Published in final edited form as:

Curr Opin Lipidol. 2009 October ; 20(5): 370–378. doi:10.1097/MOL.0b013e3283309848.

Macrophage heterogeneity in atherosclerotic plaques

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Abstract

Purpose of review—The varied behaviour of macrophages and foam cells during atherosclerosis and its clinical sequelae prompt the question whether all these activities can be the property of a single cell population.

Recent findings—Subsets of monocytes with distinct patterns of surface markers and behaviours during inflammation have recently been characterized and shown to have complementary roles during progression of atherosclerosis. A variety of macrophage phenotypes derived from these monocyte subsets in response to mediators of innate and acquired immunity have also been found in plaques. Based on functional properties and genomic signatures, they may have different impacts on facets of plaque development, including fibrous cap and lipid core formation.

Summary—Monocyte and macrophage phenotypic diversity is important in atherogenesis. More work is needed to define consistent marker sets for the different foam cell phenotypes in experimental animals and humans. Cell tracking studies are needed to establish their relationship with monocyte subtypes. In addition, genetic and pharmacological manipulation of phenotypes will be useful to define their functions and exploit the resulting therapeutic potential.

Keywords

atherosclerosis; immunity; inflammation; macrophages; monocytes

Introduction: the role of monocyte/macrophages in inflammation and atherosclerosis

Monocytes and macrophages are essential partners in innate and acquired immunity and as such play a variety of roles in atherosclerotic plaque development and its clinical sequelae (see Fig. 1) [1]. Trapping of LDL by proteoglycans in the vascular intima [2] and subsequent modification of LDL triggers recruitment of circulating monocytes in part by production of chemokines, including CCL2 [3,4]. These monocytes differentiate to macrophages, which migrate to the site of inflammation, degrading extracellular matrix (ECM) barriers as they go. Macrophages efficiently phagocytose and kill any patent infectious organisms and remove cell debris, including cells killed as ‘collateral damage’ during the inflammatory process (Fig. 1). In atherosclerosis and other xanthomas, macrophages take up modified LDL and transform into foam cell macrophages (FCMs) [1], which retain the capacity to reexport cholesterol [5]. Macrophages also help provide immunological memory of an inflammation. Macrophages, dendritic cells (and potentially other cells) present processed peptide antigens to T-lymphocytes together with major histocompatibility complexes

(MHCs) and other coactivators [6]. This may occur locally or in secondary lymphoid tissues if FCMs and/or dendritic cells exit from plaques [7]. Development of acquired immunity may require the recruitment of a separate subset of monocytes in response to a different set of chemokines, including CX₃CL1 and CCL5 [4] (Fig. 1). Macrophages can produce both proinflammatory (e.g. TNF- α , IFN- γ) and anti-inflammatory [e.g. IL-10, transforming growth factor (TGF- β)] cytokines and can therefore promote or dampen the immune response [6]. Macrophages can also mediate tissue repair [8]; in atherosclerosis, macrophages promote migration of smooth muscle cells (SMCs) from the media into the intima and their proliferation forms the fibrous cap [9]. Macrophages (particularly fibrocytes [10]) may directly contribute to ECM synthesis, whereas osteoblast-like macrophages promote calcification. FCMs also secrete angiogenic factors that attract vasa vasorum into plaques as they also do in other forms of granulomas (Fig. 1).

Patients live with coronary atherosclerosis but die of coronary thrombosis secondary to plaque disruption. In most cases, this results from rupture of a thin, highly inflamed, collagen-poor, fibrous cap, which overlies a large, collagen-poor lipid core [11]. Rupture is ultimately a mechanical failure of the weakened cap under the haemodynamic stresses of the cardiac cycle. Macrophages have been strongly implicated in plaque rupture through several mechanisms. First, macrophages are a prominent source of extracellular proteases that could degrade collagen and other ECM components in the cap and core [9]. Second, production of death ligands, reactive oxygen species including nitric oxide and proteases can cause apoptosis of SMCs [12], which in turn leads to fibrous cap thinning [13]. Third, death of FCMs provokes lipid core expansion, especially in advanced plaques [14]. Fourth, macrophages stimulate invasion into the base of the plaque of vasa vasorum, which promotes intraplaque haemorrhage leading to further growth of the core [11]. Plaque rupture often leads to nonlethal coronary thrombosis. In this case, reorganization of the thrombus occurs and is promoted by the fibrogenic action of macrophages [15].

Monocyte subsets and atherosclerosis

Circulating monocytes in the mouse exist as two equally abundant major subsets with differing cell surface marker and chemokine receptor expression patterns, that is, Ly6C^{high}CCR2⁺CX₃CR1^{low} vs. Ly6C^{low}CCR2⁻CX₃CR1^{high}. Ly6C^{high} monocytes are short-lived in the circulation and rapidly move into foci of acute inflammation, such as recent myocardial infarctions [8], and into early atherosclerotic plaques [3,4]. The circulating concentration of Ly6C^{high} monocytes increases in response to proinflammatory stimuli and hypercholesterolaemia [3,4]. Two recent studies concurred that recruitment of Ly6C^{high}CCR2⁺CX₃CR1^{low} monocytes into atherosclerotic plaques depends not only on the chemokine CCL2 but also on CX₃CL1, and that knockdown of these cytokine pathways, therefore, has an additive protective effect [16^{**}, 17^{**}]. One of these studies [16^{**}] but not the other [17^{**}] suggested that mobilization of Ly6C^{high} monocytes from the bone marrow contributes to this synergy. By contrast, Ly6C^{low} monocytes persist longer in the circulation, where they engage in so-called patrolling behaviour, interacting with the endothelium without extravasation [18]. Ly6C^{low} monocytes show delayed incorporation into inflamed and damaged tissues, including infarcted myocardium [8]. Recruitment of Ly6C^{low} monocytes into atherosclerotic plaques depends on CX₃CL1 rather than CCL2, and mobilization of Ly6C^{low} monocytes into the circulation depends on CCR5 [4,16^{**}]. Combadiere *et al.* [16^{**}] showed that antagonism of CCR5 adds to the protection against atherosclerosis in mice defective for CCL2 and CX₃CL1 pathways [16^{**}] and noted a linear relationship in these chemokine knockout studies between plaque size and circulating monocyte numbers, particularly of the Ly6C^{low} population. These experimental studies highlight the potential value of antichemokine interventions with a broad spectrum of action and prolonged time frame such as recently illustrated with lentivirus gene delivery [19].

Observations that CCL2 levels rise quickly in apolipoprotein E (ApoE) mouse plaques and CX₃CL1 only later and in more advanced plaques [20] are also consistent with the idea of sequential recruitment of the two monocyte subsets, similar to that seen after myocardial infarction [8]. Interestingly, CX₃CL1 levels are high in plaques with an unstable plaque phenotype [20], which could be related to the increased propensity of monocytes to transform into dendritic cells. Taken together, this leads to the rather attractive hypothesis that modified LDL initially draws in the Ly6C^{high} monocytes. The immune response to plaque autoantigens, including oxidized LDL, mediated preferentially by recruitment of Ly6C^{low} monocytes [21], then promotes plaque progression and instability. In effect, macrophages derived from Ly6C^{high} monocytes deal with the functions associated with innate immunity (left side of Fig. 1), whereas those derived from Ly6C^{low} monocytes participate primarily in acquired immune responses (right side of Fig. 1). Before this can be accepted, cell tracking experiments are needed to clarify the fate of monocyte subsets, and the effects of immunomodulation on plaque instability need to be tested directly.

Subpopulations of monocytes also exist in humans [18] and exhibit numerous functional differences, including their ability to scavenge oxidized LDL [22]. The abundant CD14⁺CD16⁻CCR2⁺ population in humans is the phenotypic equivalent of the Ly6C^{high} population in mice, whereas the much less abundant CD14^{low}CD16⁺CX₃CR1⁺ population is the phenotypic equivalent of Ly6C^{low} [18,23]. However, functional equivalence with the mouse subpopulations is unclear and directly extrapolating any of the findings from mouse to humans, is therefore, premature. The majority of CD14⁺CD16⁻CCR2⁺ monocytes seem anti-inflammatory, as they produce the cytokine IL-10 in response to bacterial lipopolysaccharide (LPS). Conversely, the smaller CD14^{low}CD16⁺CX₃CR1⁺ population seems proinflammatory because it produces proinflammatory mediators in response to LPS and shows an increase in plasma levels during inflammatory conditions, including atherosclerosis [24]. Puzzlingly, however, a recent genomic study concluded that circulating CD14⁺ monocytes in patients with coronary artery disease express a more anti-inflammatory gene profile [25]. There are also other minor subpopulations in humans and mice [18].

Macrophage heterogeneity and atherosclerosis

As illustrated in Fig. 1, macrophage functions can broadly be categorized by a series of dichotomies, for example, innate or acquired immunity, tissue destruction or repair, immigration or emigration, cholesterol accumulation or release, proinflammatory or anti-inflammatory. These functions could in principle be carried out by distinct subpopulations of macrophages or in some cases lineages related to different monocyte precursors as suggested above. Gordon and Taylor [26] summarized evidence for the existence of two broad types of macrophage phenotype, widely known as classically activated (or M1) and alternatively activated (or M2), each of which might be subdivided. More recently, Mosser and Edwards [27] suggested the existence of at least three phenotypes, classical, regulatory and wound healing and further hypothesized that these might simply be identifiable nodes in a rainbow of phenotypes.

Further progress depends on identifying definitive markers or the different phenotypes, ideally functional, as well as morphological or simply genomic. A phenotypic marker should be independent of activation status, irreversible or at least only slowly reversible, consistently associated with other functional genomic markers of the same phenotype and explicable by epigenetic modification.

Markers derived from in-vitro differentiated macrophages

Numerous studies have compared differentiation of monocytes or activation of differentiated macrophages under two or more conditions using candidate gene or genomic approaches.

Some studies used transformed myeloid cell lines such as human THP-1 or U937 cells or mouse RAW294.7 myeloid cells differentiated towards a macrophage-like phenotype with phorbol myristate acetate (PMA). For example, Ox-LDL uptake [28] or secreted phospholipase A2 treatment [29] was recently reported to induce several markers of dendritic cells in THP-1 monocytes. A limitation of these studies is the concern that the combined effects of transformation and PMA treatment obscure or distort normal phenotypic variation.

Human peripheral blood monocytes have been obtained and purified in a large variety of different ways (e.g. fresh blood vs. buffy coat; elutriation vs. differential centrifugation and adhesion to plastic vs. positive or negative selection on magnetic beads). Most papers have not systematically reported the effect of these interventions on the proportion of different monocyte populations that are purified, their viability or on the identity of contaminants. In animals, peripheral blood, peritoneal, alveolar and bone marrow macrophages have all been used with or without an additional eliciting stimulus. These methodological factors undoubtedly affect the gene expression patterns subsequently observed.

The conditions subsequently used to differentiate and stimulate cells also have a major impact on the resulting phenotype. Differentiation with macrophage-colony stimulating factor (M-CSF) or normal serum yields a population normally referred to simply as 'macrophages'. A wide variety of different proinflammatory regimes have been used to convert these to so-called classically activated macrophages [30]. Generally speaking, LPS treatment causes a more extensive genomic alteration than peptide mediators such as TNF- α or IL-1 β . These mediators that have activation of NF- κ B as a major part of their signalling mechanisms synergize with proinflammatory mediators that use other pathways, particularly IFN- γ . Such combined treatments change a significant proportion of the total genome [30]; functionally, they upregulate phagocytosis, bacterial killing mechanisms, production of proinflammatory mediators and extracellular proteases, consistent with the scheme in Fig. 1 [26]. Incubation of M-CSF-differentiated macrophages with IL-4 or IL-13 gives so-called alternatively activated macrophages, which show many genomic differences from classically activated cells [30,31]. Increased expressions of anti-inflammatory cytokines, IL-10 and TGF- β , suggest that alternatively activated macrophages dampen down classical activation leading to polarization of the response. Upregulation of inducible nitric oxide synthase (iNOS) during classical activation and arginase-1, which competes with iNOS for substrate, during alternative activation, suggests also an important counterregulation of nitric oxide production, enhancing the killing power of classically activated and dampening that of alternatively activated cells. There is a major shift in chemokine and chemokine receptor repertoire [30], which may allow alternatively activated macrophages to exit sites of inflammation and travel to secondary lymphoid tissues and participate in immune regulation. Enhanced production of mediators of smooth muscle and endothelial recruitment [e.g. thrombospondin-1 (TSP-1)], and vascular endothelial growth factor A (VEGFA) in alternatively activated macrophages may enhance their ability to promote granuloma formation and, ultimately, tissue repair (Fig. 1). Indeed TSP-1 deficiency was recently shown to impair fibrous cap formation in mouse atherosclerotic plaques and to promote inflammation and necrotic core formation owing to defective phagocytosis [32*]. Production of clearance receptors, such as the mannose receptor (CD206), also suggests a function of alternatively activated macrophages in tissue repair.

Incubation of isolated macrophages with oxidized or acetylated LDL has been widely used as a model for foam cell formation. Oxidized LDL increases the expression of markers of classical activation such as metalloproteinase-1 (MMP-1) [33] and iNOS, through activation of NF- κ B. However, it also induces markers of alternative activation, including arginase-I, which was shown to be due to activation of peroxisome proliferator-activated receptor- γ

(PPAR- γ) [34]. Other studies have shown activation of dendritic cell markers [28]. The ability of different components of modified LDL to activate several signalling pathways probably accounts for this rather complex behaviour in terms of macrophage phenotypes.

Incubation of either classically or alternatively activated macrophages with IL-10 or TGF- β leads to inactivated phenotypes. For example, MHC-II complexes are upregulated in both macrophage phenotypes, and in both cases expression is downregulated by IL-10. MHC-II can, therefore, be seen as a marker of macrophage activation rather than phenotype.

Other macrophage phenotypes can also be generated *in vitro*. Differentiation of monocytes with M-CSF and receptor activator of NF- κ B ligand (RANKL) yields osteoclast-like macrophages [35]. Differentiation of monocytes with granulocyte macrophage-CSF colony stimulating factor (GM-CSF) and IL-4, IL-13 or IL-21 generates populations with many characteristics of dendritic cells.

The phenotypes observed in atherosclerotic plaques may not directly correspond to those generated *in vitro*. A recent study compared monocytes differentiation with M-CSF (M-macrophages) or GM-CSF and IL-10 (GM-macrophages) [36^{*}]. GM-macrophages had rounded morphology *in vitro* and overexpressed the marker 25F9 and genes involved in cholesterol efflux. M-macrophages were elongated and overexpressed markers CD14 and CD16 together with several genes associated with alternative activation, including IL-13, MMP-12 and CD163. Based on CD14 and CD16 expression, human carotid plaques had an excess of M-like over GM-like FCMs but intermediate forms also occurred. GM-like macrophages predominated in normal artery segments but lacked 25F9 antigen, which was, therefore, an activation rather than phenotypic marker. These studies illustrate the importance of exploring the colocalization of several markers in order to define the presence of a given phenotype. In atherosclerotic plaques, of course, macrophages do not interact with individual cytokines but with a soup of mediators produced in an autocrine manner and from other tissue and inflammatory cells. Coculturing human monocytes with a mixed population of T-lymphocytes differentiates monocytes into three distinct subpopulations based on the expression of CD14, CD36 and LDL receptor (LDLR) [37]. Coculturing mouse macrophages with different defined T-helper lymphocyte populations yields different responses in terms of MMP production [38].

Ex-vivo studies with nonfoamy macrophage and foam cell macrophage generated *in vivo*

We have championed using *ex vivo* analysis of FCMs generated *in vivo*. One approach is to digest plaques directly, but many macrophages do not survive this harsh procedure [3]. To avoid this difficulty, as shown in Fig. 2, macrophages can be allowed to invade subcutaneous sponges in rabbits or mice, whereupon nonfoamy macrophages (NFMs) or FCMs are generated in the presence of normocholesterolaemia or hypercholesterolaemia, respectively [39]. FCMs and NFMs are easily isolated from the sponges and subjected to functional, morphological and genomic analyses. Combining this with gene transfer and knockdown provides a powerful platform to investigate function (Fig. 2). Finally, the main conclusions can be verified in experimental and human plaques using immunocytochemistry (ICC) and other tools. Galis *et al.* [40] used this system to show that FCMs constitutively secrete MMP-1 and MMP-3, which we later showed using gene manipulation results from steady upregulation of NF- κ B [33]. More recently, we showed that rabbit FCMs isolated in this way contain populations of cells with downregulation of arginase-I, a feature of classical activation, and upregulation of MMP-12, a feature of alternative activation. Macrophages with arginase-I downregulation or MMP-12 upregulation did not occur in early fatty streaks but were deeply situated near the lipid core in both rabbit and human plaques [39,41]. Low arginase-I increases nitric oxide production and favours atherosclerosis in rabbits [39,42]. MMP-12 overexpression promotes atherosclerosis in

rabbits [43,44], whereas MMP-12 knockout decreases atherosclerosis and reduces aneurysm formation in mice [45,46]. These observations suggest that FCMs in advanced plaques suffer both classical and alternative activation, both of which could have adverse consequences for plaque evolution (see Fig. 1).

More recently, still, we identified subpopulations of FCM in rabbit subcutaneous sponges based on differential expression of MMP-14 and its inhibitor, tissue inhibitor of MMPs (TIMP)-3 [47^{**}]. MMP-14^{high}TIMP-3^{low} FCMs were more fibroblast-like in morphology, more invasive through matrigel, proliferated more rapidly and underwent apoptosis more readily under LPS or starvation challenge than MMP-14^{low}TIMP-3^{high} FCMs. These *in vitro* properties suggest a role in lipid core formation, and consistent with this MMP-14^{high}TIMP-3^{low} macrophages were deeply situated near the lipid core [47^{**}]. MMP-14 is known to be upregulated by inflammatory cytokines, whereas TIMP-3 is upregulated by IL-4 and downregulated by IFN- γ [48], which suggests that the MMP-14^{high}TIMP-3^{low} FCMs are classically activated.

Studies on tissue sections

Immunochemical heterogeneity of human atherosclerotic plaque macrophages has been recognized for many years. Poston and Hussain [49] suggested some of this could be the result of differences between recently recruited, blood-derived macrophages compared with longer-residing cells. A variety of CD markers have been used to distinguish subpopulations of macrophages and FCMs in plaques. For example, CD14⁺ macrophages, similar to macrophages differentiated with M-CSF *in vitro*, predominate in human atherosclerotic plaques, whereas CD14⁻ macrophages, similar to GM-CSF-treated macrophages *in vitro*, are more abundant in areas devoid of disease [36^{*}]. CD16 has been proposed as a marker of plaque macrophages participating in acquired immune responses in part through CD40-CD40L [50]. The haptoglobin/haemoglobin receptor CD163 and the mannose receptor CD206 have been associated with alternatively activated macrophages phenotypes. For example, Bouhlef *et al.* [51] recently found macrophages expressing CD206 and other markers of alternative activation in human carotid plaque macrophages and noted a different distribution from FCMs and markers of classical activation. Interestingly, CD163 and CD206 did not codistribute, which suggests there may be at least three phenotypes in plaques. Boyle *et al.* [15] showed more recently that intraplaque haemorrhage provokes accumulation of a CD163^{high}human leucocyte antigen (HLA)-DR^{low} macrophage subpopulation and suggested that these NFMs are responsible for clot resolution. CD163 may, therefore, be a marker for the repair phenotype [27] but is also associated with anti-inflammatory features such as production of IL-10 and haemeoxygenase-1 [52]. Variations in mannose-binding lectin A and C distribution between superficial and deep FCMs in early plaques in humans and LDLR null mice were recently noted; lectins accumulated around sites of macrophage necrosis [53^{*}]. Interestingly, combined knockout of both lectins led to greater atherosclerosis in mice, which suggests that they are also markers for a protective macrophage phenotype.

Atherosclerotic plaques have been compared with other chronic granulomas. For example, the nodules of tuberculosis, like plaques, have central areas of necrosis surrounded by fibrous tissue and a microvascular plexus; both are subject to sporadic ulceration, haemorrhage and thrombosis and both contain leucocytes in all states of activation. Komohara *et al.* [54] found that macrophages encircling tuberculosis granulomas were predominantly CD163⁺, whereas the macrophages within granulomas were negative or only weakly positive. Similarly, nonfoamy CD163⁺ macrophages were observed in the outer layers of atherosclerotic plaques, whereas FCMs associated with the lipid-rich core in advanced lesions were negative or only weakly positive for CD163 [54]. Reminiscent of this distribution, we found islands TIMP-3⁻ FCMs surrounded by TIMP-3⁺ FCMs in the

shoulder regions of human atherosclerotic plaques [47^{**}]. MMP-7 and MMP-12 are selectively localized in macrophages juxtaposed between the necrotic core and the rupture-prone shoulders of human plaques [41]. MMP-11 is detected exclusively in advanced lesions [55]. An increase in MMP levels and loss of TIMP-3 could, therefore, be responsible for collagen degradation within the lipid core of advanced plaques.

Taken together, these observations provide evidence for the presence of several macrophage phenotypes in plaques. Recently recruited macrophages and FCMs probably have an immature, partially activated phenotype, which fits neither with the pattern of classical nor alternative activation observed *in vitro*. Mature macrophages and FCMs may become a mixture of classically and alternatively activated phenotypes with either protective or adverse consequences (see Fig. 1). Thrombus formation may provoke an additional repair phenotype.

Genetic manipulation studies

A more extensive review would be necessary to document the results of knocking out each of the potential marker genes for classical and alternative macrophage activation. Many have been discussed in other recent reviews [6,56]. Suffice it to mention here that deletion of cytokines or their receptors that favour classical activation of macrophages (e.g. IL-1 β , IFN- γ) consistently retards atherosclerosis and leads to a more stable plaque phenotype in mice [6]. A recent study added to this literature by showing that deletion of the G-protein coupled receptor G2A increased classical activation of macrophages and promoted atherosclerosis [57]. The situation with alternative activation is more surprising. Deletion of the IL-4 receptor decreases atherosclerosis [6] and dramatically reduces aneurysm formation in mice [58]. Hence, the concept that classical activation is adverse and alternative activation beneficial is probably oversimplistic.

Pharmacological modulation of macrophage phenotype

It is well established that nuclear hormone receptors for corticosteroids, estrogens, PPAR agonists and liver X receptor (LXR) agonists [59-61], and anti-inflammatory cytokines IL-10 and TGF- β [6] can all deactivate classically and alternatively activated macrophages *in vitro* and *in vivo*. These mediators have been shown to dampen inflammation in general and have beneficial effects on atherosclerosis. On the other hand, this benefit is bought at the cost of increased susceptibility to certain infections and a reduced ability to detect malignancy. Agents that selectively affect macrophage subpopulations are not widely available but might provide a different spectrum of benefits. The nuclear hormone receptors, PPAR- γ and LXR- α , are upregulated during alternative activation of macrophages [59-61]. Macrophage-specific overexpression of LXR- α greatly reduces atherosclerosis in mice, through increased cholesterol efflux and possibly also through reduced classical activation [62]. Conversely, gene knockout of PPAR- γ was recently shown to prevent alternative activation of macrophages in mice [63]. Moreover, Bouhlel *et al.* [51] recently described that PPAR- γ activation primes human monocytes to differentiate towards an anti-inflammatory phenotype that also expresses more PPAR- γ and could, therefore, experience positive feedback. PPAR- γ activation was, however, unable to reverse classical activation or switch macrophages from classic to alternative activation programmes either *in vitro* or in plaques [51]. An exciting recent study from Hughes *et al.* [64^{*}] suggested by contrast that the sphingosine-1-phosphate-receptor-1 (S1P1) agonists do have this capacity, at least *in vitro*. Activation of S1P1, a guanine nucleotide coupled receptor, decreased both LPS-induced iNOS induction and LPS repression of arginase-1, at least in part through antagonism of NF- κ B. S1P1 agonists are known to decrease atherosclerosis in mice and it will, therefore, be interesting to document their effects on foam cell phenotypes *in vivo*. Likewise, data regarding the effects on macrophage phenotypes of newer anti-inflammatory

regimes, including inhibitors of lipoprotein-associated phospholipase A2 [65**] and mitogen-activated protein kinase kinase kinase 4 [66**], are eagerly awaited.

Conclusion

Although heterogeneity of monocytes, macrophages and more recently FCMs is becoming more widely appreciated, its consequences for atherogenesis and acute coronary syndromes are only beginning to be investigated.

Defining whether several markers consistently cosegregate, as for example CD163⁺ and MHCII⁺ in the macrophages that organize thrombus [15], will help to identify distinct phenotypes. How numerous, and how interconvertible these phenotypes are needs to be clearly established, in part from cell-tracking experiments. It seems likely that chemokines and other inflammatory mediators orchestrate macrophage phenotype distributions but the details are far from elucidated. Fully identifying the environmental factors necessary to recruit and activate monocyte and macrophage subpopulations will be of utmost importance. Although there is limited evidence for reversibility of phenotypes *in vitro* [67], experiments, with lipid lowering, for example, are necessary to validate this *in vivo*. Questions surrounding the roles of different FCM phenotypes in the various aspects of plaque biology, including fibrous cap formation and destruction are as important. So far, classically activated macrophages seem the most obvious culprits in plaque disruption and hence acute coronary events because they have enhanced abilities to kill neighbouring cells and destroy the ECM. However, alternatively activated macrophages could also promote plaque progression through immune activation and by expressing a deleterious complex of mediators, including some growth factors and proteases. Elucidating the role and regulation of FCM phenotypes will undoubtedly uncover new conventional therapeutic targets or strategies for immune modulation to reduce myocardial infarction.

Acknowledgments

The authors' work is supported by the British Heart Foundation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 429–430).

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elongated and overexpressed markers CD14 and CD16 together with several genes associated with the M2 phenotype, including IL-13, MMP-12 and CD163. Human carotid plaques had an excess of M-like over GM-like FCM but intermediate forms also occurred. GM-like macrophages predominated in normal artery segments but lacked 25F9 antigen. The results point to the diversity of macrophages phenotypes in atherosclerosis and hint also at their plasticity.]

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Figure 1. Origins of macrophage diversity

Recruitment of two different populations of monocytes and action of different mediators may lead to distinct macrophage phenotypes. Uptake of modified lipoproteins converts these to foam cells that may exert a variety of functions in plaque development. GM-CSF, granulocyte macrophage-colony stimulating factor; LPS, lipopolysaccharide; M-CSF, macrophage-colony stimulating factor.

Figure 2. A platform for studying function of foamy and non-foamy macrophages generated *in vivo*

Generation of nonfoamy and foam cell macrophages in subcutaneous sponges in rabbits yields pur preparations that can be subjected to analysis or gene transfer to determine functions that can be verified by immunocytochemistry in tissue sections. FCM, foam cell macrophage; ICC, immunocytochemistry; MMP, metalloproteinase; NFM, nonfoamy macrophage.