

# Macrophages: cause or cure in atherosclerosis?

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## Abstract

Monocytes are a key cell type responsible for the generation of atherosclerosis. Monocytes/macrophages also play key roles in plaque growth and plaque instability. In contrast, recent research has demonstrated that macrophages can also suppress inflammation and promote healing and fibrosis. Some of this complexity relates to the presence of different macrophage subtypes that are induced and activated by different stimuli within the plaque. Macrophage functions include lipid uptake and oxidation with resultant foam cell formation, release of pro or anti-inflammatory cytokines, phagocytosis of dead macrophage and vessel wall cells, release of destructive enzymes such as matrix metalloproteinases, and presentation of antigen to immune cells. Each of these functions is likely to be occurring simultaneously in different subtypes within the complex environment of the plaque. While selective manipulation of monocytes/macrophages has clearly demonstrated their proatherogenic role in early plaque development, their role in advanced plaques has been more difficult to elucidate. Nonetheless, switching of a proinflammatory to an anti-inflammatory/reparative phenotype represents an attractive target for therapeutics in atherosclerosis. ■ Heart Metab; 2013;60:5–8

**Keywords:** Apoptosis; atherosclerosis; inflammation; macrophages.

## Introduction

Macrophages are an almost constant feature in atherosclerosis, present from the earliest stages to advanced plaques. Macrophage invasion in response to retained lipoproteins, lipid accumulation and oxidation, inflammatory cytokine release, and their death have been viewed as major causes of atherosclerosis, and drivers of progression of established plaques. Indeed, reduction in monocyte numbers and prevention of monocyte ingress dramatically suppress atherogenesis [1]. These roles have resulted in the widespread view that macrophages are “bad news” in plaques, and the targets of both established and new therapeutics. Like many simplistic views, recent

research has indicated that it is (at least partially) wrong. Macrophages can also be protective, particularly against ongoing inflammation and hemorrhage, which are major drivers for transition to an unstable plaque phenotype and plaque growth.

## Macrophages: not one type, but many

Research over the past decade has found that circulating monocytes and macrophages in plaques consist of different types, whose role, regulation, markers and products differ [2]. In mice, hypercholesterolemia is associated with an increase in the inflammatory monocyte subset, known as Ly6c<sup>hi</sup>, which can enter developing plaques readily. Although there are significant differ-

**ABBREVIATIONS**

**DC:** dendritic cells; **GM-CSF:** granulocyte macrophage-colony stimulating factor; **IFN:** interferon; **IL:** interleukin; **M-CSF:** macrophage colony stimulating factor; **MHEM:** macrophage phenotype induced by hemoglobin–haptoglobin complexes; **MMP:** matrix metalloproteinase; **MOX:** macrophage phenotype induced by oxidized phospholipid; **ROS:** reactive oxygen species; **TGF:** transforming growth factor; **TNF:** tumor necrosis factor; **VSMC:** vascular smooth muscle cells

ences between monocyte subsets in humans versus mice, the Ly6c<sup>lo</sup> subset is more associated with inflammation resolution [3]. Monocytes express a variety of chemokine receptors, cell surface selectins and integrins for migration and retention in atherosclerosis [1, 4]. Once within the plaque, monocytes differentiate into cells with macrophage or DC-like properties.

In atherosclerosis in humans, macrophages have been categorized into a variety of different types, based in part on the mechanisms underlying their activation and their proposed function. Therefore, a common classification of monocytes/macrophages is based on involvement in proinflammatory processes (M1, classically activated) versus those that undertake resolution of inflammation and repair (M2, alternatively activated) (see *Table 1*) [5]. To these opposing functions other differences can be added, including primary involvement with innate or acquired immunity, tissue destruction or repair, immigration or emigration, and cholesterol accumulation or release. However, in-vitro studies suggest that these forms can be

derived from the same precursors and can interchange, driven by specific growth/differentiation factors, T-helper type 1 and 2 cytokines, lipoproteins and lipids, and certain transcription factors [2] (see *Table 1*). In addition, human plaques appear to contain macrophages with M1 or M2 phenotypes, but also intermediate phenotypes. This is not surprising given the complex environment of the plaque, but means that human plaque macrophages may not correspond to the phenotypes, markers and functions seen in vitro. Further complexities occur with the lack of definitive lineage markers, a mixture of lineage and activation markers, and the lack of functional evidence for each subset in vivo (reviewed in Johnson and Newby) [2]. In addition, further macrophage subtypes are present in plaques, including those arising by exposure to Mox, which can arise from both M1 and M2 types [6], and Mhem [7], often regulated through similar pathways. For example, both Mox and Mhem are regulated by the transcription factor Nrf2 [8].

**Macrophage function in atherosclerosis**

The diversity of macrophage subtypes in atherosclerosis is reflected in a huge diversity of function. Macrophage function ranges from lipid uptake and oxidation with resultant foam cell formation, release of pro- or anti-inflammatory cytokines, phagocytosis of dead macrophage and vessel wall cells, release of destructive enzymes such as MMP, and presentation of antigen to immune cells (reviewed in Moore and Tabas) [9]. The relative importance of each function depends on the model under study, and how advanced the plaques are. For example, in early atherogenesis, the

Macrophage type	Putative function	Increased by	Activation decreased by
M1 (classically activated)	Proinflammatory, secrete MMP	M-CSF, TNF $\alpha$ , IFN $\gamma$	IL-10, TGF $\beta$
M2 (alternatively activated)	Anti-inflammatory, fibrosis, secrete IL-10 and TGF $\beta$	GM-CSF, IL-4, IL-13	IL-10, TGF $\beta$
Mox (activated by oxidized phospholipids) [6]	Decreased phagocytotic and chemotactic capacity	Oxidized phospholipids	
Mhem (activated by hemoglobin–haptoglobin complexes) [7]	Scavenge hemoglobin–haptoglobin complexes, reduced ROS release, increased survival, secrete IL-10	Hemoglobin–haptoglobin complexes, IL-10, heme oxygenase 1	

*GM-CSF*, granulocyte macrophage-colony stimulating factor; *IFN $\gamma$* , interferon  $\gamma$ ; *IL*, interleukin; *M-CSF*, macrophage-colony stimulating factor; *Mhem*, macrophage phenotype induced by hemoglobin–haptoglobin complexes; *MMP*, matrix metalloproteinase; *Mox*, macrophage phenotype induced by oxidized phospholipid; *ROS*, reactive oxygen species; *TGF $\beta$* , transforming growth factor  $\beta$ ; *TNF $\alpha$* , tumor necrosis factor  $\alpha$ .

recruitment of inflammatory macrophages clearly drives atherosclerosis; thus blocking macrophage/endothelial cell interactions or reducing monocyte numbers/function inhibits atherogenesis. Unfortunately, in many studies these manipulations are systemic, while the chemokines/chemokine receptors are not specific for macrophages, making it difficult to be sure that effects are only mediated through macrophages.

Studies in established lesions are harder both to do and to interpret. Macrophages in these lesions are exposed to cholesterol crystals that have been shown in some studies to activate inflammasomes and promote inflammation [10, 11]. Macrophages can induce apoptosis of vascular smooth muscle cells (VSMC) by both ligand-dependent pathways and cytokine secretion [12], potentially reducing the covering fibrous cap and promoting plaque rupture. Macrophages can also reduce cap thickness by release of MMPs. Indeed, cap infiltration with macrophages is one of the most consistent features of human plaques that have ruptured [13]. Macrophages and VSMC are potent phagocytes, responsible for clearing debris and apoptotic cells within the “necrotic core”. However, this “efferocytosis” is defective in advanced lesions [14], possibly by competition for phagocytic receptors by oxidized lipids [15]. Dead macrophages themselves contribute to the necrotic core, and thus macrophage death should promote core formation by both increased contribution and defective clearance. In contrast, if macrophages promote inflammation, then killing them might be protective in atherosclerosis. In fact, the literature in this area is contradictory (see Clarke and Bennett [16] for review), and recent evidence suggests that unlike VSMC death, macrophage death is not particularly proinflammatory [17]. Indeed, in most cases definitive evidence of the role of specific macrophage subtypes *in vivo* is lacking. This is partly because of the ability of different subtypes to interchange, the lack of specific markers that can be used to deplete them either from the circulation or plaque, and their rapid replenishment from the circulation [18]. It is also apparent that global increases or decreases in monocytes/macrophages both in the circulation and in the plaque can have different effects at different stages of the disease [18].

### Role of dendritic cells in atherosclerosis

DC are immune cells that process and present antigen, leading to signals to adjacent T lymphocytes. DC are present both in normal vessel walls and in plaques,

where some of them may derive from monocytes [4]. Plaque DC appear able to present antigen and activate plaque T cells [19], but also both to proliferate and form foam cells in early atherogenesis [20]. However, again their role is unclear, as targeted reduction leads to only modest effects in early lesions, or mixed effects in advanced plaques [19].

### Conclusions

So what is the answer to the question posed at the beginning – Macrophages: cause or cure in atherosclerosis? The answer is probably both. In early lesions, the evidence that inflammatory macrophages promote atherosclerosis is overwhelming, particularly when plaque development is driven by hyperlipidemia. In advanced plaques the evidence is more mixed, and the situation in humans is complex, and does not necessarily correspond to that seen in mice or in human cells *in vitro*. The beneficial effects of statins in atherosclerosis are associated with a relative reduction in cells with macrophage markers in lesions; however, the relative effects of statins on different macrophage subtypes are unclear. The challenge for potential therapeutics is harnessing the reparative properties of macrophages to resolve inflammation in advanced lesions, while simultaneously reducing the ongoing recruitment of macrophages and their differentiation into a proinflammatory subtype. ■

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