

STATE-OF-THE-ART PAPER

Monocytes in Coronary Artery Disease and Atherosclerosis

Where Are We Now?

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Despite improvements in interventional and pharmacological therapy of atherosclerotic disease, it is still the leading cause of death in the developed world. Hence, there is a need for further development of effective therapeutic approaches. This requires better understanding of the molecular mechanisms and pathophysiology of the disease. Atherosclerosis has long been identified as having an inflammatory component contributing to its pathogenesis, whereas the available therapy primarily targets hyperlipidemia and prevention of thrombosis. Notwithstanding a pleiotropic anti-inflammatory effect to some therapies, such as acetyl salicylic acid and the statins, none of the currently approved medicines for management of either stable or complicated atherosclerosis has inflammation as a primary target. Monocytes, as representatives of the innate immune system, play a major role in the initiation, propagation, and progression of atherosclerosis from a stable to an unstable state. Experimental data support a role of monocytes in acute coronary syndromes and in outcome post-infarction; however, limited research has been done in humans. Analysis of expression of various cell surface receptors allows characterization of the different monocyte subsets phenotypically, whereas downstream assessment of inflammatory pathways provides an insight into their activity. In this review we discuss the functional role of monocytes and their different subpopulations in atherosclerosis, acute coronary syndromes, cardiac healing, and recovery with an aim of critical evaluation of potential future therapeutic targets in atherosclerosis and its complications. We will also discuss technical difficulties of delineating different monocyte subpopulations, understanding their differentiation potential and function. (J Am Coll Cardiol 2013;62:1541–51) © 2013 by the American College of Cardiology Foundation

Atherosclerosis accounts for one-fifth of all deaths in the world (1). It is the leading cause of death in the United Kingdom, with more than 600,000 deaths annually due to its complications (1). Acute coronary syndrome (ACS) is an acute pathology associated with atherosclerotic plaque rupture and interruption of coronary blood supply to myocardial tissue. Acute coronary syndrome carries a high mortality rate both en route to the hospital and after receiving treatment. Acute coronary syndrome is also associated with high morbidity, especially when heart failure develops due to extensive myocardial damage. Hence, ACS forms an important field for research with an obvious need for improvement of current medical management and introduction of new therapeutic targets. Better understanding of the underlying pathophysiological mechanisms leading to plaque development and rupture is essential to meet this need.

Inflammation clearly contributes to the pathophysiology of atherosclerosis. Infectious agents, such as *Chlamydia pneumoniae*, have been detected in coronary atherosclerotic lesions (2). Human atherosclerotic plaques contain viruses as well as numerous bacterial signatures, including nucleic acids, and peptidoglycan (3). In addition, several studies suggest positive associations between oral bacterial colonization-levels and increased risk of atherosclerosis (e.g., coronary artery disease [CAD]) and cerebrovascular events (4,5), supporting the link between atherosclerosis and an inflammatory/infective pathophysiology (5,6). The low-grade inflammation associated with CAD is acknowledged in day-to-day clinical practice, with an American Heart Association/Center for Disease Control consensus report (7) recommending measurement of C-reactive protein in asymptomatic subjects at intermediate risk for future coronary events (10-year risk of 10% to 20%) and in selected patients after an ACS (7). In fact, JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) trial indicated that statin therapy might reduce numbers of adverse cardiovascular events in subjects with normal low-density lipoprotein (LDL) range but high C-reactive protein concentrations.

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**Abbreviations
and Acronyms**

- ACS** = acute coronary syndrome
- CAD** = coronary artery disease
- DC** = dendritic cells
- Ig** = immunoglobulin
- IL** = interleukin
- LDL** = low-density lipoprotein
- MCP** = monocyte chemoattractant protein
- MI** = myocardial infarction
- MMP** = matrix metalloproteinase
- ROS** = reactive oxygen species
- TGF** = transforming growth factor
- TNF** = tumor necrosis factor
- VEGF** = vascular endothelial growth factor

Although it is pertinent to note that LDL levels were lower in intervention compared with placebo groups, an anti-inflammatory role of LDL lowering cannot be excluded (8). The ongoing CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) and CIRT (Cardiovascular Inflammation Reduction Trial) trials directly investigate the potential role of anti-inflammatory therapies in reducing vascular events, both on a secondary and primary basis, respectively. Further evidence for the importance of inflammation in cardiovascular disease is seen from the ruptured plaque histology with abundance of macrophages, a thin fibrous cap, and smooth muscle cell loss due to apoptosis (9).

The innate immune system plays a major role in the initiation and propagation of atherosclerosis, with monocytes/macrophages being the key players in this process (10). Monocyte involvement in the development of atherosclerotic plaques was reported in the 1970s, with monocyte accumulation demonstrated in porcine atherosclerotic lesions (11).

Three indirectly related processes, which involve monocytes, have been identified in atherosclerosis (Fig. 1). Monocytes have been shown to play a role:

1. During the long-term process of initiation and formation of an atherosclerotic plaque, presumed to be accelerated by different risk factors, including smoking, hypertension, hyperglycemia, and critically hyperlipidemia;
2. During the acute inflammatory phase that follows destabilization, rupture of the atherosclerotic plaque, and acute thrombus formation in ACS; and
3. During healing, where they reside in the myocardial tissue in the hypoxic phase during an acute coronary event and might promote myofibroblast accumulation, angiogenesis, and myocardial healing and remodeling, thus showing a protagonist or antagonist influence in post-ACS recovery.

In the present review, we critically examine these 3 roles of monocytes and evaluate data on the modulation of monocyte function indicating the future direction of novel therapeutic interventions. Several characteristic features of monocytes are important in explaining this multitude of actions, namely, varying subpopulations, high plasticity and trafficking capacity, and existence of multiple reservoirs and hematopoietic maturation sites.

**How Can the Diversity of
Monocyte Functions Be Explained?**

Monocytes account for 3% to 8% of peripheral blood leukocytes. They are mononuclear cells often characterized by typical kidney-like shaped nuclei, but they are more accurately described by their expression of various surface receptors (12). They are the main component of the innate immune system that is responsible for counteracting exogenous bacterial, viral, and fungal infections mainly by phagocytosis (13). However, they are also involved in endogenous inflammatory processes. Monocytes contribute to atherogenesis through promoting leukocyte recruitment to plaques, and their roles are also mediated by activation of downstream signaling pathways, such as nuclear factor kappa-B pathway (14). Indeed, monocytes have been directly implicated in a number of chronic inflammatory conditions, including glomerulonephritis, rheumatoid arthritis, lung fibrosis, and atherosclerosis (15,16).

Cell surface receptor expression allows discrimination between monocyte subpopulations and was first described in murine models. Palframan et al. (17) and Geissmann et al. (18), with *CX3CR1* knockout mice, demonstrated that peripheral blood monocytes differ in *CX3CR1*, *CCR2*, and *CD62L* expression. Monocytes expressing *CCR2*, *CD62L*, and low levels of *CX3CR1* seemed to be preferentially recruited to inflamed sites by virtue of their recognition of *CCL2* (monocyte chemoattractant protein [MCP]-1). Conversely, the *CX3CR1*^{high} monocytes could migrate into non-inflamed sites (18) or migrate later during the recovery period after an acute inflammation (19).

In humans, “classical” monocytes, which represent 80% to 85% of the total population of circulating blood monocytes, can be identified by high expression of *CD14* and lack of *CD16* expression (also referred as *Mon1*). They are considered inflammatory mediators and represent the predominant subpopulation identified in atherosclerotic plaques (20). These monocytes also express *CCR2*, *CD62L*, and *CD64* (21). The migration of this subpopulation depends strongly on *MCP-1* secreted by resident macrophages (22).

Another human monocyte subset is defined as the *CD14+CD16++* cells, and it is referred to as “non-classical” monocytes or *Mon3* population. They express high levels of *CX3CR1* but do not express *CCR2* or *CD62L* (23,24). This subtype depends on fractalkine (or *CX3CL1*, a soluble chemokine-like domain) for attraction and recruitment to endothelial surfaces. Fractalkine is expressed on activated endothelial cells as a transmembrane-anchored adhesion receptor, thus attracting and arresting monocytes from the circulation into the atherosclerotic plaque. Indeed, *CX3CR1* knockout mice fed on a high-fat diet showed a significant reduction in monocyte recruitment to the vascular wall and reduced atherosclerotic plaque formation. In fact, genetic deletions of *CCL2*, *CX3CL1*, or their cognate receptors, *CCR2* and *CX3CR1*, markedly reduced atherosclerotic lesion size in murine models of atherosclerosis

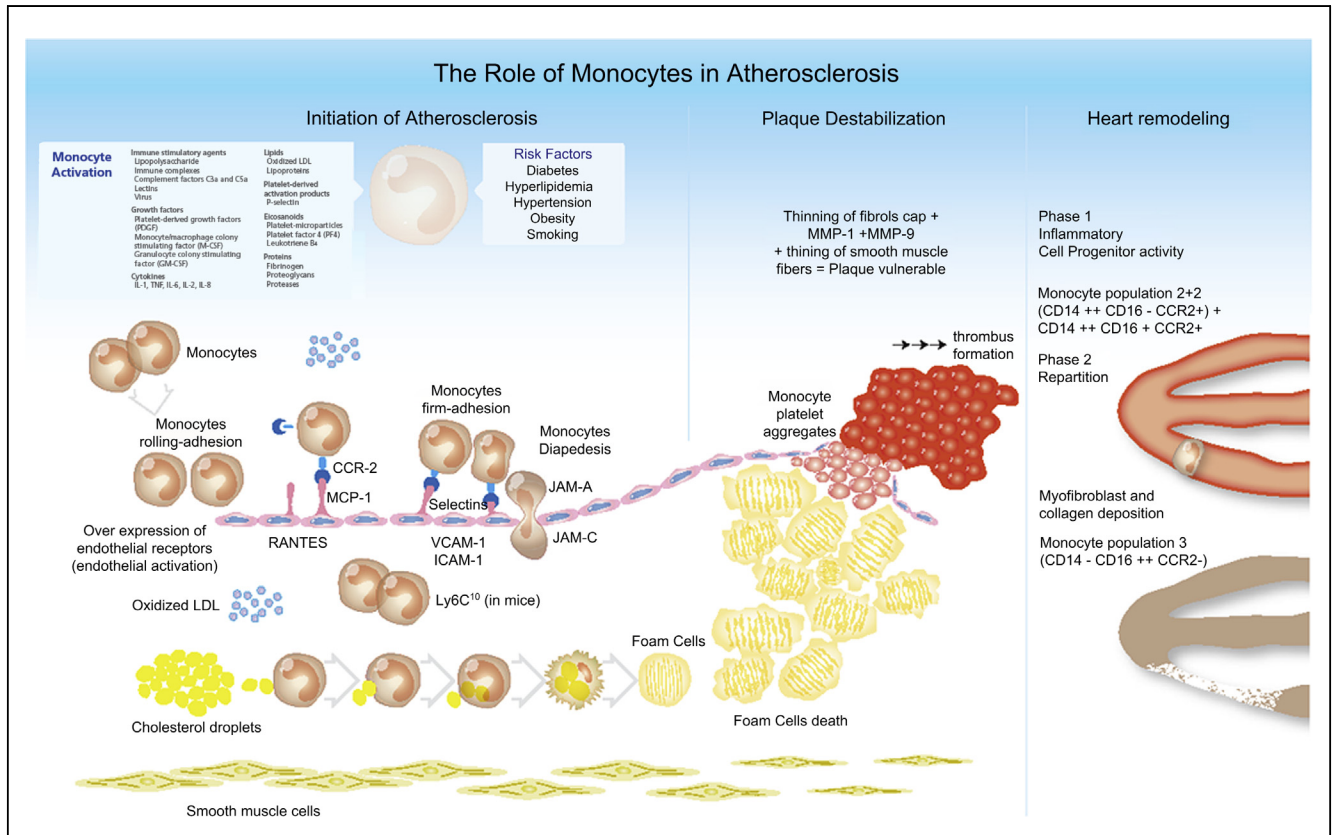


Figure 1 Triple Role of Monocytes at Different Stages of the Atherosclerotic Process

The 3 panels depict the functions of monocytes. Monocytes patrolling in the circulation are activated by different factors. They traffic to the damaged/activated endothelium. The dysfunctional endothelium over-express monocyte chemoattractant protein-1 ligand and adhesion molecules (vascular cell adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1) on its surface. After rolling and attachment to the endothelium, the monocytes cross the endothelial surface (diapedesis). In the subendothelial space monocytes differentiate to macrophages via macrophage colony stimulating factor. The macrophages ingest oxidized low-density lipoprotein (LDL) via scavenger receptors, especially CD36, forming “foam” cells. These undergo a process of apoptosis/necrosis that perpetuates the formation of further lipid-laden macrophages. The **middle panel** depicts events during an acute coronary syndrome with plaque rupture, thinning of the fibrous cap on plaque surface, and monocyte platelet aggregates. The **right panel** illustrates cardiac repair, acutely with attraction of monocytes CD14++CD16–CCR2+ (Mon1) and later in the phase 2 of remodeling where CD14+CD16++CCR2– (Mon3) (and potentially CD14++CD16+CCR2+ (Mon2) cells) alters the extracellular matrix remodeling by myofibroblast deposition and angiogenesis, leading to thinning of the infarcted cells. CCR = CC chemokine receptor; JAM = junctional adhesion molecule; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; RANTES = regulated upon activation, normal T-cell expressed and secreted.

(25). When the 3 chemokine receptors CCR5, CCR2, and CX3CR1 were blocked, the maximal reduction in the atherosclerotic plaque formation was evident, suggesting that all monocyte subpopulations are involved in atherogenesis (26). Table 1 summarizes the characteristics of different monocyte subsets in humans and in mice.

Differences between monocyte subpopulation functions have been exemplified in an elegant in vivo mouse study by Auffray et al. (27), who postulated that non-classical/resident monocytes constantly patrol healthy tissues through long-range crawling along the endothelium. During acute inflammation these monocytes use CX3CR1 and the integrin lymphocyte function-associated antigen-1 to “home” on the inflamed tissues on an “as required basis.” In humans, Cros et al. (28) indicated that CD14 low (i.e., CD14+CD16++) “non-classical” monocytes also have similar patrolling properties and are involved in the innate local surveillance of tissues and the pathogenesis of autoimmune diseases.

More recently, a third human monocyte subpopulation has been identified as “intermediate” CD14++CD16+ (also called Mon2) cells (29). They are reported to be a predominant type of monocytes expressing Tie-2 (an angiopoietin receptor), which has been implicated in angiogenesis. The presence of the 3 distinct monocyte subsets was recently confirmed by gene microarray analysis (21,29). The “intermediate” subset has the highest expression of major histocompatibility complex class II molecules, whereas “non-classical” CD14+CD16++ monocytes are characterized by high expression of cytoskeletal re-arrangement genes, inflammatory cytokines, and CD294 (21).

The varying nomenclature used to describe monocytes poses a problem in unified interpretation of animal and human data. Because most of the current knowledge in the field originates from murine models, effort is directed toward establishment of parallels between monocyte subsets across species. Scarce information on their functions adds to the

Table 1 Comparison of Different Monocyte Subpopulations Phenotype and Functions

Characteristic		Inflammatory/Classical	Newly Described Intermediate Population	Resident/Nonclassical
Human		CD14++CD16-CCR2+ CX3CR1 ^{lo} ; CD62L+ CD115+ (Mon 1)	CD14+CD16+CCR2+, CX3CR1 ^{hi} , CD62L-, CD115+ (Mon2)	CD14+CD16++CCR2- VCAM ^{hi} CCR2 ^{lo} CD64low (Mon3)
Mice		Ly6-C ^{hi} , CCR2+, CXCR1 ^{lo} , CD62L+, CD115+, CD11-, MHC class II -ve	No distinct model is available; however, has characteristics of Ly6-C ^{hi} models	Ly6Clow, CX3CR1hi, CD62L-, CD115, MHC- class II +ve
Recruitment		Early in acute inflammation	Early in acute inflammation	Late in acute inflammation
Reservoirs		Spleen	None known	Unknown
Function	Acute	Accumulate in injured myocardium and perform inflammatory and proteolytic function	? Inflammation	Assumed role in granulation tissue formation and angiogenesis, later mobilization
	Chronic	Accumulate in atherosclerotic plaque in response to activated endothelium.	Given high expression of CD163 and CD204 scavenger receptors then accumulation in atherosclerotic plaque is possible	Accumulate in atherosclerotic plaque via CCR5 and CX3CR1

MHC = major histocompatibility complex; VCAM = vascular cell adhesion molecules.

complexity of drawing reliable conclusions on the physiological and pathological roles of monocyte subsets in humans. For example, features of the so-called “intermediate” subsets are increasingly demonstrated and recognized in a wide range of pathological conditions. An “intermediate” pattern of the subset might be partly due to some overlap with other subsets (particularly with “non-classical” monocytes), when their definition is solely based on their CD14 and CD16 expression. More accurate delineation of CD14++CD16+ and CD14+CD16++ monocytes can be achieved by additional marker CCR2 (29), with “intermediate” monocytes being “CD14++CD16+CCR2+” and “non-classical” monocytes being “CD14+CD16++CCR2-.” Of note, CD14++CD16+ monocytes show the highest of all monocyte expression of many surface receptors, particularly those involved in reparative processes (e.g., CXCR4, Tie2, vascular endothelial growth factor [VEGF] receptors type 1 and 2). This, together with the evidence of specific enrichment of this subset in bone marrow, indicates that it is unlikely to just represent an “intermediate” state between the other 2 subsets but rather a unique and distinct monocyte subpopulation. Although it is not yet entirely clear whether specific monocyte subsets are pre-determined to differentiate into particular types of tissue macrophages and dendritic cells (DC), published data suggest existence of common features and links between CD14++CD16- (Mon1) and CD14++CD16+ (Mon2) monocytes and M1 and M2 polarized macrophages, respectively. Also in vivo, Ly-6C^{lo} and Ly-6C^{hi} monocytes from mice differentiate more readily into M2-like cells and M1-like macrophages, respectively, but macrophage development also depends on surrounding microenvironment and interaction(s) with other cell types, such as lymphocytes (30,31). Accordingly, the assignment of numerical dominators (i.e., Mon1, Mon2, and Mon3) for human monocyte subsets might not be appropriate until more data are available on the relationships between the subsets.

Monocyte Trafficking and Developmental Plasticity

High trafficking ability is another characteristic feature of monocytes. A hallmark of monocyte trafficking is the

capacity of monocytes to traverse from the circulation into areas of injury/inflammation, aiming for resolution of infection and contribution to the restoration of the tissue integrity via differentiation of different types of tissue macrophages and DC. However, presumably “reparative” properties of the monocytes might fail and lead to a disease state, with atherosclerosis being an example. Indeed, monocytes are precursors of lipid-laden “foam” cell macrophages, which are a critical component of atherosclerotic plaques. Even mature monocyte-derived macrophages do not lose their mobility entirely, under certain circumstances (which are not entirely understood) monocyte/macrophages, including “foam cells” can migrate from the vascular wall back into the circulation (31). In several studies monocyte migration from atherosclerotic plaques led to plaque regression under experimental conditions (32,33). In a mouse model, statin therapy augmented the egression of the plaque macrophages via removing the inhibitory effect on CCR7 and independently of lipid levels (34), possibly representing an additional pleiotropic effect of statins.

Monocytes are also characterized by an extremely high developmental plasticity, being able to differentiate under appropriate stimulation into different cell types ranging from epithelial, endothelial, cartilage cells to functional fibroblasts, cardiomyocytes, and neuronal cells. Most of this work has been done under experimental conditions, and the in vivo and (more importantly) clinical relevance is only beginning to emerge (as discussed in the following text). More information on the role of monocytes in atherosclerotic plaque development and progression can be found in the [Online Appendix](#), including [Online Table 1](#).

Monocytes in the ACS

Acute coronary syndrome refers to a clinical spectrum ranging from those for ST-segment elevation myocardial infarction (STEMI) to non-STEMI or unstable angina (35,36). Acute coronary syndrome is almost always associated with rupture of an atherosclerotic plaque and partial or complete thrombosis of the infarct-related artery. Most cases of ACS occur from disruption of a previously non-occlusive

but unstable (vulnerable) plaque (37,38). The characteristic features of a vulnerable plaque include a thin fibrous cap, a higher predominance of macrophages in the cap, smaller collagen content, and a large, lipid-rich necrotic core with overlying thrombus and platelet aggregates (39).

Monocytes promote destabilization of the fibrous cap leading to the plaque rupture. This is mainly orchestrated by matrix metalloproteinases (MMPs) (40,41). Activated macrophages produce a wide range of lytic enzymes, including MMPs (e.g., MMP-1, -2, -3, -8, -9, and -14) (42). Increased expression and activity of MMPs has been noted in vulnerable plaque regions, whereas elevated serum MMPs have been demonstrated in patients with ACS (43).

Plaque rupture could lead to downstream occlusion of coronary flow to the myocardium, the principle mechanism of STEMI. Monocytes have a role in thrombus propagation contributing to the coagulation cascade during the acute event. Patients with ACS show features of procoagulant monocyte activation with exposure of tissue factor (44). Monocyte-platelet aggregates, and markers of monocyte and platelet activation involved in regulation of their function are also increased in ACS patients, persisting even after one month of the acute event (45). Also, microparticles derived from monocytes are abundant in ACS and support faster fibrin formation (46).

Monocyte adherence to extracellular matrix and extravasation to the injured tissue induces the expression of a multitude of cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 and IL-6 (potent inflammatory cytokines); platelet-derived endothelial cell growth factor (a potent chemoattractant and mitogen for fibroblasts); transforming growth factor (TGF)- α and - β (which contributes to fibrosis, by stimulating extracellular matrix release, primarily collagen, from myocardial fibroblasts); macrophage colony-stimulating factor (cytokine necessary for macrophage survival), and insulin-like growth factor (47,48).

Monocytes are also thought to participate in tissue injury. Downstream occlusion of blood supply to the myocardium (ischemia) followed by pharmacological or interventional revascularization therapy (reperfusion) results in the ischemia-reperfusion cascade. During hypoxia, reactive oxygen species (ROS) leakage from mitochondria is increased (49). The ROS-modified biomolecules formed during ischemia stimulate infiltration of inflammatory cells, including monocytes, thus mediating an acute inflammatory response leading to cell injury and necrosis. Recently, a new model of “innate autoimmunity” for ischemia/reperfusion injury has been introduced, which integrates mechanisms of both intrinsic ischemic cell injury and initiation of an extrinsic innate immune response (50). This hypothesis that the intrinsic changes associated with cell injury are augmented by a second wave of the innate immune system involvement largely represented by monocytes (e.g., mediated by the complement system, immunoglobulin [Ig] M and their receptors) helps to explain the continued loss of

myocytes despite reperfusion and, in some in vitro studies, despite elimination of inflammatory cells (51).

Within the subintimal space, monocytes mature into DC and macrophages, each with its separate polarity, and inflammatory functions that have further effect on tissue necrosis (30). Macrophages are currently considered to comprise 2 types: the classically activated (pro-inflammatory M1 type); and the alternatively activated M2 acting as anti-inflammatory cells (47). The M1 macrophages promote inflammation and extracellular matrix destruction. The IL-1 β secretion from M1 induces MMP-9 and TGF- β secretion and stimulates fibroblast proliferation (47). Macrophage phagocytosis of dying cells also triggers TGF- β production (52). Of interest, in murine models, monocyte tissue residence time was found only to be approximately 20 h, with persistently high rates of recruitment to infarcted myocardium days after the acute event and disproportionately slower rate of exiting from infarcted tissues at a maximum rate of 13%/day (53).

A mouse model of myocardial infarction (MI) has demonstrated substantial functional differences between monocyte subsets in the course of the infarction. For instance, mice Ly-6C^{hi} monocytes (considered to be equivalent to CD14⁺⁺CD16⁻CCR2⁺ human cells) are mobilized early after MI onset and show distinct phagocytic properties. In contrast Ly-6C^{lo} monocytes (considered to be the equivalent of human CD14⁺CD16⁺⁺CCR2⁻ cells) showed anti-inflammatory properties and were critical for myocardial healing and reverse remodeling in MI, promoting myofibroblast accumulation, angiogenesis, and the deposition of collagen (19). The production of these subsets seemed to follow a biphasic mode, with an early release of Ly-6C^{hi} followed by a later production of Ly-6C^{lo} (19).

At the other end of the monocyte differentiation spectrum, levels of circulating DC (both plasmacytoid as well as myeloid DC) have been found to be significantly reduced in patients after an ACS, compared with healthy control subjects (54). It is thought that this is due to increased recruitment of DC to the infarcted tissue. This hypothesis was supported with immunohistochemistry findings indicating an increase in the DC and T-cell infiltration of peri-infarct zone (55).

Monocytes and Cardiac Remodeling

Despite advances in medical and interventional therapy for ACS, many patients still develop heart failure. The process of post-MI myocardial recovery depends on numerous factors at the cellular and molecular levels. After the initial tissue damage induced by hypoxia, an acute inflammatory response ensues with recruitment of leukocytes to the infarcted areas (19). Subsequent release of ROS, phagocytosis, fibroblast accumulation, angiogenesis, and tissue formation occurs, ultimately leading to cardiac remodeling and recovery modulated by the activity of these recruited cells. The role of inflammation is important. For example, an increase of the

pro-inflammatory cytokine TNF- α with a corresponding decrease of anti-inflammatory cytokine IL-10 is associated with adverse/reverse ventricular remodeling (56). Together, leukocytes degrade extracellular matrix constituents and macromolecules released by the injured cells and aid clearance of dead cardiomyocytes and their debris.

Historically, monocytoysis has been associated with left ventricular dysfunction post-MI, with recruited monocytes releasing multiple cytokines, such as IL-1 α and -1 β , IL-6, and TNF- α , which are negatively associated with myocardial healing and development of heart failure (57). The balance between removing dead myocytes and prompt initiation of regeneration might determine patient outcomes.

Monocyte injection and their cardiac recruitment through MCP-1, IGF-1 secretion, enhanced remodeling with improved post-MI cardiac contractility in mice (58). Monocytes are also involved in myocardial fibrosis and post-infarction scar formation, whereas their release of angiogenic factors (e.g., VEGF) promotes angiogenesis in and around the healing tissue.

Of note, prolonged Ly-6C^{hi} monocytoysis early after MI onset could impair myocardial healing in a murine model (59,60). By contrast, depletion of Ly6C^{hi} monocytes early post-infarct led to increased areas of debris and necrotic tissue with impaired ventricular healing (19). This accords with results of a previous murine study where post-infarct macrophage depletion led to ventricular dilation and myocardial wall thinning with concurrent decrease in neovascularization, myofibroblast, and collagen depositions (61). Recently, depletion of DC led to a similar result, with sustained expression of inflammatory cytokines such as IL-1 β , TNF- α , IL-18, and MMP-9 as well as a decrease in IL-10 within the infarcted area in a mouse model. Interestingly, there was an increase in Ly6C^{hi} monocytes in DC-depleted mice (62). It could be postulated that this is due to interruption of DC-induced “negative feedback” on differentiation signaling that controls monocyte subpopulations/macrophage interaction. Hence, depleting DC might lead to uncontrolled monocyte differentiation and disruption of this phagocytic system, thus illustrating the intricate balance and cross talk between different components.

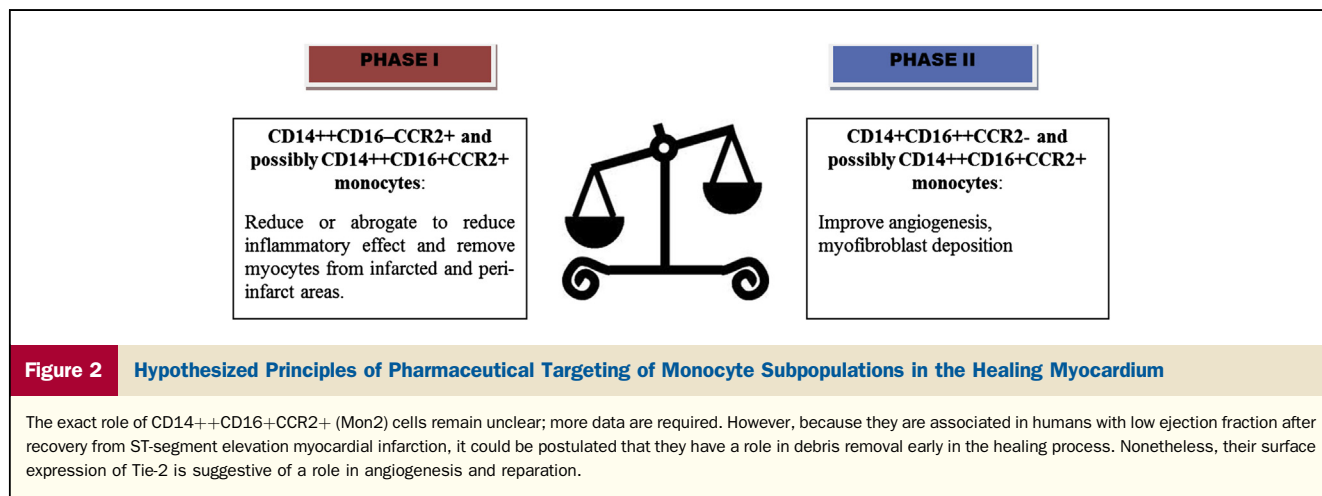
However, our knowledge of the effect of monocytes on cardiac remodeling and clinical outcome in humans is still limited. Although similarities exist in MI pathophysiology between species, the inter-species differences between monocyte subsets are substantial (including that monocytes account for 50% of murine leukocytes vs. a much smaller proportion in humans). This might make extrapolation of animal data to humans challenging from a clinical and pharmaceutical perspective (63).

The effect of monocytes on ventricular healing in humans has been investigated in 1 small study, where numbers of CD14+CD16- monocytes were negatively associated with myocardial salvage after STEMI and poor clinical outcome (64). The CD16+ monocytes (analyzed as a mixture or “intermediate” and “non-classical” monocytes) had no effect

on ventricular remodeling, which contrasted with previous animal data (19). In a recent study where levels of the 3 human monocyte subsets in STEMI were analyzed separately a prominent (over 2.5-fold) up-regulation of the CD14++CD16+CCR2+ (Mon2) (described elsewhere in the published data as intermediate monocytes) subset in acute STEMI was observed with no changes in CD14+CD16++CCR2- (Mon3) cells (45). This was accompanied by a significant change in phenotype of the “intermediate” subset (increase in CD14 and CCR2 expression, and a reduction in CD16 expression). These observations of distinctive changes related to this subset together with existing evidence of their pro-reparative and pro-angiogenic phenotype and anti-inflammatory properties are suggestive of their possible specific roles in cardiac recovery, but sufficiently powered data in this respect are lacking.

Interest in the “intermediate” monocytes has been echoed in a number of clinical studies that indicated that their high levels have been associated with poor clinical outcome both in terms of future MI in stable coronary artery disease (the HOM SWEET HOME [Heterogeneity of Monocytes in Subjects Who Undergo Elective Coronary Angiography—The Homburg evaluation] study) (65) and lower left ventricular ejection fraction in patients post-STEMI (66) or as recurrence of coronary events in patients with chronic kidney disease and in stroke patients (67,68). The biological roles of this subset are complex, and expression of receptors with a putative role in angiogenesis and repair (e.g., VEGF receptor-2, CD163, and CXCR4, which was found to be relevant to STEMI) are highest in this subset (69). One might speculate that the intermediate monocyte subset might play a role in myocardial reparation post-MI. However, the cause of such associations remains unclear, and the exact function of the intermediate monocytes is still under investigation. Currently our knowledge only extends to very limited functional studies and a number of descriptive surface and genetic markers (to mention a few: CCR5, VEGF receptor 2, HLA-DR, ENG, CLEC10A, ACE, GFRA2) (21,70). It is still unclear whether this subpopulation is a separate and independent entity from Mon1 and Mon2 and, hence, has its own differentiating pathway or whether it is an intermediate “stop” for classical monocytes as they shift toward a non-classical monocytes phenotype.

Of importance, “classical” monocytes are featured by a distinct proinflammatory phenotype, and their high counts are associated with poor myocardial recovery and worse outcome after MI (71). Although inflammatory stress associated with these cells is usually mentioned in the context of their potentially detrimental effects, the role of these cells is by far more complex and includes a number of potentially beneficial properties, including: phagocytic activity; and regulation of extracellular matrix turnover. An appropriate balance in numbers and functional activity as well as in timing in relation to MI onset is probably the key in relation to the role of the cells in acute coronary



catastrophes. However, this perspective is based on numerical quantification and presence of monocytes as opposed to assessment of function, not to mention that the role of the “intermediate” and “non-classical” monocytes is still to be deciphered.

The role of progenitor cells in wound healing and cardiac remodeling is currently the object of intense scientific interest. Infusion of mesenchymal cells into the mouse myocardium 48 h after MI induction reduced overall myocardial macrophage/monocyte levels. This included pro-inflammatory M1-type macrophages, whereas alternatively activated M2-type macrophages were significantly increased both in the circulation and the heart (72). Delivery of the mesenchymal cells resulted in: reduced cardiac expression of IL-1 β ; reduced expression of IL-6; increased anti-inflammatory IL-10 expression without changes in angiogenesis in the infarct area; and improved cardiac systolic function (72). This could suggest that mesenchymal cells might regulate the switch in monocyte/macrophage phenotype toward M2 polarization and favorable remodeling post-infarction.

Of interest, Kuwana *et al.* (73) described CD14+ monocyte-derived mesenchymal progenitors that can differentiate into a variety of mesenchymal cell types as well as having phagocytic function, thus providing a possible cellular source for tissue regeneration including wound healing and potentially heart remodeling. Also, monocyte-derived progenitor cells have been implicated in cardiac allograft vasculopathy, likely due to their ability to differentiate into smooth muscle cells and promote intimal hyperplasia (74). However, it is important to note that this group of patients routinely receive anti-rejection medication, and it remains to be seen whether this has an effect on the abundance of monocyte-derived progenitor cells (74).

To address the potential role for inflammation in tissue damage post-MI, steroid therapy was used in the 1970s and 1980s in acute MI but has not produced the anticipated beneficial effect and failed to show an improvement in clinical outcome post-STEMI (75,76). This might be partly

due to creating an imbalance between the different monocyte subpopulations eradicating both the inflammatory as well as the reparative subtypes, altering the downstream differentiation of monocytes or potentially disturbing the cross talk between DC, monocytes, and macrophages. Hence, targeting specific subpopulations at appropriate phases of cardiac healing/remodeling is a theoretical alternative to improve outcome of inflammatory events post-MI (Fig. 2).

Interestingly, successful stem cell therapy in STEMI has been based on intracoronary administration of bone marrow mononuclear cells, which include a large proportion of monocytes (Table 2). However, none of the studies used purified monocyte-derived progenitor cells but rather used a total pool of mononuclear progenitors.

Perhaps delivery or modulation of specific monocyte subpopulations at different stages of healing will form the basis of future regenerative cell therapy after MI. Indeed, Leor *et al.* (77) have shown that administration of activated human macrophages to the ischemic myocardium in rats accelerated vascularization and repair of the infarcted myocardium with improved cardiac remodeling and systolic function.

Where Are We Now?

No doubt monocytes play an important role in the pathophysiology of CAD and its complication. Blood monocytes are easier to detect and characterize, compared with tissue macrophages. As medical practice moves into the 21st century with more emphasis on the prediction and prophylaxis of future acute events, monocyte numbers and functions might become a very attractive biomarker. However, the key monocyte parameters with a potential to become prognostic markers and treatment targets are still to be defined and validated.

Indeed, there are still gaps in our knowledge of how monocytes differentiate into specific type of macrophages/DC in coronary plaques and later within infarcted tissue and

Table 2 Summary of Clinical Trials Using Bone Marrow Mononuclear Cells in Acute Coronary Syndrome Patients With Summary of Trial Findings

Study/First Author (Ref. #)	Methodology	Study Period	Study Population (Intervention/Control Group)	Results
BALANCE (78)	BMNC in the culprit lesion/vessel	3, 12, and 60 months	60 (30/30)	Compared with control group, patients treated with BMNC exhibited: improved contractility of infarcted area; reduced mortality; improved exercise
REPAIR-AMI (79)	BMPC infusion into culprit vessel 3-7 days post-AMI	12 months	204	In patients vs. placebo: BMPC administration was a significant predictor of a favorable outcome; cumulative endpoints of death, recurrence of MI, or revascularization or hospital stay for heart failure significantly reduced
BOOST (80)	BMNC intracoronary 4 days post-AMI	6 and 8 months	60 (30/30)	Increased global EF in intervention group (6% at 6 months) Effect between intervention group and control group lost at 18 months; however, improved EF retained among transmural infarct group
Janssens et al. (81)	BM stem cells intracoronary 24 h post-AMI	4 months	67 (33/34)	No improvement in EF
ASTAMI (82)	BMNC intracoronary	3 years	100 (50/50)	The results indicate that intracoronary BMNC treatment in AMI is safe in the long term; a small improvement in exercise time in the BMNC group was found, but no effects of treatment on global LV systolic function
Traverse et al. (83)	BMNC intracoronary at day 3-10	6 months	40 (30/10)	No difference in EF between groups; BMNC group had improved remodeling with significantly lower LVEDV

AMI = acute myocardial infarction; ASTAMI = Autologous Stem Cell Transplantation in Acute Myocardial Infarction trial; BALANCE = Bone Marrow Cell Transplantation in Patients with Acute Myocardial Infarction study; BM = bone marrow; BMNC = bone marrow mononuclear cells; BMPC = bone marrow progenitor cells; BOOST = Bone marrow transfer to enhance ST-elevation infarct regeneration trial; EF = ejection fraction; LV = left ventricle; LVEDV = left ventricular end diastolic volume; PC = progenitor cells; REPAIR-AMI = Reinfusion of Enriched Progenitor Cells And Infarct Remodeling in Acute Myocardial Infarction trial.

their role in regulating post-infarct reparative processes. The preferential differentiation of certain monocyte populations into particular macrophage types and other cells (e.g., myofibroblasts) has been suggested but needs further evidence.

The understanding of the function of intermediate monocytes is still in its early days. This venture will be blighted with practical difficulties: namely, the isolation of the small amounts of intermediate monocytes from whole blood, then maintaining cell viability (without differentiation) to allow characterization of their function and downstream inflammatory pathway activation. A multitude of murine/animal models could be used, but ultimately development of new high-resolution molecular in vivo imaging techniques to tag and track monocytes is needed. This will provide valuable information on mechanisms and magnitude of mobilization of individual monocyte subsets to the myocardium and their differentiation and might shed further light on intimate aspects of monocyte activities that might prove to be new therapeutic targets.

The role of the microenvironment and local factors in driving monocyte differentiation both in the sub-endothelial space and in the infarcted tissue also need to be further elucidated. Furthermore, detailed exploration of monocyte action via interaction and in coordination with other cells, such as lymphocytes and platelets, and in relation to different progenitor and stem cells is deserved.

The expression of chemokine receptors on the surface of infarcted cardiomyocytes is essential to be studied, even on

animal models (e.g., porcine hearts, the most closely related species anatomically and physiologically to human hearts). If therapeutic agents could modulate the expression of MCP-1, fractalkine, or CCR5, then monocytes subpopulations could be attracted to infarcted tissues earlier, leading to concurrent removal of debris, angiogenesis, fibroblast deposition, and better myocardial recovery.

Study limitations. Inflammation might be the “new kid on the block” in atherosclerosis and ACS. However, it is important not to forget the complex interaction between platelets, the clotting pathways, as well as other components of the immune system, including T-cells at the time of an ACS. More information is needed on the effect of the myriad of antiplatelet therapies administered during ACS on monocytes, their function and differentiation potential. Most of our current information is provided from peripheral sampling in murine, in vivo, or human studies. Hence, “direct” coronary sampling and “live” visualization of the ruptured and unstable plaque, via hybrid intracoronary spectroscopy and intravascular ultrasound will soon be available in the research and clinical arena, providing valuable local interrogation of lipid cores, fibrous cap, monocytes, and their microenvironment in different clinical settings. Ventricular remodeling post-infarct is greatly influenced by the neuro-hormonal cascade, namely aldosterone. Hence, the interaction(s) of this system with inflammation at the time of infarction and ensuing period are yet to be fully elucidated.

Conclusions

Multiple essential roles are played by monocytes during the various stages of atherosclerosis, from its initiation to the progression and development of its complications and later on, during myocardial healing and remodeling. These different roles can provide a fertile ground for pharmacological modulation of atherogenesis, stabilization of the atherosclerotic plaque, or more importantly in myocardial healing and post-infarction remodeling.

Monocyte-mediated pathways are not limited to the cardiovascular system, and inhibition of any of the surrounding milieu of cellular mechanisms could actually alter this finely tuned balance in other inflammatory systems leading to deleterious side effects. Hence, direct targeting of specific monocyte subpopulations at different sites and stages of MI would seem to be the best option. In fact, stem cell therapy relying largely on monocyte subpopulations renders an attractive potential. Given that most of our current knowledge in the field comes from murine models, further clinical studies are clearly required to improve our understanding of monocyte pathophysiology in human cardiac damage post-ACS and the subsequent remodeling and recovery of the myocardium.

Whichever way we appraise our current knowledge about origin and progression of atherosclerosis, we are drawn to the same common origin: monocytes, their subpopulations, their function, and their differentiation. With multiple therapeutic agents targeting “the clot” during ACS, an alternate and most attractive target for therapy lies in inflammation and particularly specific monocytes subpopulations with their diverse phenotypes and sentinel role in both the innate and adaptive immune system.

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Key Words: atherosclerosis ■ inflammation ■ macrophages ■ monocyte subsets.

 **APPENDIX**

For supplementary text and a table, please see the online version of this article.