Differential role of monocyte subsets in atherosclerosis

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Summary
Endothelial dysfunction and inflammation of the arterial wall continuously drive the development of atherosclerosis. Details regarding the sequential involvement of different monocyte subsets in the pathology of this disease have recently emerged. This review concentrates on major monocyte subpopulations in mouse and men and specifically addresses their phenotype, function and recruitment during primary atherosclerosis as well as their contribution to angiogenesis and tissue regeneration secondary to plaque rupture.

Keywords
Atherosclerosis, chemokines, monocytes

Introduction
Monocytes are mononuclear phagocytes of myeloid origin that represent about 6% of total leukocytes in adults. After maturation and release from bone marrow monocytes do not proliferate and circulate for a relative short period of a few days, followed by accumulation at sites of inflammation, tissue extravasation with differentiation into macrophages or dendritic cells. Monocytes can secrete inflammatory cytokines and are coated by multiple surface receptors (e.g. integrins, G-protein coupled and Toll-like receptors) allowing locomotion towards distinct gradients but also direct immune effects and uptake of particles (1). Monocytes play also a key role in homeostatic conditions by scavenging of toxic compounds and elimination of apoptotic cells (1). Although monocyte plasticity is functionally restricted mainly to conversion into tissue macrophages and dendritic cells, a sub-fraction with pluripotent plasticity exists and may differentiate at appropriate culture conditions into epithelial, endothelial, neuronal and liver cells (2). Beside inflammation, monocytes are involved also in angiogenesis and tissue remodelling after injury.

Atherosclerosis as a chronic inflammatory disease of the arterial wall is closely related to subendothelial lipoprotein retention, endothelial activation and migration of immune cells to the inflamed intima which result in formation of fatty streaks and subsequent atheromas (3, 4). Monocytes are prominently involved in initiation, progression and complication of the atherosclerotic lesions. They enter atherosclerotic plaques and transform to macrophage-like, lipid-loaded foam cells. Blocking monocyte pass into developed plaques (e.g. through improving lipid profile by intensive cholesterol lowering) promotes reduction in macrophage content and plaque stabilisation (4, 5). Notably, monocyte-like cells featuring dendritic cells can also emigrate from the lesion (6). Thus, a robust monocyte influx and possibly an inadequate “clearance” of dendritic-like cells in atherosclerotic plaques may represent one complex mechanism for disease progression and exacerbation.

Many published studies reviewed below have addressed phenotype heterogeneity and differential function of monocytes in murine models of atherosclerosis as well as in patients with cardiovascular diseases and in healthy subjects with subclinical atherosclerosis.

Heterogeneity of mouse monocytes in atherosclerosis

Studies examining homing and differentiation of mouse monocytes \textit{in vivo} have identified two major subsets (summarised in Table 1) as divided by their expression profile of Gr1/Ly6C and chemokine receptors: a prevailing subset of Gr1\(^+\)/Ly6C\(^{\text{high}}\)CX3CR1\(^{\text{low}}\)CCR2\(^+\) cells that are preferentially recruited to inflamed tissues and a subset of Gr1\(^+\)/Ly6C\(^{\text{low}}\)CX3CR1\(^{\text{high}}\)CCR2\(^+\) cells that are characterised rather by CX3CR1-dependent homing to non-inflamed tissues (7–10). The Gr1\(^+\) monocytes are often referred to as “inflammatory”, while the Gr1\(^-\) monocytes as “resident” or “patrolling”. Monocytes of both subsets can differentiate into macrophages and dendritic cells, and Gr1\(^+\) cells can also convert into Gr1\(^-\) cells \textit{in vivo} (9, 10). The Gr1\(^-\) monocytes are negative for CCR2 but intensively express CX3CR1 and CCR5 (8–10). The Gr1\(^-\) monocytes further patrol the microvascular lumen of healthy tissues through long-range CX3CR1-dependent endothelial crawling, which allows their rapid extravasation at sites of inflammation, where they can sub...
Monocyte subsets in atherosclerosis

Human monocyte diversity

Based on the expression intensity of the lipopolysaccharide (LPS) receptor CD14 and the FcγRII receptor CD16, human monocytes have been divided into three major subsets: “classical” CD14++CD16−, “intermediate” CD14+CD16+ and “non-classical” CD14−CD16+ monocytes (23, 24). These three subsets differ significantly in phenotype and function (Table 1). The classical CD14++CD16− subset (about 85% of total monocytes) is a possible human counterpart for mouse Gr1+ cells and highly express CCR2, FcγRI, L-selectin, scavenger receptor class A (ScR-A) and VEGFR1 (25–30). In contrast, the non-classical CD14−CD16+ cells (near 10% of total monocytes) are smaller, less dense and mainly correspond to the mouse Gr1− cells. They display higher levels of HLA-DR, CD11c, CX3CR1 and CCR5 but much lower amount of CCR2 as compared to the classical subset (25–29). The intermediate CD14+CD16+ subset (about 5% of all monocytes) can be clearly discriminated from the non-classical CD14−CD16+ subset by the expression of CCR2, thus conceivably reflecting one additional counterpart of mouse Gr1+ monocytes (31). Nonetheless, this subset has some unique features which put it separately from the other two subsets. For instance, CD14+CD16+ monocytes are enriched in bone marrow and have maximal of all monocyte subsets expression of Tie2, endoglin, MHC class II and HLA-DR (31–34).

Functionally, classical monocytes are professional phagocytes that ingest native low-density lipoprotein (LDL), generate reactive oxygen species (ROS) and secret cytokines in response to LPS (35). In contrast, non-classical monocytes do not generate ROS and are weak phagocytes taking-up preferentially oxidised LDL (ox-LDL) but substantially secrete inflammatory cytokines (tumour necrosis factor [TNF]-α, interleukin [IL]-1β and CCL3) after Toll-like receptor-dependent activation by viruses and nucleic acids (35–37). Thus, non-classical monocytes might serve as patrolling immune cells that selectively remove virally infected or injured cells and detoxify ox-LDL. The lower phagocytosis activity of CD14−CD16+ cells as compared to classical monocytes was efficiently used to enumerate the number and ratio of both subsets by their differential uptake of magnetic nanoparticles (38). Interestingly, using an in vitro vessel wall model has further shown that a fraction of dendritic-like cells reverse transmigrated after initial transendothelial passage of human monocytes into the sub-endothelial collagenous matrix, whereas the matrix-resident cells resembled macrophages (6). Hence, while CD14−CD16+ monocytes as classical macrophage/foam cell precursors may stay within the atherosclerotic lesion, CD14−CD16+CD11c− monocytes as feasible dendritic precursors may be able to emigrate (at least in part) out of the lesion after certain in situ maturation. The inter-

Table 1: Differential expression of crucial markers on mouse and human monocyte subsets.

<table>
<thead>
<tr>
<th>Monocyte subset</th>
<th>Major markers</th>
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<tbody>
<tr>
<td>Mouse</td>
<td></td>
</tr>
<tr>
<td>Gr1/ly6C&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Gr1, CCR2, CCR5&lt;sup&gt;+&lt;/sup&gt;, CX3CR1&lt;sup&gt;+&lt;/sup&gt;, CD62L</td>
</tr>
<tr>
<td>Gr1/ly6C&lt;sup&gt;−&lt;/sup&gt;</td>
<td>CX3CR1, CCR5, LFA-1, CD11c</td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>CD14++CD16−</td>
<td>CD14, CCR2, FcγRI, CD62L, ScR-A, VEGFR1</td>
</tr>
<tr>
<td>CD14−CD16+</td>
<td>CD14, CD16, CCR2, Tie2, CD105, MHC II, HLA-DR</td>
</tr>
<tr>
<td>CD14−CD16−</td>
<td>CD16, HLA-DR, CD11c, CX3CR1, CCR5</td>
</tr>
</tbody>
</table>

Sequentially initiate an early immune response and differentiate into macrophages (11). Mice deficient for either CX3CR1 or CX3CL1 display reduced numbers of Gr1+ monocytes, whereas the Gr1+ subset remains unaffected (12). Understanding the differential mobilisation, recruitment and survival/emigration of monocyte subsets during the pathogenesis of atherosclerosis, at least in suitable mouse models, is of particular importance for development of successful therapeutic strategies. Atherosclerotic ApoE<sup>−/−</sup> mice kept on a high-fat diet revealed monocytopoiesis with a rise of Gr1+ monocytes that efficiently homed to plaques (13). The Gr1− monocytes were either unaffected or increased and their count correlated with lesion size (14, 15). Of note, Gr1+ monocytes did not seem to require CX3CR1 for plaque recruitment in this setting, but instead used CCR5, which is overexpressed in ApoE<sup>−/−</sup> mice and also acquired expression of the dendritic cell antigen CD11c (10). By comparison, Gr1+ monocytes required CX3CR1 in addition to CCR2/CCR5 for trafficking to plaques and the CX3C axis was essential for monocyte/foam cell survival inside the plaque (Fig. 1), which contrasts with other inflammatory settings (12, 15). The role of CCR2 and CCL5/CXCR5 in the recruitment of both subsets is consistent with the observation of decreased monocyte counts and attenuated atherogenesis in CCR2<sup>−/−</sup> and CX3CR1<sup>−/−</sup> mice (16–18). Similarly, blockade of CCR5 with Met-RANTES further decreased circulating monocyte counts and almost fully protected mice against atherosclerosis (19). Moreover, a combined inhibition of CCL2, CX3CR1, and CCR5 in ApoE<sup>−/−</sup> mice was associated with decrease in circulating monocytes and a marked reduction in atherosclerosis (14). Thus, CCL2, CX3CR1 and CCR5 play additive, monocyte-related role in atherogenesis. After an acute myocardial infarction as a result of occlusive atherothrombotic complication, the healing myocardium sequentially mobilised both monocyte subsets as revealed by experiments in ApoE<sup>−/−</sup>, CCR2<sup>−/−</sup> and CX3CR1<sup>−/−</sup> mice (20). While Gr1+ monocytes dominated at early phases by displaying phagocytic, proteolytic and inflammatory functions in damaged tissue, the Gr1− monocytes dominated rather during the chronic phase by promoting myocardial healing and angiogenesis (20).

Parallel to chemokine receptors, adhesion molecules of the selectin and integrin family are crucial in monocyte rolling and firm adhesion to the endothelium. Differences in their expression on the monocyte subsets exist as well (Table 1). For instance, Gr1− monocytes do not express L-selectin but show higher levels of the β2-integrin LFA-1 that cooperates with CX3CR1 during endothelial crawling (7, 9, 21). In contrast, Gr1+ monocytes were differentially recruited to sites of endothelial dysfunction and thrombosis by a predominant PSGL-1/P-selectin mediated interaction (9, 22).
mediate monocyte subset does not produce ROS but shows highest secretion of TNF-α and IL-1β in response to LPS (37).

**Monocyte subsets in cardiovascular pathology**

Epidemiological data have defined leukocytosis as an independent risk factor and predictor of future cardiovascular events (39). Ongoing differential analysis on monocyte subsets by flow cytometry dissects this knowledge, thus allowing a detailed risk assessment together with a view towards novel therapeutic options. However, there is no standard flow cytometric protocol up to now for quantification of monocyte subsets and some clinical studies have analysed CD16+ monocytes (CD14++CD16+ and CD14+CD16++) as one population, thus preventing a reliable conclusion on the role of individual CD16+ subsets (Table 2).

So far, high CD14+CD16++ monocyte counts were associated with higher body mass index (BMI) and increased intima-media...
Monocyte subsets in angiogenesis and tissue remodelling

As mentioned above, monocytes possess pluripotent plasticity and can acquire also endothelial features (2). Previous evidence has revealed that cultured monocytes can develop an endothelial-like phenotype and form capillary-like tubes in vitro (49, 50). The contribution of the CCL2/CCR2 axis in arteriogenesis and endothelial regeneration has also been well established (51, 52). Tissue macrophages might be further instrumental in compensatory myocardial neovascularisation by drilling angiogenic channels as demonstrated in a transgenic mouse model of ischaemic cardiomyopathy where CCR2+ monocytes are attracted to the myocardium by local overexpression of CCL2 (53). In this setting, local hypoxia might be another important pro-angiogenic cofactor, and colonisation of the tunnels by CXCR4+ progenitor cells is well conceivable, since their CXCL12-dependent recruitment along hypoxic gradients was newly described (54).

Recent studies have identified another interesting subset of human monocytes expressing the angiopoietin receptor Tie2 (31, 33, 34). These Tie2-expressing monocytes (TEMs) are mainly intermediate CCR2+CD161+ monocytes with significant contribution to tumour angiogenesis and growth. TEMs failed to differentiate into endothelial cells or to incorporate into vessels. Thus, their proangiogenic activity is probably related to secretion of angiogenic cytokines. Vice versa, monocytes may activate Tie2 on human umbilical vein endothelial cells by secreting angiopoietin-1, thus sup-

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects (n)</th>
<th>Disease</th>
<th>Target subsets</th>
<th>Measure</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rogacev et al. (40)</td>
<td>622</td>
<td>no</td>
<td>CD14+CD16++</td>
<td>(per μl)</td>
<td>(+) BMI and IMT</td>
</tr>
<tr>
<td>Wildgruber et al. (38)</td>
<td>18</td>
<td>CAD</td>
<td>CD14+CD16++</td>
<td>(%)</td>
<td>↑ in CAD</td>
</tr>
<tr>
<td>Schlitt et al. (42)</td>
<td>247</td>
<td>CAD</td>
<td>pooled CD16+</td>
<td>(%)</td>
<td>↑ in CAD</td>
</tr>
<tr>
<td>Kashiwagi et al. (43)</td>
<td>73</td>
<td>CAD</td>
<td>pooled CD16+</td>
<td>(%)</td>
<td>(+) vulnerable plaques</td>
</tr>
<tr>
<td>Timmerman et al. (41)</td>
<td>30</td>
<td>no</td>
<td>CD14++CD16+</td>
<td>(%)</td>
<td>↓ after exercise</td>
</tr>
<tr>
<td>Heine et al. (47)</td>
<td>94</td>
<td>CKD (dialysis)</td>
<td>CD14++CD16+</td>
<td>(per μl)</td>
<td>(+) cardiovascular events</td>
</tr>
<tr>
<td>Rogacev et al. (48)</td>
<td>119</td>
<td>CKD</td>
<td>CD14++CD16+</td>
<td>(per μl)</td>
<td>(+) cardiovascular events</td>
</tr>
<tr>
<td>Hristov et al. (44)</td>
<td>80</td>
<td>CAD</td>
<td>CD14++CD16+</td>
<td>(%)</td>
<td>↑ at high-risk/FH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD14+CD16+</td>
<td></td>
<td>(+) number of risk factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD14+CD16++</td>
<td></td>
<td>↓ at high-risk/FH/smoking</td>
</tr>
<tr>
<td>Tsujioka et al. (46)</td>
<td>80</td>
<td>CAD</td>
<td>CD14++CD16+</td>
<td>(per μl)</td>
<td>(-) LVEF after AMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pooled CD16+</td>
<td></td>
<td>no correlation</td>
</tr>
<tr>
<td>Ura et al. (34)</td>
<td>46</td>
<td>stroke</td>
<td>CD14++CD16+</td>
<td>(%)</td>
<td>(-) outcome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lower mortality</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD14++CD16++</td>
<td></td>
<td>(+) outcome</td>
</tr>
</tbody>
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AMI, acute myocardial infarction; BMI, body-mass-index; CAD, coronary artery disease; CKD, chronic kidney disease; FH, family history; IMT, intima-media-thickness; LVEF, left ventricular ejection fraction; (+), positive relation; (-), negative relation.
porting endothelial cell survival (55). Whether human TEMs are also involved in neovascularisation of ischaemic tissue, where the mouse Gr1+ cells are sequentially recruited to support regeneration and angiogenesis, remains yet open. Further data outlined expression of VEGFR1 and endoglin mainly on classical and intermediate monocytes (24, 30, 32).

The therapeutic infusion of freshly isolated CD14+ monocytes for accelerating blood flow recovery after ischaemia has been controversially discussed (56, 57). As monocytes are abundant and easy to separate, their local therapeutic use in neovascularisation could be of interest rather in pathological settings such as diabetes mellitus, where the function of circulating CD34+ progenitor cells, including also putative angioblasts, is overall compromised (56, 58). Several published studies have reported that cultured angiogenic cells, also denoted as “endothelial progenitor cells” (EPCs), are reliable tools in regenerative cardiology (59–61). They are usually cultured out from peripheral blood mononuclear cells and display myeloid (CD45+, CD14+, CD16+, CD11b+) along with endothelial (VEGFR2+, CD31+, Tie2+, Ulex-lectin binding) features, which partially correspond to classical and intermediate monocytes (60, 61). Strategies to modulate their number and to augment their regenerative function are being under investigation (62–64).

Together, the differential contribution of monocytes to neovascularisation of ischaemic tissue remains not yet clear. Most probably, there is additive subset interplay in angiogenesis and further efforts are intended to identify monocyte populations with highest pro-angiogenic capacity.

Concluding remarks

It is very important to understand monocyte biology under homeostatic conditions versus inflammation/atherosclerosis and angiogenesis, but also during different stages of the disease (as summarised in [Fig. 1]). In the context of subclinical and primary atherosclerosis with formation, growth and complication of atherosclerotic plaques, monocyte subsets seem to associate in general with plaque vulnerability. A shift in monocyte ratio may refer to imbalance between lesion influx and egression, rather than to imbalanced mobilisation from the bone marrow. Conversely, monocytes may also contribute to tissue regeneration secondary to plaque rupture (e.g. neovascularisation after infarction), obviously in different manner and possibly by a substantial input of the CD16+ subsets as well. Clinical follow-up studies in larger patient cohorts are strongly required to accurately correlate monocyte counts with cardiovascular outcomes. Differential monitoring of monocyte subsets may be also of interest to identify subjects with subclinical atherosclerosis. Thus, there is an urgent need for a standardised flow cytometric assay that can be applied uniformly. Novel therapeutic strategies to reduce CD16+ monocytes during advanced atherosclerosis and to re-direct their accumulation selectively into ischaemic tissue secondary to plaque complication may be exciting for the future.

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Conflict of interest

None declared.

References


