they could induce atherosclerotic lesions in the aortae of rabbits fed a cholesterol-rich diet and that the extent of lesion formation was dependent upon the dose of cholesterol fed to the animal and the duration of feeding [12]. The importance of hyperlipidemia in driving atherosclerotic lesion development was confirmed in other large animal models, notably nonhuman primates [13]. However, it was only with the development of the apolipoprotein E (ApoE)^{-/-} mouse model of atherosclerotic plaque development in 1993, and the subsequent development of the LDL receptor^{-/-} mouse model in 1994, that experimentalists could begin to use powerful genetic technologies and bone marrow transplantation techniques to directly address the role of specific genes and signaling pathways in atherosclerotic plaque initiation and progression [reviewed in 14]. Despite their many advantages for mechanistic studies, murine models of atherosclerosis have significant shortcomings, especially the lack of plaque rupture and subsequent arterial thrombosis and infarction [15]. While animal experiments have shown a pathological role for macrophage activation and differentiation in atherosclerosis, an elusive goal has been to show that the same mechanisms are important in human cardiovascular disease and ideally that they can be targeted for therapeutic benefit [16].

Circulating Monocyte Subsets

Human Monocyte Subsets

Human monocyte subsets in peripheral blood can be distinguished on the basis of the expression of CD14 and CD16 by surface receptor staining [17]. The analysis of multiple cell surface markers, augmented by transcriptomic studies, has been used to define 'classical' CD14⁺⁺CD16⁻ monocytes (approx. 90% of all circulating monocytes) and a less abundant 'nonclassical' CD14⁺CD16⁺⁺ monocyte population [18]. An important observation relevant to the study of atherogenesis is the finding that monocyte subsets defined by CD14 and

CD16 staining exhibit differential expression of adhesion molecules and chemokine receptors known to be important for monocyte adhesion and recruitment [19]. Notably, CD14⁺⁺CD16⁻ monocytes express the chemokine receptor CCR2, while CD14⁺CD16⁺⁺ monocytes do not express it, but both subsets express the CX3CR1 chemokine receptor, which seems to be important both for the patrolling behavior of monocytes along endothelial cell surfaces [20] and in monocyte/macrophage survival [21]. It remains unclear whether there is differential recruitment of each monocyte subset into human atherosclerotic plaques, as is thought to happen in mice [22, 23], although there are a number of reports linking elevated monocyte subset counts (monocytosis) with elevated plasma lipid levels, increased carotid intima medial thickness and elevated risk of cardiovascular disease.

Murine Monocyte Biology

The classic experiments of Ralph van Furth and Zanvil Cohn [24] demonstrated that nearly all murine macrophages present at a site of inflammation are derived from circulating monocytes rather than proliferation of cells present in situ. Reminiscent of the situation in human peripheral blood, FACS analysis allows identification of at least two murine monocyte subsets, Gr1⁺Ly6C^{high} 'inflammatory monocytes' and Gr1⁻Ly6C^{low} 'patrolling monocytes' [20, 25]. Of direct relevance to atherogenesis, ApoE^{-/-} mice develop a Gr1⁺Ly6C^{high} monocytosis, in part due to an expansion of bone marrow progenitor populations and increased mobilization of monocytes from bone marrow via the CCR2 receptor [26]. In murine systems, differential labeling of circulating monocyte subsets has been achieved by intravenous injection of fluorescent latex microspheres. By combining in vivo labeling of monocytes in ApoE^{-/-} and chemokine receptor double-knockout animals with aortic arch transplantation from ApoE^{-/-} donor mice, Tacke et al. [22] were able to show that both CCR2 and CX3CR1 receptors are important for recruitment of the Gr1⁺Ly6C^{high} monocyte population into established atherosclerotic lesions. Using the bead labeling technique, Tacke et al. [22] showed a 73% decrease in the recruitment of CCR2^{-/-}Ly6C^{high} monocytes into the atherosclerotic plaque compared to wild-type Ly6C^{high} monocytes, while Ly6C^{low} monocyte recruitment into plaques was unaffected by CCR2 deficiency. The authors concluded that this was due to both decreased CCR2-dependent mobilization of Ly6C^{high} monocytes from bone marrow as well as decreased CCR2-dependent recruitment from the circulation into

established atherosclerotic plaques [22, reviewed in 25]. Interestingly, CX3CR1 was also required for recruitment of Ly6C^{high} but not Ly6C^{low} monocytes, despite the fact that these cells express significantly higher levels of the CX3CR1 receptor.

While both monocyte subsets are recruited to the atherosclerotic plaque, there are no definitive data tracing the origin of plaque macrophages. Therefore, it is not known if the phenotype of a macrophage is affected by the monocyte subset from which it is derived.

The Macrophage Differentiation Spectrum in vitro

Monocytes respond to their environment by differentiating into a range of heterogeneous macrophages and dendritic cell-like cells. This differentiation is dependent on interaction with a milieu of local factors that ultimately lead to changes in transcription factor activation and hence changes in gene expression [27]. The first stage in monocyte-macrophage differentiation is dependent on growth factors, including granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF). Thereafter, a variety of other stimuli cause polarization into diverse phenotypic states that have been classified by means of in vitro experiments as classically activated M1 cells and alternatively activated M2 cells [27].

The M1/M2 Paradigm in vitro

M1 cells are thought of as being proinflammatory. Their activation is dependent upon interferon (IFN)- γ , a cytokine produced in vivo by T helper 1 (Th1) and CD8⁺ T cells and natural killer cells. This initial stimulus is then followed by a second activation signal, either from other proinflammatory cytokines such as tumor necrosis factor (TNF)- α or from Toll-like receptor activation by either exogenous stimuli such as microbial lipopolysaccharide (LPS) or endogenous stimuli such as minimally modified LDL and oxidized LDL (oxLDL) [28]. M1 macrophages are characterized by a proinflammatory secretome [including interleukin (IL)-1, IL-12 and TNF], prolific generation of bacteriocidal molecules (reactive nitrogen and oxygen species) and enhanced phagocytic capacity [28, 29].

Conversely, the M2 macrophage classification encompasses a spectrum of predominantly anti-inflammatory phenotypes, and in vitro experiments have led to its further subdivision into M2a, M2b and M2c cells. M2a activation is caused by cytokines IL-4 and IL-13, produced

primarily by Th2 cells. These macrophages secrete factors such as fibronectin and insulin-like growth factor, which cause increased deposition of extracellular matrix (ECM) and suggest a role in tissue repair [28]. They have been referred to as ‘wound healing macrophages’ [30]. M2b cells are generated by stimulation with IL-1 β or LPS, as well as immune complexes binding to Fc γ receptors. They secrete predominantly anti-inflammatory mediators, notably IL-10. This is a potent anti-inflammatory cytokine that reduces the secretion of proinflammatory mediators by surrounding cells, including M1 macrophages, and impairs their activity as antigen-presenting cells [28]. Similarly, M2c macrophages, induced by IL-10 and transforming growth factor- β as well as glucocorticoid signaling, also secrete high levels of IL-10. This has led to both M2b and M2c cells being referred to as ‘regulatory macrophages’ [30].

M1/M2 Markers

The evidence for macrophage phenotypic diversity is dependent on the use of cell type-specific markers, with relatively few useful monoclonal antibodies having been identified. A variety of these markers have been used in human and murine macrophages, including nuclear receptors, cell surface markers and differing secretomes. Some commonly used markers are listed in table 1.

It is important to note that many in vitro and in vivo studies have used different markers to identify cells as M1 or M2, with limited evidence that all of these different markers are associated with the same macrophage phenotype. Therefore, cells labeled as M1 or M2 in one study may be phenotypically and functionally different from those labeled as such in a different study using different markers. Furthermore, there is much work in this field with both murine and human macrophages, and we know that there is significant interspecies variability between murine and human macrophage biology. For example, many markers of M2 activation have been shown to be murine-specific (table 1), with no identifiable human homologues. Indeed, transcriptional profiling of human and mouse macrophages shows that approximately 50% of macrophage polarization markers are species-specific [31]. The differences in macrophage biology in mouse and man have recently been reviewed [32] and illustrate the difficulty in making direct comparisons between the two. However, as discussed below, and despite these considerations and caveats, there is evidence that the in vitro classification of macrophage phenotype, or the ‘M1/M2 paradigm’, has some relevance to in vivo atherosclerotic plaque phenotype.

Table 1. Examples of markers used to identify M1 and M2 macrophages

M1	M2
Secretome IL-12, TNF- α , MCP-1	Secretome IL-10, CCL2, <i>Ym1</i> Cell surface Scavenger receptors (e.g. MR), IL1R2
Transcription factors NF κ B	Transcription factors PPAR- γ , <i>FIZZ1</i>
Intracellular enzymes iNOS, <i>ArgII</i>	Intracellular enzymes <i>ArgI</i>

Murine-specific markers are shown in italics. MCP-1 = Monocyte chemoattractant protein-1; CCL2 = chemokine ligand 2; Ym1 = chitinase 3-like 3 lectin; MR = mannose receptor; IL1R2 = IL-1 receptor type II (decoy receptor); NF κ B = nuclear factor- κ B; PPAR- γ = peroxisome proliferator-activated receptor- γ ; FIZZ1 = found in inflammatory zone 1; Arg = arginase; iNOS = inducible nitric oxide synthase.

Macrophage Phenotype within Atherosclerotic Plaques

The atherosclerotic plaque contains a wide range of local factors, some of which are discussed below, including growth factors, cytokines and lipid mediators that have profound effects on monocyte differentiation into heterogeneous macrophage populations. This heterogeneity in atherosclerosis has long been recognized [33], and macrophages expressing markers of M1 and M2 activation have been demonstrated in murine and human plaques [34, 35]. Furthermore, macrophages are known to be plastic cells that retain their ability to respond to their microenvironment and change their phenotype once differentiated. Therefore, changes in the plaque microenvironment are likely to profoundly alter the function of macrophages within it. Indeed, there is recent evidence that in ApoE^{-/-} mice, initially the macrophages in early-stage (‘fatty streak’) plaques are of the M2 type, but as the plaque progresses in size and complexity, they become M1-like [36].

Growth Factors

The growth factors M-CSF and GM-CSF are key components of monocyte-to-macrophage differentiation and are also known to influence macrophage phenotype [37]. Both have been detected in human atherosclerotic lesions [38, 39]. Under basal conditions, M-CSF circulates at de-

tectable levels in vivo, and in vitro, it is constitutively expressed by certain cell types, including endothelial cells, fibroblasts and smooth muscle cells [40, 41]. In contrast to M-CSF, GM-CSF expression is low under basal conditions. However, inflammatory stimuli such as TNF and IL-1 enhance its expression in human arterial smooth muscle cells [42], and oxLDL can do the same in endothelial cells and macrophages [43].

In vitro incubation of monocytes with M-CSF or GM-CSF has contrasting effects on phenotype; M-CSF-differentiated cells secrete a cytokine profile that is more typical of M2 macrophages when stimulated with LPS, whereas those differentiated with GM-CSF have a secretome that is more typical of M1 macrophages [37]. This suggests that with plaque progression, an increase in GM-CSF expression in vivo contributes to the observed increase in M1-like cells.

Differences in the ratio of M-CSF to GM-CSF are known to alter macrophage gene expression, and this has been shown through in vitro experiments to be plastic. Pretreatment of cultured murine macrophages with either M-CSF or GM-CSF, followed by incubation with the other CSF, leads to a partial phenotypic switch towards gene and cytokine expression typical of the second factor [37]. This plasticity has also been demonstrated in human macrophages in vitro with the sequential addition of factors that promote differentiation towards both M1 and M2 phenotypes [44]. Isolated human macrophages were differentiated towards an M1 phenotype with proinflammatory stimuli, including GM-CSF and IFN- γ , and then subsequently exposed to anti-inflammatory stimuli, including M-CSF and glucocorticoid. This microenvironmental switch induced a complete reversal of phenotype, with the loss of M1 markers and upregulation of M2 markers. Similar plasticity was seen in macrophages initially differentiated towards the M2 phenotype.

Plaque Cytokines

Within the plaque, macrophages are exposed to a complex array of cytokines derived from T cells and other macrophages, among other cell types [extensively reviewed elsewhere, e.g. 45, 46]. The advanced plaque, especially in humans, is known to be an environment that contains many Th1-type lymphocytes, with consequently high-level expression of proinflammatory cytokines, including IFN- γ [47], which favor M1 polarization. Indeed, selective inhibition of Th1 differentiation retards atheroma formation in ApoE^{-/-} mice, demonstrating the importance of Th1-cell derived cytokines in atherogenesis [48].

As noted above, the current literature on cytokine expression in the murine atherosclerotic plaque suggests that in early lesions, default differentiation is towards an M2 phenotype, before the plaque progresses and M1 phenotypic differentiation begins to predominate. This is supported by in vivo murine studies showing that IL-4 cytokine knockout mice, with defective M2 differentiation, have reduced fatty streak (early-lesion) formation [49], but less marked differences are observed in advanced plaque burden [45]. Further in vivo evidence comes from studying the macrophage content of murine plaques at different stages of development. Early plaques in the ApoE^{-/-} mouse were rich in IL-4, with undetectable levels of IFN- γ . They were infiltrated primarily by M2 macrophages, identified by the murine-specific M2 marker arginase I. In contrast, lesions from older mice contained high levels of IFN- γ , with IL-4 being almost absent. These plaques contained significant numbers of M1 macrophages, characterized by arginase II expression, as well as M2 cells [36]. Interestingly, this study also confirmed the plastic nature of macrophage differentiation in vitro, in response to the sequential addition of IFN- γ and IL-4 and vice versa. A complete reversal of phenotype, as judged by the expression of either arginase I or arginase II, was demonstrated in vitro, although no direct evidence for this in vivo was presented.

Markers of both M1 (monocyte chemoattractant protein-1) and M2 (mannose receptor) macrophages have also been found in advanced human lesions [34]. Staining for these markers demonstrated that M1 and M2 markers are found in cells in distinct locations within the plaque. M1-like cells tended to be within the lipid core, whereas M2-like cells were found in areas overlying the core, closer to the fibrous cap [50]. Furthermore, the M2-like cells were located in areas with high levels of IL-4 expression, consistent with the idea that macrophage differentiation is influenced by microenvironmental cytokines and also that while the advanced plaque may be Th1/M1 dominant, Th2/M2 phenomena may be focally important. Indeed, the possible importance of a focal Th2/M2 interaction has been demonstrated in a recent study using intraperitoneal murine filarial infection to induce a Th2-driven inflammatory response; this caused rapid in situ proliferation of resident tissue M2 macrophages with interestingly relatively little contribution from recruitment and differentiation of blood monocytes [51].

Taken together, the above work with growth factors and cytokines suggests that the phenotypic state of a

Fig. 1. Macrophage differentiation and polarization within the atherosclerotic plaque. Upon entering the plaque, monocytes differentiate into macrophages. In response to a variety of microenvironmental stimuli, macrophages are then polarized either to a phenotypic state within the M1/M2 activation spectrum or to a phenotypic state that does not fit within this spectrum, as exemplified by the Mox and M4 phenotypes here. Several key factors promoting polarization towards each end of the spectrum are listed. TGF- β = Transforming growth factor- β ; CXCL4 = CXC chemokine ligand 4 (also known as platelet factor 4).

plaque macrophage is determined by both the stage of plaque development during which it enters and the location within the plaque that it occupies.

Lipids and Lipoproteins

Lipid and lipoprotein mediators have been shown to have pleiotropic and seemingly contrasting effects on macrophage differentiation. Global deletion of cholesterol efflux transporters in transgenic mice leads to increased macrophage cholesterol content, including in the plasma membrane. This is associated in vivo with accelerated atherosclerotic plaque formation and in vitro with an increase in the secretion of proinflammatory cytokines, suggesting enhanced polarization towards an M1 phenotype [52]. However, in vitro exposure of murine macrophages to oxLDL leads to increased transcription and activity of the enzyme arginase I, a marker of M2 differentiation, likely due to the effects of lipoproteins in addition to cholesterol uptake, such as their content of activators of peroxisome proliferator-activated receptor- γ , an M2-polarizing factor [53]. Furthermore, it is also known that pure cholesterol itself does not necessarily alter the phenotype of macrophages [54]. Therefore, while macrophage cholesterol uptake and foam cell formation are prominent within atherosclerotic plaques, the exact functional roles of these foam cells are not clear.

Limitations of the M1/M2 Paradigm

The contrasting M1/M2 phenotypes are based heavily on in vitro culture conditions in which a limited number of external factors are applied to monocyte/macrophages; in vivo, these cells are exposed to a much wider range of microenvironmental mediators simultaneously. Furthermore, there remain gaps in knowledge regarding the in vivo concentration of these mediators and their exact cellular sources. Therefore, a strict M1/M2 classification is unlikely to exactly represent in vivo macrophage phenotype, and a continuum of activation states is more likely (fig. 1). Indeed, it has been suggested that there is an infinite number of potential macrophage phenotypes [27], and as discussed below, there have been several recent studies that question the M1/M2 paradigm altogether.

Outside of the M1/M2 Paradigm

Murine macrophages exposed to oxidized phospholipid in vitro have recently been shown to differentiate into a phenotype not fitting into the M1/M2 paradigm, termed the 'Mox' phenotype [55]. These cells expressed a range of 'Mox-specific' genes, including the marker heme oxygenase-1, and functionally had impaired phagocytic capacity relative to M1 and M2 macrophages. Furthermore, the Mox macrophages were generated in vitro from fully differentiated M1 and M2 macrophages, providing further evidence for macrophage plasticity under experimental

conditions. The macrophages expressing Mox-specific markers were subsequently demonstrated *in vivo* in murine plaques and were found to localize separately from M1 and M2 cells, suggesting that *in vivo* plaque macrophage phenotype is also plastic and sensitive to the microenvironment. Perhaps the best direct evidence for *in vivo* macrophage plasticity has been provided not from the study of atherosclerosis but from models of murine tissue injury, in which, for example, a recent cell labeling study demonstrated *in vivo* phenotypic switching of M1 to M2 cells [56].

Macrophage differentiation outside of the M1/M2 paradigm has also been demonstrated in human cells [57]. Surface antigen markers CD68 and CD14 were used to define macrophages differentiated with either M-CSF (CD68⁺/CD14⁺) or GM-CSF (CD68⁺/CD14⁻) *in vitro*. M-CSF/GM-CSF-induced macrophage gene expression did not correlate accurately with the M1/M2 paradigm, with macrophages from each of the groups expressing a range of markers associated with both M1 and M2 differentiation. *In vitro* M-CSF-treated macrophages expressed several genes associated with the M2 phenotype, including IL-13, but also proinflammatory mediators such as monocyte chemoattractant protein-1, a marker of classical M1 activation. Large numbers of these M-CSF-like macrophages were subsequently demonstrated *in vivo* through immunohistochemical studies of established human carotid plaques.

Similarly, *in vitro* differentiation of macrophages with M-CSF and CXC chemokine ligand 4 (platelet factor 4) generates cells which have significantly reduced expression of the scavenger receptor CD163. These have been classified as 'M4 macrophages', and similar macrophages with reduced CD163 expression have subsequently been found in human atherosclerotic lesions, suggesting that the M4 macrophage may be relevant to *in vivo* plaque biology [58].

These studies demonstrate the diversity of macrophage activation within the atherosclerotic plaque, which cannot be fully explained by the *in vitro* M1/M2 classification.

Macrophage Dysfunction and the Vulnerable Plaque

Activated proinflammatory macrophages secrete a multitude of mediators, including cytokines, chemokines and growth factors, that contribute to the nonresolving inflammatory process in atherosclerosis. They activate overlying endothelial cells, promote the influx and retention of other inflammatory cells and cause VSMCs to mi-

grate from the media to the intima of affected arteries. The variety and functions of these mediators are beyond the scope of this review and have been reviewed in detail elsewhere [e.g. 46]. This discussion will focus on the contribution of macrophages to fibrous cap thinning and plaque necrosis, key processes in the formation of the 'vulnerable plaque'.

Fibrous Cap Thinning

Human and murine plaques are covered by a fibrous cap that contains collagens, elastin and proteoglycans and helps to prevent the exposure of thrombogenic plaque contents to the vascular lumen. It is secreted primarily by VSMCs, which proliferate and take on a matrix-secretory phenotype during plaque progression. When the cap is breached, intraluminal exposure to plaque contents leads to sudden intraluminal thrombosis, which is thought to be the pathological mechanism underlying most acute occlusive vascular events in humans. The most common reason for this scenario to occur is plaque rupture, which happens at sites with a thin fibrous cap typically overlying the necrotic core. These sites are predominantly found at the shoulder regions of plaques and are associated with reduction and remodeling of ECM proteins including collagen and elastin [59].

These vulnerable sites have few VSMCs, a result of VSMC apoptosis in advanced plaques, and also a high macrophage content [60]. *In vitro*, macrophages have been shown through coculture experiments to induce VSMC apoptosis. Cell-cell proximity and the interaction of Fas-L with its receptor on VSMCs activates apoptosis, which is augmented synergistically by M1-specific cytokines including TNF- α and IFN- γ [61]. *In vivo*, a reduction in the number of VSMCs is thought to lead to a reduction in ECM secretion in the advanced plaque, which predisposes it to rupture. Furthermore, the apoptotic VSMCs produce procoagulant factors, including thrombin and tissue factor, that promote arterial thrombosis in the event of rupture [62].

In advanced lesions, the existing cap ECM is also pathologically remodeled and weakened by the action of macrophage-derived protease enzymes, including matrix metalloproteinases (MMPs). *In vivo* study of MMP function is unfortunately limited by the fact that experimental murine lesions do not rupture. However, using plaque collagen content as a surrogate marker, it has been shown that macrophage-specific deletion of MMP-13 in the ApoE^{-/-} mouse increases plaque collagen content, which is independent of lesion size and macrophage content [63]. Interestingly, MMP-13 has been shown to be M1

specific in murine macrophages [55]. This supports a role for M1-type macrophages in collagen depletion and hence fibrous cap thinning.

Clinically, increased circulating levels of MMP-9 in patients with documented coronary artery disease are associated with a higher risk of cardiovascular death [64], and statins are known to reduce circulating levels of MMP-9 [65], which may account for some of their atheroprotective effects. There is currently significant interest in inhibitors of MMPs and other plaque protease enzymes as potential therapeutic targets [reviewed in 66].

The Necrotic Core

Advanced vulnerable plaques are characterized by a large necrotic core containing many sick or frankly apoptotic cells, primarily macrophages. Contributing to core formation is a combination of macrophage apoptosis and ineffective phagocytic clearance of apoptotic cells by healthy macrophages, also known as efferocytosis. This combination contributes markedly to the nonresolving inflammatory process that typifies atherosclerotic plaques and is an essential part of plaque progression [15]. Numerous mechanisms have been suggested to explain macrophage apoptosis in advanced atherosclerotic lesions, including a lack of growth factors, the presence of toxic cytokines and oxidative stress. One theory which has been proposed and developed by Tabas and colleagues to explain the underlying mechanism of macrophage apoptosis in advanced plaques is the endoplasmic reticulum (ER) stress model [reviewed in 67]. Many intraplaque factors are known to cause ER stress, including oxidized lipids. Macrophages endocytose these lipids, and some of the intracellular cholesterol that is consequently derived is trafficked to the ER. This leads to ER stress signaling, also known as the unfolded protein response, with consequent upregulation of a number of genes, including several involved in apoptosis such as caspase enzymes and the transcription factor CHOP [67]. In human plaques, apoptosis and CHOP expression are increased in advanced vulnerable lesions with thin fibrous caps or evidence of rupture [68]. This suggests that macrophage apoptosis related to the ER stress signaling pathway is relevant to plaque necrosis and the development of vulnerable plaques.

Therapeutic Opportunities

Recent results from mouse models of atherosclerosis have revived hopes that clinical regression of the disease can be achieved [reviewed in 11], although it should be

noted that the arteries examined in these models are rarely the coronaries, with obvious translational implications. In some of the models, a drastic reduction in non-high-density lipoprotein cholesterol or a significant elevation of high-density lipoprotein resulted not only in reduced macrophage content of plaques but also tilting of the phenotypic profile of the plaque macrophages from M1 to M2 [35, 69], which supports the notion of macrophage plasticity in response to changes in the microenvironment summarized above.

The reduced content of macrophages in murine regression has been ascribed to a stimulation of an emigration pathway [70] or to reduced recruitment with ongoing apoptosis [71] and suggests that one therapeutic strategy would be to promote emigration by the upregulation of chemokine receptors on macrophages (such as CCR7) shown to be involved or the downregulation of macrophage retention molecules, such as netrin-1 [72]. Another would be to block recruitment, a strategy shown to be achievable not just by genetic means (e.g. the ablation of CCR2 in monocytes [73]), but also by the application of a virally derived protein with chemokine receptor blocking ability [74, 75]. It is also possible to regulate apoptosis, but depending on the age of the plaque, there might be adverse consequences; for example, in advanced disease this may contribute to necrotic core expansion and greater plaque vulnerability as suggested by the work of Moore and Tabas [15].

Perhaps more approachable would be small-molecule modifiers of the inflammatory or polarization states of the macrophages, since in each case of murine plaque regression that has been examined from this aspect, there was an increase in M2 and reduction in M1 characteristics, either change expected to be beneficial. However, direct plaque therapies to achieve this have the major limitation that general effects on the immune system need to be minimized. Nonetheless, for a promising agent, given the progress in nanotechnology that has already achieved carriers for toxic drugs to treat cancers [76], it should be possible to target it to plaques. Indeed, the prospect of molecular imaging of atherosclerosis for diagnosis [77], which involves the targeting of imaging agents to plaques, implies that this technology may not only be borrowed for therapeutic use, but can also be combined in a 'theranostic' approach. Furthermore, for the delivery of agents directly to plaques, another approach that has been suggested is to add them to stent coatings. This would not be relevant to those patients in the primary prevention group but could rapidly dampen the inflammatory state of macrophages in a diseased

area in those experiencing acute coronary artery syndromes.

One current view of the 'problem' with plaque macrophages is that while the initial processes described in this review are part of their normal functioning in a hostile environment, there is a failure to resolve the inflammatory state. One factor contributing to this is thought to be impaired efferocytosis of dead or dying macrophages by their healthier neighbors [15], suggesting that enhancement of this function of macrophages may be desirable. A growing number of molecular factors are involved in the efferocytosis process, and some of them may be promising specific targets for manipulation. Alternatively, more global promoters of efferocytosis, such as IL-10 and LXR agonists, may have potential application, especially if they can be delivered to plaques along the lines noted above, in order to avoid either systemic inflammatory immunosuppression (IL-10) or hepatic steatosis (LXR agonist).

Another approach to resolving the plaque inflammatory state is suggested by the work of Wan et al. [78], in which conferring the ability in mice to synthesize long-chain n-3 polyunsaturated fatty acids (normally obtained from fish in the diet) resulted in less atherosclerosis. The inspiration for this approach comes from the work of Serhan and colleagues, who have shown that these fatty acids can be converted into compounds dubbed 'resolvins', which in nonatherosclerotic settings have promoted inflammation resolution [reviewed in 79]. Related phenomena (decreased atherosclerosis progression, enhanced regression) have also been reported for another type of fatty acid, conjugated linoleic acid, though this is a member of the n-6 class [80].

Finally, although this review has been focused on macrophages, as noted earlier, their phenotypic state is heavily influenced by a number of factors secreted by neighboring cells. In particular, the M2 phenotype can be

induced in culture by IL-4 and IL-13, which, in vivo, presumably come from Th2 lymphocytes. One indirect approach to tilting the M1/M2 balance of plaque macrophages would be to change the ratio of Th1 to Th2 lymphocytes, which has recently been reviewed [81].

Conclusion

Macrophage phenotype is heterogeneous, plastic and profoundly affected by local factors within the microenvironment. The relevance of this to atherosclerotic plaque progression, the generation of vulnerable plaques and ultimately the clinical events associated with plaque rupture is in the early stages of investigation. Further work should focus on increasing our in vivo knowledge, particularly to elucidate the exact phenotypes present in early and late, as well as 'vulnerable', human lesions. The effects of manipulating macrophage phenotype within the murine plaque will also be of great interest. As discussed above, we hope this will ultimately lead to the development of novel pharmacological agents aimed at reducing the burden of cardiovascular disease.

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References

- 1 McGill HC Jr, McMahan CA, Gidding SS: Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation* 2008;117:1216–1227.
- 2 Davies MJ, Thomas A: Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death. *N Engl J Med* 1984;310:1137–1140.
- 3 Falk E, Shah PK, Fuster V: Coronary plaque disruption. *Circulation* 1995;92:657–671.
- 4 Mallat Z, Taleb S, Ait-Oufella H, Tedgui A: The role of adaptive T cell immunity in atherosclerosis. *J Lipid Res* 2009;50(suppl):S364–S369.
- 5 Mayerl C, Lukasser M, Sedivy R, Niederegger H, Seiler R, Wick G: Atherosclerosis research from past to present – on the track of two pathologists with opposing views, Carl von Rokitansky and Rudolf Virchow. *Virchows Arch* 2006;449:96–103.
- 6 Barrett TB, Benditt EP: Sis (platelet-derived growth factor B chain) gene transcript levels are elevated in human atherosclerotic lesions compared to normal artery. *Proc Natl Acad Sci USA* 1987;84:1099–1103.
- 7 Ross R: The pathogenesis of atherosclerosis – an update. *N Engl J Med* 1986;314:488–500.
- 8 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL: Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915–924.

- 9 Williams KJ, Tabas I: The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol* 1995;15:551–561.
- 10 Hansson GK: Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685–1695.
- 11 Williams KJ, Feig JE, Fisher EA: Rapid regression of atherosclerosis: insights from the clinical and experimental literature. *Nat Clin Pract Cardiovasc Med* 2008;5:91–102.
- 12 Steinberg D: Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy: part I. *J Lipid Res* 2004;45:1583–1593.
- 13 Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801–809.
- 14 Knowles JW, Maeda N: Genetic modifiers of atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 2000;20:2336–2345.
- 15 Moore KJ, Tabas I: Macrophages in the pathogenesis of atherosclerosis. *Cell* 2011;145:341–355.
- 16 Libby P, Ridker PM, Hansson GK: Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011;473:317–325.
- 17 Passlick B, Flieger D, Ziegler-Heitbrock HW: Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* 1989;74:2527–2534.
- 18 Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB: Nomenclature of monocytes and dendritic cells in blood. *Blood* 2010;116:e74–e80.
- 19 Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, Kourilsky P, Wong SC: Gene expression profiling reveals the defining features of the classical, intermediate, and non-classical human monocyte subsets. *Blood* 2011;118:e16–e31.
- 20 Woollard KJ, Geissmann F: Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol* 2010;7:77–86.
- 21 Landsman L, Bar-On L, Zernecke A, Kim KW, Krauthgamer R, Shagdarsuren E, Lira SA, Weissman IL, Weber C, Jung S: CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood* 2009;113:963–972.
- 22 Tacke F, Alvarez D, Kaplan TJ, Jakubczik C, Spanbroek R, Llodra J, Garin A, Liu J, Mack M, van Rooijen N, Lira SA, Habenicht AJ, Randolph GJ: Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest* 2007;117:185–194.
- 23 Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, Pittet MJ: Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J Clin Invest* 2007;117:195–205.
- 24 van Furth R, Cohn ZA: The origin and kinetics of mononuclear phagocytes. *J Exp Med* 1968;128:415–435.
- 25 Ingersoll MA, Platt AM, Potteaux S, Randolph GJ: Monocyte trafficking in acute and chronic inflammation. *Trends Immunol* 2011;32:470–477.
- 26 Combadiere C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z: Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation* 2008;117:1649–1657.
- 27 Gordon S, Martinez FO: Alternative activation of macrophages: mechanism and functions. *Immunity* 2010;32:593–604.
- 28 Martinez FO, Sica A, Mantovani A, Locati M: Macrophage activation and polarization. *Front Biosci* 2008;13:453–461.
- 29 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M: The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25:677–686.
- 30 Mosser DM, Edwards JP: Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–969.
- 31 Martinez FO, Gordon S, Locati M, Mantovani A: Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006;177:7303–7311.
- 32 Murray PJ, Wynn TA: Obstacles and opportunities for understanding macrophage polarization. *J Leukoc Biol* 2011;89:557–563.
- 33 Poston RN, Hussain IF: The immunohistochemical heterogeneity of atheroma macrophages: comparison with lymphoid tissues suggests that recently blood-derived macrophages can be distinguished from longer-resident cells. *J Histochem Cytochem* 1993;41:1503–1512.
- 34 Bouhrel MA, Derudas B, Rigamonti E, Dievart R, Brozek J, Haulon S, Zawadzki C, Jude B, Torpier G, Marx N, Staels B, Chinetti-Gbaguidi G: PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab* 2007;6:137–143.
- 35 Feig JE, Parathath S, Rong JX, Mick SL, Vengrenyuk Y, Grauer L, Young SG, Fisher EA: Reversal of hyperlipidemia with a genetic switch favorably affects the content and inflammatory state of macrophages in atherosclerotic plaques. *Circulation* 2011;123:989–998.
- 36 Khallou-Laschet J, Varthaman A, Fornasa G, Compain C, Gaston AT, Clement M, Dussiot M, Levillain O, Graff-Dubois S, Nicoletti A, Caligiuri G: Macrophage plasticity in experimental atherosclerosis. *PLoS One* 2010;5:e8852.
- 37 Fleetwood AJ, Lawrence T, Hamilton JA, Cook AD: Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent macrophage phenotypes display differences in cytokine profiles and transcription factor activities: implications for CSF blockade in inflammation. *J Immunol* 2007;178:5245–5252.
- 38 Brocheriou I, Maouche S, Durand H, Brauersreuther V, Le Naour G, Gratchev A, Koskas F, Mach F, Kzyshkowska J, Ninio E: Antagonistic regulation of macrophage phenotype by M-CSF and GM-CSF: implication in atherosclerosis. *Atherosclerosis* 2011;214:316–324.
- 39 Plenz G, Reichenberg S, Koenig C, Rautenberg J, Deng MC, Baba HA, Robenek H: Granulocyte-macrophage colony-stimulating factor (GM-CSF) modulates the expression of type VIII collagen mRNA in vascular smooth muscle cells and both are codistributed during atherogenesis. *Arterioscler Thromb Vasc Biol* 1999;19:1658–1668.
- 40 Filonzi EL, Zoellner H, Stanton H, Hamilton JA: Cytokine regulation of granulocyte-macrophage colony stimulating factor and macrophage colony-stimulating factor production in human arterial smooth muscle cells. *Atherosclerosis* 1993;99:241–252.
- 41 Hamilton JA: Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol* 2008;8:533–544.
- 42 Stojakovic M, Krzesz R, Wagner AH, Hecker M: CD154-stimulated GM-CSF release by vascular smooth muscle cells elicits monocyte activation – role in atherogenesis. *J Mol Med (Berl)* 2007;85:1229–1238.
- 43 Liao F, Berliner JA, Mehrabian M, Navab M, Demer LL, Lusis AJ, Fogelman AM: Minimally modified low density lipoprotein is biologically active in vivo in mice. *J Clin Invest* 1991;87:2253–2257.
- 44 Porcheray F, Viaud S, Rimaniol AC, Leone C, Samah B, Dereuddre-Bosquet N, Dormont D, Gras G: Macrophage activation switching: an asset for the resolution of inflammation. *Clin Exp Immunol* 2005;142:481–489.
- 45 Kleemann R, Zadelaar S, Kooistra T: Cytokines and atherosclerosis: a comprehensive review of studies in mice. *Cardiovasc Res* 2008;79:360–376.
- 46 Tedgui A, Mallat Z: Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 2006;86:515–581.
- 47 Frostegard J, Ulfgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, Hansson GK: Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis* 1999;145:33–43.
- 48 Buono C, Binder CJ, Stavrakis G, Witztum JL, Glimcher LH, Lichtman AH: T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. *Proc Natl Acad Sci USA* 2005;102:1596–1601.

- 49 George J, Shoenfeld Y, Gilburd B, Afek A, Shaish A, Harats D: Requisite role for interleukin-4 in the acceleration of fatty streaks induced by heat shock protein 65 or *Mycobacterium tuberculosis*. *Circ Res* 2000;86:1203–1210.
- 50 Chinetti-Gbaguidi G, Baron M, Bouhlel MA, Vanhoutte J, Copin C, Sebti Y, Derudas B, Mayi T, Bories G, Tailleux A, Haulon S, Zawadzki C, Jude B, Staels B: Human atherosclerotic plaque alternative macrophages display low cholesterol handling but high phagocytosis because of distinct activities of the PPARgamma and LXRalpha pathways. *Circ Res* 2011;108:985–995.
- 51 Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, MacDonald AS, Allen JE: Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science* 2011;10:1284–1288.
- 52 Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, Welch C, Tall AR: Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J Clin Invest* 2007;117:3900–3908.
- 53 Gallardo-Soler A, Gomez-Nieto C, Campo ML, Marathe C, Tontonoz P, Castrillo A, Corraliza I: Arginase I induction by modified lipoproteins in macrophages: a peroxisome proliferator-activated receptor-gamma/delta-mediated effect that links lipid metabolism and immunity. *Mol Endocrinol* 2008;22:1394–1402.
- 54 Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kurikose G, Fisher EA, Marks AR, Ron D, Tabas I: The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol* 2003;5:781–792.
- 55 Kadl A, Meher AK, Sharma PR, Lee MY, Doran AC, Johnstone SR, Elliott MR, Gruber F, Han J, Chen W, Kensler T, Ravichandran KS, Isakson BE, Wamhoff BR, Leitinger N: Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. *Circ Res* 2010;107:737–746.
- 56 Lee S, Huen S, Nishio H, Nishio S, Lee HK, Choi BS, Ruhrberg C, Cantley LG: Distinct macrophage phenotypes contribute to kidney injury and repair. *J Am Soc Nephrol* 2011;22:317–326.
- 57 Waldo SW, Li Y, Buono C, Zhao B, Billings EM, Chang J, Kruth HS: Heterogeneity of human macrophages in culture and in atherosclerotic plaques. *Am J Pathol* 2008;172:1112–1126.
- 58 Gleissner CA, Shaked I, Erbel C, Böckler D, Katus HA, Ley K: CXCL4 downregulates the atheroprotective hemoglobin receptor CD163 in human macrophages. *Circ Res* 2009;106:203–211.
- 59 Skjot-Arkil H, Barascuk N, Register T, Karsdal MA: Macrophage-mediated proteolytic remodeling of the extracellular matrix in atherosclerosis results in neoepitopes: a potential new class of biochemical markers. *Assay Drug Dev Technol* 2010;8:542–552.
- 60 Virmani R, Burke AP, Kolodgie FD, Farb A: Vulnerable plaque: the pathology of unstable coronary lesions. *J Interv Cardiol* 2002;15:439–446.
- 61 Boyle JJ, Weissberg PL, Bennett MR: Tumor necrosis factor-alpha promotes macrophage-induced vascular smooth muscle cell apoptosis by direct and autocrine mechanisms. *Arterioscler Thromb Vasc Biol* 2003;23:1553–1558.
- 62 Flynn PD, Byrne CD, Baglin TP, Weissberg PL, Bennett MR: Thrombin generation by apoptotic vascular smooth muscle cells. *Blood* 1997;89:4378–4384.
- 63 Deguchi JO, Aikawa E, Libby P, Vachon JR, Inada M, Krane SM, Whittaker P, Aikawa M: Matrix metalloproteinase-13/collagenase-3 deletion promotes collagen accumulation and organization in mouse atherosclerotic plaques. *Circulation* 2005;112:2708–2715.
- 64 Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L: Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 2003;107:1579–1585.
- 65 Kalela A, Laaksonen R, Lehtimäki T, Koivu TA, Høyhty M, Janatuinen T, Pollanen P, Vesalainen R, Saikku P, Knuuti J, Nikkari ST: Effect of pravastatin in mildly hypercholesterolemic young men on serum matrix metalloproteinases. *Am J Cardiol* 2001;88:173–175, A6.
- 66 Johnson JL: Matrix metalloproteinases: influence on smooth muscle cells and atherosclerotic plaque stability. *Expert Rev Cardiovasc Ther* 2007;5:265–282.
- 67 Tabas I: The role of endoplasmic reticulum stress in the progression of atherosclerosis. *Circ Res* 2010;107:839–850.
- 68 Myoishi M, Hao H, Minamino T, Watanabe K, Nishihira K, Hatakeyama K, Asada Y, Okada K, Ishibashi-Ueda H, Gabbiani G, Bochaton-Piallat ML, Mochizuki N, Kitakaze M: Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. *Circulation* 2007;116:1226–1233.
- 69 Feig JE, Rong JX, Shamir R, Sanson M, Vengrenyuk Y, Liu J, Rayner K, Moore K, Garabedian M, Fisher EA: HDL promotes rapid atherosclerosis regression in mice and alters inflammatory properties of plaque monocyte-derived cells. *Proc Natl Acad Sci USA* 2011;108:7166–7171.
- 70 Trogan E, Feig JE, Dogan S, Rothblat GH, Angeli V, Tacke F, Randolph GJ, Fisher EA: Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc Natl Acad Sci USA* 2006;103:3781–3786.
- 71 Potteaux S, Gautier EL, Hutchison SB, van Rooijen N, Rader DJ, Thomas MJ, Sorci-Thomas MG, Randolph GJ: Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of ApoE^{-/-} mice during disease regression. *J Clin Invest* 2011;121:2025–2036.
- 72 Ly NP, Komatsuzaki K, Fraser IP, Tseng AA, Prodan P, Moore KJ, Kinane TB: Netrin-1 inhibits leukocyte migration in vitro and in vivo. *Proc Natl Acad Sci USA* 2005;102:14729–14734.
- 73 Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF: Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest* 2007;117:902–909.
- 74 Bursill CA, Choudhury RP, Ali Z, Greaves DR, Channon KM: Broad-spectrum CC-chemokine blockade by gene transfer inhibits macrophage recruitment and atherosclerotic plaque formation in apolipoprotein E-knockout mice. *Circulation* 2004;110:2460–2466.
- 75 White GE, McNeill E, Christou I, Channon KM, Greaves DR: Site-directed mutagenesis of the CC chemokine binding protein 35K-Fc reveals residues essential for activity and mutations that increase the potency of CC chemokine blockade. *Mol Pharmacol* 2011;80:328–336.
- 76 Wang AZ, Langer RS, Farokhzad OC: Nanoparticle delivery of cancer drugs. *Annu Rev Med* 2012;63:185–198.
- 77 Owen DR, Lindsay AC, Choudhury RP, Fayad ZA: Imaging of atherosclerosis. *Annu Rev Med* 2011;62:25–40.
- 78 Wan JB, Huang LL, Rong R, Tan R, Wang J, Kang JX: Endogenously decreasing tissue n-6/n-3 fatty acid ratio reduces atherosclerotic lesions in apolipoprotein E-deficient mice by inhibiting systemic and vascular inflammation. *Arterioscler Thromb Vasc Biol* 2010;30:2487–2494.
- 79 Ariel A, Serhan CN: Resolvins and protectins in the termination program of acute inflammation. *Trends Immunol* 2007;28:176–183.
- 80 Toomey S, Harhen B, Roche HM, Fitzgerald D, Belton O: Profound resolution of early atherosclerosis with conjugated linoleic acid. *Atherosclerosis* 2006;187:40–49.
- 81 Hansson GK, Hermansson A: The immune system in atherosclerosis. *Nat Immunol* 2011;12:204–212.