Atherosclerosis is a lipoprotein disorder with inflammatory, vascular, and hemodynamic determinants. Total white blood cell count and subpopulations including granulocytes, monocytes, T cells, and bone marrow-derived precursors have been implicated in atherosclerotic cardiovascular disease (CVD). Although these associations may inform biological processes, they have not, to date, permitted specific mechanistic understanding of subsets of leukocytes involved nor have they advanced risk prediction and therapeutic targeting or monitoring in the clinic. Although short-lived in circulation, blood monocytes are proposed to play a specific role in atherosclerosis because (1) blood monocytes are the major source of tissue macrophages; (2) monocyte-derived macrophages and dendritic cells are the predominant leukocytes in atherosclerotic lesion; and (3) macrophages/dendritic cells have a variety of important proinflammatory (inflammatory recruitment/activation of other leukocytes and vascular cells, foam cell formation and lipid accumulation, fibrous cap erosion, and plaque rupture) and anti-inflammatory (phagocytosis, effectorcytosis, and leukocyte egress) actions in experimental atherosclerotic lesions. Despite these mechanistic insights from human pathology and experimental studies in rodents, there have been disparate findings in studies of total monocyte counts and CVD.

There are several challenges to our understanding of the role of monocytes in atherosclerosis. First, circulating monocytes are heterogeneous due to subtype differentiation in bone marrow. The regulation and rate of subpopulation recruitment as well as their roles in atherosclerosis are uncertain. Second, monocyte subpopulations are typically defined by differential expression of specific cell surface antigens including receptors that modulate their function, for example, chemokine receptors chemokine (C-C motif) receptor 2 (CCR2), CX3C chemokine receptor 1 (CX3CR1), and C-C chemokine receptor type 5 (CCR5), as well as adhesion molecule integrin receptors very late antigen-4 (also called integrin α4β1) and LFA-1 (integrin αLβ2). Markers of monocyte subpopulations have evolved with time and differ between humans and mice leading to dynamic and sometimes confusing classifications as well as uncertainty in directly comparing human and mouse studies. Third, the plasticity of monocytes is well documented with differentiation of monocytes to macrophage subtypes or dendritic cells depending on local tissue cues, which may differ in atherosclerosis at various stages of disease. Finally, the fate and survival of specific monocyte subpopulations after entry into plaque is poorly understood. Which monocytes and how they differentiate to inflammatory macrophages, foam cells, or dendritic cells is poorly understood. Indeed, the relative contribution of blood monocytes versus expansion of local macrophages and dendritic cells to these lesion cells is also uncertain. Thus, monocyte heterogeneity, plasticity, and fate represent significant challenges to ascribing roles within lesions to specific monocyte subpopulations (Figure).

Recent classifications of monocytes (classical, intermediate, and nonclassical) strive to use morphology and immunomarker profiles without inferring function, but much literature has ascribed, perhaps incorrectly, specific “proinflammatory” or “anti-inflammatory” functions to monocyte subsets. Human monocytes have been defined on the basis of morphology, cytochemistry, and, more recently, by flow cytometry, particularly using antibodies to cell-surface markers such as CD14 (endotoxin coreceptor) and CD16 (FcγRIII receptor). This technology enables the identification of monocyte subsets and their classification based on differential expression of CD14 and CD16 as classical (CD14++ CD16−; cells with high CCR2 and low CX3CR1 expression), intermediate (CD14++ CD16+ cells), and nonclassical (CD14+CD16++; cells with no CCR2 and high CX3CR1 expression). The latter 2 subsets are sometimes combined as CD16+ monocytes and account for 10% to 20% of all circulating monocytes. Although different cell-surface markers are required, a parallel classification of mouse monocytes based on Ly6C and CD43 expression with Ly6C+CD43+ considered equivalent to CD14++CD16− and Ly6C+CD43++ thought to be equivalent to CD14+CD16++.

CD16+ monocytes have been designated “proinflammatory” in some literature because they are efficient producers of inflammatory cytokines, whereas they secrete little anti-inflammatory interleukin-10. Furthermore, CD16+ monocyte counts are elevated in numerous inflammatory conditions such as sepsis, asthma, and inflammatory bowel disease and are reported as associated with atherosclerosis as well as CVD and stroke. Indeed, many properties support the relevance of CD16+ monocytes in atherosclerosis and...
CD14+CD16− monocyte numbers. Notably, however, the CD14+CD16− monocyte subset did not associate with the extent of carotid atherosclerosis measured by intimal-media thickness (IMT) at baseline. Surprisingly, although CD16+ monocytes were not significant predictors of CVD events after adjusting for risk factors, the percentage of CD14+CD16+ monocytes was inversely associated with baseline carotid IMT. Finally, they also found that there was no difference in CCR2, CX3CR1, and CCR5 chemokine receptor expression intensity on any of the monocyte subsets between incident cases and control subjects.

There are several strengths of this article. A relatively large sample, nested in a prospective cohort study which included both CVD outcomes and a measure of atherosclerosis burden, was used. Furthermore, laboratory techniques were robust, including use of multiple controls with rigorous quality checks of monocyte markers and receptor expression by flow cytometry as well as experiments to validate use of frozen materials. The statistical analytic approach considered multiple confounders of CVD including traditional risk factors and circulating C-reactive protein. Finally, the combination of carotid IMT and CVD event data within the same study population is a unique strength raising novel hypotheses. However, there are several weaknesses and unanswered questions. First, the study sample may have been underpowered. The effect sizes for the number of CD14+CD16+ (1.66; 95% CI, 1.02–2.72), CD16+ (1.44; 0.87–2.39), and total monocytes (1.61, 0.98–2.66) on CVD events were very similar raising concern for power to detect and ascribe unique risk to specific monocyte subsets, particularly less abundant CD16+ monocytes. Furthermore, the association of percent, but not number, of CD16+ cells with carotid IMT may have arisen due to statistical artifact and requires replication. Combining data for intermediate and nonclassical CD16+ cells may not be supported by their functional roles in vivo.

Monocyte subsets in atherosclerosis. Human monocytes are classified as classical (CD14++CD16−; high CCR2 and low CX3CR1 expression), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++; no CCR2 and high CX3CR1 expression). Classical CD14++CD16− monocytes are increased by a high-fat diet and are rapidly recruited to atherosclerosis (through CCR2 and CX3CR1), whereas nonclassical CD14+CD16++ patrol the endothelium and are more slowly recruited to lesions (through CX3CR1 and CCR5). Whether specific monocyte subsets preferentially differentiate to macrophage subtypes, foam cells, or dendritic cells is uncertain. Thus, their specific roles in atherosclerosis progression, lesion stability, and plaque rupture, and ultimately clinical events are uncertain. Berg et al19 report that the classical CD14++CD16− subset are independent predictors of incident cardiovascular events.

### Monocyte Classification

<table>
<thead>
<tr>
<th>Blood</th>
<th>Vessel Wall</th>
<th>Lesion</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic</td>
<td>CD14++/CD16−</td>
<td>CD14++/CD16+</td>
<td>CD14+/CD16++</td>
</tr>
<tr>
<td>Intermediate</td>
<td>CD14+/CD16+</td>
<td>CD14++/CD16+</td>
<td>CD14+/CD16++</td>
</tr>
<tr>
<td>Non-classical</td>
<td>CD14+/CD16++</td>
<td>CD14+/CD16++</td>
<td>CD14+/CD16++</td>
</tr>
</tbody>
</table>

- **Blood**: CCR2
- **Vessel Wall**: CCR1
- **Lesion**: CX3CR1
- **Clinical**: Atherosclerosis

**Figure.** Monocyte subsets in atherosclerosis.
complex question and provide the foundation for addressing unanswered hypotheses relating to monocyte subsets in atherosclerotic CVD.

What are the biological implications of the study? First, these novel clinical data suggest that classical CD14+/CD16− monocytes play a causal role in human CVD. Conflicting findings for CD16+ relative to prior work might point to modulation of monocyte functions in established renal and vascular disease states, although they may also reflect study design differences and biases. The lack of association between monocyte CCR2, CX3CR1, and CCR5 expression with CVD outcomes raises important questions: does this represent true biology or is this a technical artifact due to lack of sensitivity of the antibodies for the receptors? Are there other chemokines/Receiveders and cytokines/effectors of greater importance for monocyte function in clinical CVD? The apparent distinct associations with clinical events versus IMT for CD16− versus CD16+ raise intriguing questions regarding the role of specific monocytes in progression of atherosclerosis versus distinct roles in plaque rupture and clinical complications. This pattern might relate to differential functions of specific chemokines in chronic atherosclerosis relative to acute CVD, for example, CXCL12 might point to modulation of monocyte functions in established CVD.

These novel clinical data suggest that classical CD14+/CD16− monocytes are causal in human CVD. The apparent distinct associations with clinical events versus IMT for CD16− versus CD16+ raise intriguing questions regarding the role of specific monocytes in progression of atherosclerosis versus distinct roles in plaque rupture and clinical complications. This pattern might relate to differential functions of specific chemokines in chronic atherosclerosis relative to acute CVD, for example, CXCL12 might point to modulation of monocyte functions in established CVD.

Although provocative, the findings of Berg et al. have limited clinical applications. For clinical prediction, larger studies are required that replicate and extend these findings and to establish monocyte subtypes as independent predictors of CVD events and in reclassification and discrimination of those at risk. Future studies, particularly of novel therapies, may establish value for monocyte subpopulation targeting in directly modulating cells that cause CVD or as biomarkers of efficacy of anti-inflammatory therapeutics in CVD. Basic and translational progress are required to better define monocyte subsets (within and across species); their relationship to macrophage, foam cells, and dendritic cells in lesions; and their specific functions in atherosclerosis and its clinical complications. Future clinical studies of monocyte subsets in CVD should focus on very large prospective samples with homogeneous outcomes, subgroup analyses, and multiple measures of subclinical atherosclerosis. These studies should examine each monocyte subtype separately, apply novel markers as available, and compare findings with those for total monocytes, neutrophils, T-cells, bone marrow progenitors as well as inflammatory biomarkers and traditional risk factors. Integration with human genetic data and pathway analyses should provide the greatest opportunity for mechanistic insight in teasing out the effect of specific monocyte subsets and their molecular effectors in human atherosclerotic CVD.

**Disclosures**

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**References**


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