

REVIEW

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Clinical significance of monocyte heterogeneity

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Abstract

Monocytes are primitive hematopoietic cells that primarily arise from the bone marrow, circulate in the peripheral blood and give rise to differentiated macrophages. Over the past two decades, considerable attention to monocyte diversity and macrophage polarization has provided contextual clues into the role of myelomonocytic derivatives in human disease. Until recently, human monocytes were subdivided based on expression of the surface marker CD16. “Classical” monocytes express surface markers denoted as CD14⁺⁺CD16⁻ and account for greater than 70% of total monocyte count, while “non-classical” monocytes express the CD16 antigen with low CD14 expression (CD14⁺CD16⁺⁺). However, recognition of an intermediate population identified as CD14⁺⁺CD16⁺ supports the new paradigm that monocytes are a true heterogeneous population and careful identification of specific subpopulations is necessary for understanding monocyte function in human disease. Comparative studies of monocytes in mice have yielded more dichotomous results based on expression of the Ly6C antigen. In this review, we will discuss the use of monocyte subpopulations as biomarkers of human disease and summarize correlative studies in mice that may yield significant insight into the contribution of each subset to disease pathogenesis.

Keywords: Monocyte; CD14; CD16; Ly6C; Macrophage; Cardiovascular; Atherosclerosis; Autoimmune Disease; Human; Mouse

Introduction

Until the late 1980s, monocytes were considered to represent a single population of circulating hematopoietic cells derived from the common myeloid progenitor cell in the bone marrow. The seminal work of Passlik et al. demonstrated that distinct monocyte subsets could be identified based on the expression of the surface antigen CD16 [1]. “Classical” monocytes do not express the CD16 antigen (CD14⁺⁺CD16⁻), while “non-classical” monocytes are smaller in size and express CD16 on the cell surface (CD14⁺CD16⁺⁺) [1]. The expression pattern of the two surface markers lends some insight into their function. CD14 acts as a co-receptor for toll-like receptor 4 and mediates lipopolysaccharide (LPS) signaling, while the CD16 antigen is identified as FcγRIIIa and participates in innate immunity [2,3]. The subsequent two decades have yielded considerable insight into the role of each cell population in human disease; however, the recent emergence of an intermediate monocyte population denoted as CD14⁺⁺CD16⁺ has shifted focus away from this simple classification system

[4-8] (Figure 1). As of 2010, three distinct monocyte populations outlined by Ziegler-Heitbrock et al. are officially recognized: CD14⁺⁺CD16⁻ (classical), CD14⁺⁺CD16⁺ (intermediate), and CD14⁺CD16⁺⁺ (non-classical) [4]. We will adhere to the official nomenclature for this review article.

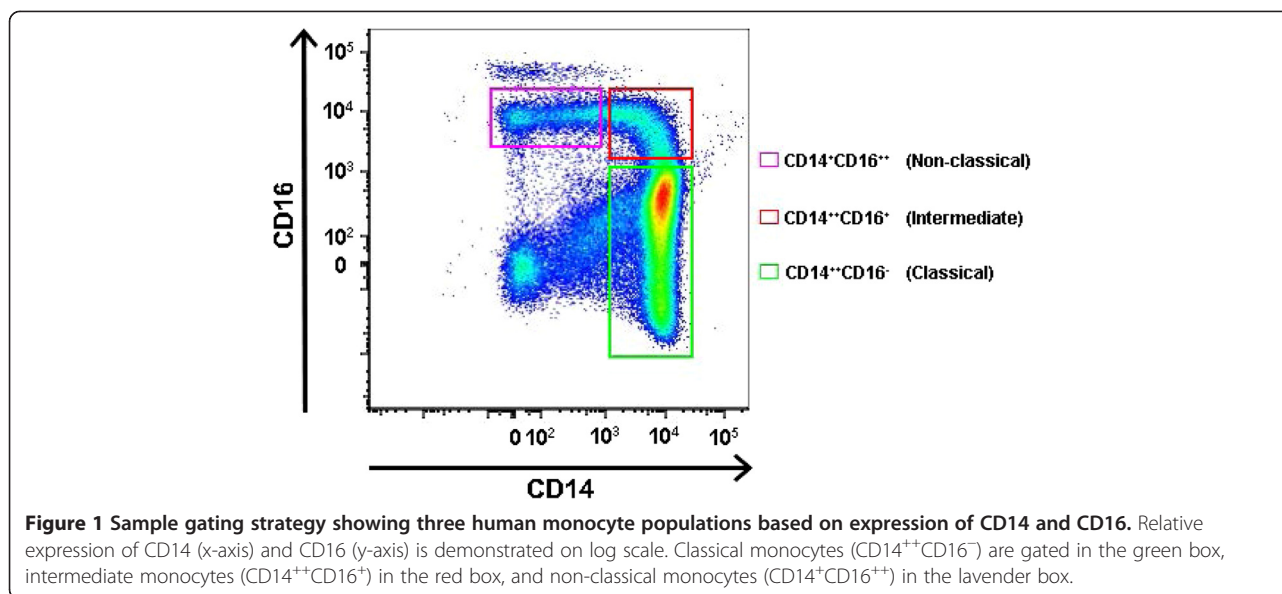
Classical monocytes represent the largest population of monocytes and are important scavenger cells [4]. Non-classical monocytes were often referred to as pro-inflammatory, secondary to their mobilization in disease and secretion of important inflammatory cytokines [7,9-11] (i.e. TNF-α). The new tripartite view of monocyte subpopulations has shifted attention from the inflammatory characteristics of non-classical monocytes and supports a more significant role for intermediate monocytes in inflammation. Further, unique functions in angiogenesis, production of reactive oxygen species, and patrolling behavior have been attributed to CD16⁺ monocytes [12-18]. In the present review, we will summarize the contribution of monocyte heterogeneity in human disease and draw across multiple common pathologies to characterize each monocyte subtype for clinical relevance. We will focus on the impact of monocytes in cardiovascular disease, including myocardial infarction, atherosclerosis, and stroke, chronic kidney disease, and autoimmune disease. Finally, we will

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identify distinct murine monocyte populations and link their function in preclinical animal models to human monocyte function.

Review

Monocyte ontogeny and heterogeneity

Genetic profiles, antigen expression, and cytokine production of each monocyte population provide a foundation for hierarchical clustering and classification. Cros et al. showed that CD14⁺CD16⁺⁺ monocytes (non-classical) are segregated independently from intermediate and classical monocytes based on principal component analysis [14]. Functionally, CD14⁺⁺CD16⁻ and CD14⁺⁺CD16⁺ monocytes are more phagocytic than non-classical monocyte [14]. Conversely, other investigators have provided genetic and functional evidence to suggest that CD16⁺ monocytes cluster together and are more distinct from classical CD16⁻ monocytes [7,16,19-21]. Wong et al. showed significantly different genetic profiles between intermediate and classical monocytes, which supported independent findings in humans and rhesus monkeys [13,19,22]. Interestingly, cytokine production in response to LPS *in vitro* reveals a more complex relationship. Intermediate monocytes produced significantly more tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) compared to the other populations; however, production of interleukins 6, 8, and 10 were approximate in intermediate and classical monocytes when compared to non-classical monocytes [14,19].

Despite this controversy, stereotypic functions of classical and non-classical monocytes are beginning to emerge. CD14 is a pattern recognition receptor and classical monocytes are critical components of innate immunity, while CD16⁺ (intermediate and non-classical) monocytes are preferentially mobilized during inflammation and lend to

their designation as “pro-inflammatory” [16,23,24]. The significant functional overlap of intermediate monocytes with classical and non-classical monocytes lends credence to the present hypothesis that CD14⁺⁺CD16⁻ monocytes arise from the bone marrow and give rise to CD14⁺⁺CD16⁺ and CD14⁺CD16⁺⁺ monocytes [4,13,24]. CD14⁺⁺CD16⁻ monocytes primarily produce monocyte chemotactic protein-1 (MCP-1/CCL2) and express its cognate receptor CCR2, as do intermediate monocytes [14,22]. CCR2 is critical for monocyte emigration from the bone marrow and monocyte homing [25-29]. Likewise, CD62L (SELL), expressed on classical monocytes, is an important early marker of progenitor cell commitment to specific progeny [30,31]. Population specific genetic profiles reveal a progressive increase in genes associated with maturation in intermediate and non-classical monocytes when compared with classical monocytes [19,24]. Finally, in response to macrophage colony stimulating factor (M-CSF), intermediate monocytes are initially expanded in the peripheral blood followed by a delayed increase in non-classical monocyte frequency [24,32,33]. Though not dogmatic, general recognition that classical monocytes arise from the bone marrow and give rise to intermediate and non-classical monocytes has provided a platform for understanding monocyte maturation and methods of replenishment in disease states. Table 1 outlines the expression of surface markers for classical, intermediate, and non-classical monocytes.

Presently, murine monocytes are evenly distributed by their relative expression of the Ly6C antigen and are, for the most part, functionally distinct [34-39]. Ly6C^{high} monocytes co-express CCR2 and CD62L with low expression of the fractalkine receptor CX₃CR1, whereas Ly6C^{low} monocytes have elevated expression of CX₃CR1 with lower expression of CCR2 [39,40]. This expression

Table 1 Profile of human and mouse monocyte subpopulations

	Human			Mouse	
	CD14 ⁺⁺ CD16 ⁻	CD14 ⁺⁺ CD16 ⁺	CD14 ⁺ CD16 ⁺⁺	Ly6C ^{high}	Ly6C ^{low}
CCR2 ^{a,b,c}	++	+	-	++	+
CX ₃ CR1 ^{a,b,c}	+	++	+++	+	++
CD62L ^{a,c}	++	+/-	-	++	-
CXCR1 ^{a,c}	++	+/-	-	ND	ND
CXCR2 ^{a,c}	++	+/-	-	ND	ND
PSGL-1 ^d	++	+	+	++	+
CD11a ^{e,f}	+	+	++	+	++
CCR5 ^{a,b,c}	+	++	-	+	+
ACE ^g	+	++	+	ND	ND
HLA-DR ^a	-	++	+	ND	ND
CD11b ^a	+	+	+/-	+	+
CD11c ^{e,h,i}	-	+	+	-	+
CD43 ^f	-	+	++	+	++
MHCII ^{a,e,h}	+	++	+	-	+

^aWong et al. *Blood* 2011; ^bRogacev et al. *Eur Heart J* 2011; ^cAncuta et al. *JEM* 2003; ^dAn et al. *Circulation* 2008; ^eIngersoll et al. *Blood* 2009; ^fZawada et al. *Blood* 2011; ^gUlrich et al. *Nephrol Dial Transplant* 2010; ^hSunderkotter et al. *J Immunol* 2004; ⁱTanaka et al. *Clin Exp Immunol* 1999.

pattern supports the present view that Ly6C^{high} monocytes are closely related to human classical (CD14⁺⁺CD16⁻) monocytes and conversely, Ly6C^{low} monocytes are analogous to the non-classical CD14⁺CD16⁺⁺ population [14,19,24,40,41]. Bone marrow cells are enriched for the Ly6C antigen and Ly6C^{high} monocytes are capable of giving rise to Ly6C^{low} monocytes and other derivatives in some animal models [35,42-46]. However, Ly6C^{high} monocytes are preferentially recruited in inflammation and are the major source of cytokines such as TNF- α and IL-1 β [36,37,40]. This departure from the functional description of classical human monocytes requires resolution and may be a function of current analytical techniques. For example, separation techniques leverage the expression of human monocyte markers to label each monocyte with differentially expressing antibodies. The binding of these labeling antibodies to poorly expressed surface antigens may preclude their activation and underestimate cytokine production and their response to various stimuli including LPS [16]. Additionally, expression patterns of classic markers for human monocytes (CD14, CD16, CD86, etc.) and murine markers (Ly6C, CCR2, CX3CR1) are not directly analogous between species. CCR2, for example, is preferentially expressed on Ly6C^{high} murine monocytes and has provided evidence for their functional similarity to classical human monocytes. However, CCR2 is expressed on Ly6C^{low} monocytes as well, but is poorly expressed on human CD16⁺ monocytes [41]. Further, these simple murine monocyte profiles (inflammatory vs. pro-inflammatory) likely limit our ability to understand individual murine monocyte function and hinder translation to human studies. Ingersoll et al. compared human CD16⁻ and CD16⁺ monocytes with

their murine counterpart to show that multiple pro-inflammatory and phagocytic antigens (TREM-1, CD64 and CD36) were highly expressed on Ly6C^{low} monocytes and approximated relative expression in CD16⁻ human monocytes [41]. However, antigen expression patterns and genomic data mostly confirm the present grouping of Ly6C^{high} monocytes with CD14⁺⁺CD16⁻ human monocytes [14,40,41]. Several recent reviews attempt to resolve this controversy [24,39,40].

Monocytes phenotypes in cardiovascular disease

Expansive remodeling and leukocyte infiltration of the left ventricle in response to coronary artery occlusion is predictive of poor outcomes [40,47]. Monocyte mobilization during the evolving infarction and recovery period follow a sequential pattern. Tsujioka et al. studied the mobilization of CD16⁻ and CD16⁺ monocytes during the natural progression of acute myocardial infarction (AMI) in a Japanese patient population [48]. Circulating CD16⁺ monocytes were diminished in patients diagnosed with AMI at the time of admission and rose significantly over the first week after diagnosis. The mobilization of CD16⁺ monocytes was preceded by increased CD14⁺⁺CD16⁻ monocytes in the first days following AMI admission. Circulating monocyte populations did not differ between groups; however, peak levels of CD14⁺⁺CD16⁻ monocytes were a negative predictor of myocardial salvage and no relationship between CD16⁺ monocytes and infarction size was noted in this study [48]. Other human studies and murine models of myocardial infarction support these findings [50-53]. Ly6C^{high} monocytes, corresponding to classical

human monocytes, accumulate in the myocardium immediately after AMI and are followed by a recovery period denoted by expansion of Ly6C^{low} monocytes (analogous to human non-classical monocytes) [50,54]. However, characterization of human monocyte mobilization in Tsujioka et al. is difficult to interpret, as CD16⁺ monocytes were not distinguished into intermediate and non-classical populations [48]. Recently, monocyte subtypes were analyzed in a cohort of patients admitted for AMI and compared to patients with stable coronary artery disease (CAD) and a second cohort of healthy controls [55]. Patients admitted for AMI had a 60-80% increase in total monocyte count and a disproportionate increase (3-fold) in intermediate (CD14⁺⁺CD16⁺) monocytes when compared with patients admitted with CAD and healthy controls. The intermediate monocyte population remained elevated for the subsequent 7 days after AMI and trended to background control levels by day 30. The authors go on to show that return of the intermediate monocyte population to normal frequency correlated with decreased troponin levels and return of left ventricular ejection fraction [55]. These smaller cohort studies are supported by a large prospective cohort study of persons undergoing angiography. Intermediate monocyte frequency strongly correlated with a composite outcome of cardiovascular death, non-fatal AMI, and non-hemorrhagic stroke in 951 patients. A planned secondary analysis revealed that patients with the highest frequency of intermediate monocytes had a 40% risk of experiencing a cardiovascular event within three years of study enrollment [49]. Collectively, clinical studies suggest an intriguing role for monocyte differentiation as a diagnostic and predictive biomarker in patients suffering AMI.

Similar to the findings observed in patients suffering AMI, a characterization study of human monocyte mobilization in the days following a stroke revealed an expansion of intermediate monocytes by day 2, while the classical monocyte population was unperturbed [56]. Non-classical CD14⁺CD16⁺⁺ monocyte frequency inversely mirrored intermediate monocyte frequency and reached the nadir by day 2 after stroke. The classical monocyte population was lower at the time of admission for stroke in surviving patients and correlated with improved prognosis. Intermediate monocyte mobilization over the subsequent 48 hours following stroke also correlated with stroke severity and increased mortality rates, which held significance following adjustment for age and stroke severity scoring [56,57]. A second cohort of 36 stroke patients supported the increased frequency of intermediate monocytes in the peripheral blood in the days to weeks following infarction; however, Kaito et al. showed that classical monocyte numbers increased immediately following presentation for stroke [58]. A positive correlation with stroke severity score in this study was also observed and may be explained by a strong association with

progressing infarction [58]. Thus, assessment of monocyte subsets following admission for stroke may show a time-dependent predictive value in outcomes and survival.

Monocytes in chronic cardiovascular inflammation

Atherosclerosis is the result of complex interactions between circulating leukocytes and the vascular wall, which are likely propagated by chronic secretion of growth factors and inflammatory cytokines. The lack of true homology between murine and human monocyte subpopulations limit the direct application of findings in murine atheroma to that observed in human cohorts. Ly6C^{high} monocytes, the murine correlate of human classical monocytes, drive atherosclerosis in transgenic animal models based on the genetic deletion of apolipoprotein E or the LDL receptor [26,37,45,59-61]. Ly6C^{high} monocytes preferentially bind to the activated endothelium in atherogenic ApoE knockout mice and infiltrate the developing atheroma to give rise to lesional macrophages and, eventually, foam cells [26,37]. Comparatively, CD16⁺ monocytes are primarily implicated in human atherosclerosis. Increased frequency of non-classical monocytes, in association with other markers of dyslipidemia, has been demonstrated in multiple human studies [24,62,63]. Non-classical monocytes are mobilized in patients with hypercholesterolemia and their frequency in the peripheral blood appears to be directly correlated with total cholesterol and triglyceride level and inversely proportional to HDL concentration [62-64]. Recent work attempting to differentiate the contribution of CD16⁺ monocytes to atherosclerosis show that intermediate monocyte frequency is closely related to severity of angina and may contribute to atherosclerosis [65,66]. These findings have implications on therapeutic efficacy since monocyte subpopulations have variable expression of scavenger receptors that participate in cholesterol uptake and metabolism. For instance, an early randomized trial targeting hypercholesterolemia showed that statin treatment (fluvastatin) increased CD16⁺ monocytes [63]. Other studies have not identified a similar increase in non-classical monocytes following initiation of a statin (rosuvastatin, atorvastatin, and pivastatin), which may suggest class-dependent effects on monocyte frequency [67,68].

To compound the difficulty of interpreting these studies, the vast majority of human studies failed to include the intermediate monocyte population in their analysis. Recently, intermediate monocyte frequency was identified as a positive predictor of major cardiovascular event in two large European cohorts of 438 and 951 patients [49,69]. Further, intermediate monocyte frequency negatively correlated with HDL and Apo-1 [49,69], which is likely consistent with previous clinical studies demonstrating an association between CD16⁺ monocytes and markers of dyslipidemia. These findings are particularly intriguing since intermediate monocytes may possess significant inflammatory properties and

exaggerate oxidative stress when compared with non-classical monocytes. Intermediate monocytes express CCR2 and CCR5, which are critical for monocyte homing and trans-endothelial migration into the atherosclerotic plaque [26,27,61,70]. Genetic deletion of CCR2 or CCR5 in ApoE knockout mice significantly reduces atherosclerosis and monocyte mobilization [71-75]. Further exploration of the relationship between intermediate (CD14⁺⁺CD16⁺) monocytes and atherosclerosis will significantly advance our understanding of pro-atherogenic conditions and aid in the design of novel cell-directed therapies for the treatment of atherosclerosis.

Obesity represents a chronic inflammatory state that increases the risk for heart disease and stroke by greater than fifty percent [76,77]. Several groups have independently demonstrated that the frequency of non-classical and intermediate monocytes positively correlates with WHO obesity classification and fat mass [24,64,78-80]. In fact, a cohort of 105 subjects with WHO class II and III obesity had an increased frequency of non-classical and intermediate monocytes in the peripheral blood compared to healthy controls; a finding that was not observed in WHO class I subjects [64]. A modest 5% reduction in fat mass significantly reduced non-classical monocyte frequency and improved glycemic control in obese diabetic patients [64]. Not surprisingly, the vast majority of positive correlative studies between non-classical monocyte frequency and cardiovascular disease lose statistical significance in post-hoc analysis correcting for BMI. These epidemiologic observations in human cohorts provide evidence for a direct link between adiposity, inflammation, and monocyte maturation.

Animal models of metabolic disease may provide some clues to the connection between obesity and vascular inflammation. ApoE knockout (ApoE^{-/-}) mice provided a high fat western diet develop morbid obesity and have evidence of chronic inflammation. Loss of ApoE expression in hematopoietic cells appears to drive monocyte proliferation, mobilization, and differentiation in the development of atherosclerosis [81]. ApoE^{-/-} mice on high fat diet (HFD) developed a time-dependent exponential increase in Ly6C^{high} monocyte frequency in the peripheral blood and spleen compared to wildtype mice on HFD [37]. Ly6C^{high} monocytes rapidly incorporated into the developing atheroma and accounted for the vast majority of lesional macrophages [37,45]. However, local macrophage turnover within atheroma may drive foam cell formation independent of monocyte recruitment [36]. Several studies have demonstrated that ApoE interacts with the ATP-binding cassette transporters (i.e. ABCA1) to coordinate cholesterol efflux and monocyte recruitment [81-83]. Loss of ABCA1, and other cholesterol transport proteins, accelerates lesion formation

and likely participates in myeloid cell proliferation and function.

Recent attention to the role of adventitial adipose tissue, termed perivascular adipose tissue or PVAT, provides a novel look at the relationship between adiposity and vascular inflammation. Perivascular adipocytes secrete growth factors and adipokines that stimulate an inflammatory response by recruiting monocytes and other leukocytes to the vascular wall [84-88]. Precursor and differentiated adipocytes accumulate near atheroma in the perivascular adventitia of conduit vessels and expand with obesity [86]. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is highly secreted by perivascular adipocytes and is a potent mitogen for circulating monocytes [85,89]. Genetic deletion of MCP-1 in mice impedes macrophage infiltration and lipid deposition following arterial injury [85]. Interestingly, MCP-1 is sequentially expressed in the adventitia followed by the media and intima after coronary artery injury, suggesting a central role for perivascular adipocytes in monocyte recruitment [90]. These data provide evidence that perivascular adiposity, along with visceral and subcutaneous fat, participate in vascular inflammation and monocyte chemotaxis. How perivascular adipocytes interact with circulating hematopoietic cells and isolating their effect on leukocyte recruitment from other fat stores in vascular inflammation are important future areas of investigation.

Monocyte subpopulations in kidney disease

Chronic kidney disease (CKD) is closely related with cardiovascular health and commonly exists as a co-morbid condition in persons with cardiovascular disease. Persons with CKD often suffer from atherosclerosis and are at increased risk for mortality following an acute cardiovascular event [91,92]. Patient cohorts have uniformly demonstrated that increased intermediate (CD14⁺⁺CD16⁺) monocyte frequency is associated with cardiovascular event rate and increased mortality [70,78,93-95]. A study of 119 patients with CKD demonstrated an increased frequency of intermediate monocytes in the peripheral blood of hemodialysis-dependent CKD patients compared to CKD patients with adequate native renal function [70]. Depletion of intermediate monocytes during hemodialysis was associated with an increased cardiovascular event-free period and reduced mortality in patients with end-stage renal disease [70,96]. Classical and non-classical monocytes have failed to show a correlation with mortality in CKD patients regardless of the need for hemodialysis. Subgroup analysis identified intermediate monocytes expressing angiotensin-converting enzyme (ACE) were significantly higher in CKD patients with severe atherosclerosis and may yield additional prognostic value [94-96]. Increased ACE expression in intermediate monocytes has also been demonstrated in patients with abdominal aortic aneurysm and appeared to be independent of CKD classification [97]. While the role

of ACE (CD143) on circulating monocytes remains unknown, the renin-angiotensin system is critical for monocyte maturation and function and targeting this signaling axis may be of therapeutic benefit in monocyte-driven human disease [98,99]. Along this line of inquiry, a small cohort of dialysis-dependent CKD patients was randomized to an angiotensin receptor 1 blocker (losartan) or other anti-hypertensive medication and monocyte populations were evaluated. The losartan group showed a reduction of CD16⁺ monocytes in their peripheral blood after two months of therapy compared to patients receiving antihypertensive medication that did not target the renin-angiotensin axis [100]. The authors demonstrate that the CD16⁺ monocytes produced TNF- α *in vitro*; however their gating strategy failed to distinguish intermediate and non-classical monocytes within the CD16⁺ population. While CD14⁺⁺CD16⁺ monocytes appear to be an important biomarker of CKD severity, their role in the evolution of CKD is yet to be determined.

Monocytes and autoimmune disease

Monocyte-mediated inflammation has long been targeted in the treatment of rheumatoid arthritis. Attention to monocyte heterogeneity has expanded our understanding of rheumatologic diseases and provided new interventions for patients. Most groups have demonstrated an expansion of CD16⁺ monocytes in patients with rheumatoid arthritis with the recent delineation of CD16⁺ subpopulations strongly suggesting that CD14⁺⁺CD16⁺ monocytes are the principle inflammatory effectors [21,101,102]. CD16⁺ monocytes are found in the synovial fluid of RA patients and are associated with joint destruction [101]. Elevation of the CD16⁺ monocyte frequency in RA patients was recently demonstrated to be primarily an expansion of the intermediate population [21]. Sequestering the inflammatory properties of CD14⁺⁺CD16⁺ monocytes may inhibit joint injury and signify response to therapy. Monoclonal antibodies directed against TNF- α (Infliximab, Adalimumab, Etanercept) have been extremely effective in the treatment of RA and may target, in part, intermediate and non-classical CD16⁺ monocytes. A comparison study of anti-TNF- α antibodies demonstrated a reduction in CD14⁺⁺HLADR⁺ monocytes in RA patients taking infliximab that the authors suggest may account for the increased sepsis event rate noted in this study population [103]. HLADR is an important third marker (along with CD86) that is highly expressed on intermediate monocytes and less so on non-classical monocytes [104]. A larger cohort of RA patients (111 total) showed a slight, but non-significant increase in intermediate monocyte frequency in early RA that was profoundly increased in RA patients with long-standing disease [105]. In response to treatment with methotrexate, the authors identified that CD16 expression on CD14⁺⁺

monocytes was a negative predictor of response after 14 weeks of therapy. Both RA patients with and without a history of treatment with immune modulating agents showed a negative correlation between intermediate monocyte frequency during treatment and disease activity scores [105]. These clinical studies suggest that intermediate monocyte frequency may predict response to therapy and side effect profile in RA patients using immunosuppressive therapy. However, cytokine production has not been linked to monocyte subpopulation frequency in RA patients during active disease or following treatment. Therefore, the use of monocyte markers as a biomarker for therapeutic response remains limited.

Similar to RA patients, CD16⁺ monocytes are preferentially expanded in persons with both Crohn's Disease (CD) and ulcerative colitis [11,106,107]. Grip et al. showed a spike in intermediate monocyte frequency in CD patients with active disease, which was marked by increased expression of CCR2 on the intermediate monocyte population. Classical and intermediate monocytes expressed similar levels of CCR2 during active disease and the expression pattern of CCR2 on the intermediate monocyte population in CD patients was significantly elevated compared to the expression of CCR2 on matched healthy controls [11]. These findings are intriguing and suggest that monocytes, within a sub-classification system, may have alternative phenotypic signatures in response to inflammation. Further, this data supports the independent finding that MCP-1 (CCL2) levels are increased in patients with inflammatory bowel disease and correlate with disease severity [108,109]. Recently, Thiesen et al. showed enhanced migration of classical monocytes isolated from CD patients in response to MCP-1 (CCL2) and increased CD14 expression in macrophages isolated from inflamed intestinal mucosa of CD patients [110]. The CD14⁺⁺HLADR^{low} macrophages isolated from the colon of CD patients expressed high levels of CD62L which supports a classical monocyte origin, however, these macrophages also expressed the fractalkine receptor CX₃CR1 which is more characteristic of non-classical monocytes [110]. Other groups have found a more pronounced infiltration of CD16⁺ monocytes in actively inflamed tissue from CD patients that corresponded with increased staining for TNF- α [107]. Resolution between these findings may again point back to an integral role for intermediate monocytes in active CD.

Gaps and future directions in understanding monocyte heterogeneity

Recent adherence to the tripartite description of monocytes based on expression of CD14 and CD16 along with standardized gating strategies has enabled investigators to more thoroughly explore the nature of monocyte differentiation and function in human disease. However, the recognition of an intermediate population that most truly represents an

inflammatory monocyte casts a shadow on previous studies demonstrating expansion of classical and non-classical monocytes during inflammation. Thus, a re-examination of previous clinical studies and human cohorts with a uniform gating strategy and consistent use of approved nomenclature is warranted.

Though evidence of monocyte mobilization in human disease has now been demonstrated, the question of whether their rise is consequential of the illness/inflammation or directly contributes to the inflammatory process remains largely unanswered. In particular, does the increase in CD16⁺ monocyte (intermediate and non-classical) frequency observed in cases of both acute and chronic inflammation result from increased differentiation or do they arise independently in response to inflammatory signaling cascades. Human studies addressing these questions will likely focus on chronic diseases with periodic episodes of acute inflammation (i.e. autoimmunity), as several groups have pointed out that classical monocytes appear to exhibit a progressive increase in CD16 expression during acute illness [107,111,112]. A particularly interesting subset of these patients includes those with disease primarily affecting a single organ such as RA or Crohn's Disease. Comparative studies of monocyte frequency between the peripheral blood and diseased organ have generated promising data supporting the maturation of these monocytes in the peripheral blood prior to infiltration. Further, does the spleen function as a monocyte reservoir for mobilization during periods of acute inflammation as has been observed in mice [45]? Supportive *in vitro* characterization of all monocyte populations, especially the intermediate subset, will help define the inflammatory signature of each population and may lead to the designation of a true pro-inflammatory monocyte. Specific therapeutic targeting of the offending monocyte population may limit the need for treatments that indiscriminately target all monocytes and the important side effect profile associated with these types of therapies. Finally, could preferential recruitment of a less inflammatory monocyte population limit inflammation and promote healthy repair?

Conclusions

Recognition of three monocyte populations with diversified and heterogeneous responses in health and disease has increased mechanistic insight into the pathogenesis of inflammation as it relates to both chronic and acute illness. The more dichotomous murine monocyte populations, denoted as Ly6C^{high} and Ly6C^{low}, hinder the ability to directly translate animal findings to humans. Careful mechanistic and lineage tracing studies in mice and humans will enhance our understanding of monocyte function in health and disease. At present, the intermediate monocyte population appears to represent an inflammatory monocyte population and therefore harnessing the inflammatory capacity of

intermediate monocytes may promote a healthy response to injury or inflammation. Selective depletion of intermediate monocytes requires a highly specific surface marker regimen. Therefore, uniform adoption of the recommended gating strategies and nomenclature for proper identification of each monocyte subpopulation is necessary to provide a clear picture of their role in human disease [4,5,24].

Abbreviations

ABCA1: ATP-binding cassette transporter-1; ACE: Angiotensin-converting enzyme; AMI: Acute myocardial infarction; Apo-1: Apolipoprotein-1; ApoE: Apolipoprotein-E; CAD: Coronary artery disease; CCR2: C-C motif receptor-2; CCR5: C-C motif receptor-5; CD: Crohn's disease; CD14: Cluster of differentiation 14; CD16: Cluster of differentiation 16; CD62L/SELL: Cluster of differentiation 62 L; CD86: Cluster of differentiation 86; CKD: Chronic kidney disease; CX₃CR1: Fractalkine receptor; HDL: High density lipoprotein; HFD: High fat diet; HLADR: Human leukocyte antigen-DR; IL-1β: Interleukin-1β; LDL: Low density lipoprotein receptor; LPS: Lipopolysaccharide; Ly6C: Lymphocyte antigen 6C; MCP-1/CCL2: Monocyte chemoattractant protein-1/ chemokine (C-C Motif) ligand-2; M-CSF: Macrophage-colony stimulating factor; PVAT: Perivascular adipose tissue; RA: Rheumatoid arthritis; TNF-α: Tumor necrosis factor-α.

Competing interests

The authors have no conflicts of interest to resolve or competing interests to disclose.

Authors' contribution

BS and DI contributed equally to drafting, editing, and approving all aspects of this review.

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References

1. Passlick B, Flieger D, Ziegler-Heitbrock HW: Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* 1989, **74**:2527–2534.
2. Clarkson SB, Ory PA: CD16. Developmentally regulated IgG Fc receptors on cultured human monocytes. *J Exp Med* 1988, **167**:408–420.
3. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC: CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990, **249**:1431–1433.
4. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB: Nomenclature of monocytes and dendritic cells in blood. *Blood* 2010, **116**:e74–e80.
5. Heimbeck I, Hofer TP, Eder C, Wright AK, Frankenberger M, Marei A, Boghdadi G, Scherberich J, Ziegler-Heitbrock L: Standardized single-platform assay for human monocyte subpopulations: lower CD14 + CD16++ monocytes in females. *J Int Soc Anal Cytol* 2010, **77**:823–830.
6. Skrzeczynska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J: Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. *Scand J Immunol* 2008, **67**:152–159.

7. Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, Espevik T, Ziegler-Heitbrock L: **The proinflammatory CD14 + CD16 + DR++ monocytes are a major source of TNF.** *J Immunol* 2002, **168**:3536–3542.
8. Ancuta P, Rao R, Moses A, Mehle A, Shaw SK, Lusinskas FW, Gabuzda D: **Fractalkine preferentially mediates arrest and migration of CD16+ monocytes.** *J Exp Med* 2003, **197**:1701–1707.
9. Schlitt A, Heine GH, Blankenberg S, Espinola-Klein C, Doppeide JF, Bickel C, Lackner KJ, Iz M, Meyer J, Darius H, Rupprecht HJ: **CD14 + CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels.** *Thromb Haemost* 2004, **92**:419–424.
10. Fingerle G, Pforte A, Passlick B, Blumenstein M, Strobel M, Ziegler-Heitbrock HW: **The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients.** *Blood* 1993, **82**:3170–3176.
11. Grip O, Bredberg A, Lindgren S, Henriksson G: **Increased subpopulations of CD16(+) and CD56(+) blood monocytes in patients with active Crohn's disease.** *Inflamm Bowel Dis* 2007, **13**:566–572.
12. Mayer A, Hiebl B, Lendlein A, Jung F: **Support of HUVEC proliferation by pro-angiogenic intermediate CD163+ monocytes/macrophages: a co-culture experiment.** *Clin Hemorheol Microcirc* 2011, **49**:423–430.
13. Zawada AM, Rogacev KS, Rotter B, Winter P, Marell RR, Fliser D, Heine GH: **SuperSAGE evidence for CD14+/CD16+ monocytes as a third monocyte subset.** *Blood* 2011, **118**:e50–e61.
14. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, Puel A, Biswas SK, Moshous D, Picard C, Jais JP, D'Cruz D, Casanova JL, Trouillet C, Geissmann F: **Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors.** *Immunity* 2010, **33**:375–386.
15. Randolph GJ, Sanchez-Schmitz G, Liebman RM, Schakel K: **The CD16(+)(FcgammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting.** *J Exp Med* 2002, **196**:517–527.
16. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC: **The three human monocyte subsets: implications for health and disease.** *Immunol Res* 2012, **53**:41–57.
17. De Palma M, Murdoch C, Venneri MA, Naldini L, Lewis CE: **Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications.** *Trends Immunol* 2007, **28**:519–524.
18. Murdoch C, Tazzyman S, Webster S, Lewis CE: **Expression of Tie-2 by human monocytes and their responses to angiopoietin-2.** *J Immunol* 2007, **178**:7405–7411.
19. Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, Kourilsky P, Wong SC: **Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets.** *Blood* 2011, **118**:e16–e31.
20. Frankenberger M, Sternsdorf T, Pechumer H, Pforte A, Ziegler-Heitbrock HW: **Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis.** *Blood* 1996, **87**:373–377.
21. Rossol M, Kraus S, Pierer M, Baerwald C, Wagner U: **The CD14(bright) CD16 + monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population.** *Arthritis Rheum* 2012, **64**:671–677.
22. Kim WK, Sun Y, Do H, Autissier P, Halpern EF, Piatak M Jr, Lifson JD, Burdo TH, McGrath MS, Williams K: **Monocyte heterogeneity underlying phenotypic changes in monocytes according to SIV disease stage.** *J Leukoc Biol* 2010, **87**:557–567.
23. Rogacev KS, Zawada AM, Heine GH: **CKD-associated atherosclerosis and monocyte heterogeneity.** *Kidney Int* 2012, **81**:599. author reply 599–600.
24. Zawada AM, Rogacev KS, Schirmer SH, Sester M, Bohm M, Fliser D, Heine GH: **Monocyte heterogeneity in human cardiovascular disease.** *Immunobiology* 2012, **217**:1273–1284.
25. Strauss-Ayali D, Conrad SM, Mosser DM: **Monocyte subpopulations and their differentiation patterns during infection.** *J Leukoc Biol* 2007, **82**:244–252.
26. Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, Garin A, Liu J, Mack M, van Rooijen N, Lira SA, Habenicht AJ, Randolph GJ: **Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques.** *J Clin Invest* 2007, **117**:185–194.
27. Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF: **Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites.** *J Clin Invest* 2007, **117**:902–909.
28. Si Y, Tsou CL, Croft K, Charo IF: **CCR2 mediates hematopoietic stem and progenitor cell trafficking to sites of inflammation in mice.** *J Clin Invest* 2010, **120**:1192–1203.
29. Qu C, Edwards EW, Tacke F, Angeli V, Llodra J, Sanchez-Schmitz G, Garin A, Haque NS, Peters W, van Rooijen N, Sanchez-Torres C, Bromberg J, Charo IF, Jung S, Lira SA, Randolph GJ: **Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes.** *J Exp Med* 2004, **200**:1231–1241.
30. Kohn LA, Hao QL, Sasidharan R, Parekh C, Ge S, Zhu Y, Mikkola HK, Crooks GM: **Lymphoid priming in human bone marrow begins before expression of CD10 with upregulation of L-selectin.** *Nat Immunol* 2012, **13**:963–971.
31. Xu H, Manivannan A, Crane I, Dawson R, Liversidge J: **Critical but divergent roles for CD62L and CD44 in directing blood monocyte trafficking in vivo during inflammation.** *Blood* 2008, **112**:1166–1174.
32. Weiner LM, Li W, Holmes M, Catalano RB, Dovnarsky M, Padavic K, Alpaugh RK: **Phase I trial of recombinant macrophage colony-stimulating factor and recombinant gamma-interferon: toxicity, monocytosis, and clinical effects.** *Cancer Res* 1994, **54**:4084–4090.
33. Korkosz M, Bukowska-Strakova K, Sadis S, Grodzicki T, Siedlar M: **Monoclonal antibodies against macrophage colony-stimulating factor diminish the number of circulating intermediate and nonclassical (CD14(++)CD16 (+)/CD14(+)CD16(++)) monocytes in rheumatoid arthritis patient.** *Blood* 2012, **119**:5329–5330.
34. Getts DR, Terry RL, Getts MT, Muller M, Rana S, Shrestha B, Radford J, Van Rooijen N, Campbell IL, King NJ: **Ly6c + "inflammatory monocytes" are microglial precursors recruited in a pathogenic manner in west Nile virus encephalitis.** *J Exp Med* 2008, **205**:2319–2337.
35. Rivollier A, He J, Kole A, Valatas V, Kelsall BL: **Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon.** *J Exp Med* 2012, **209**:139–155.
36. Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, Iwamoto Y, Liao R, Zirlik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK: **Ly-6Chi monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium.** *Circ Res* 2014, **114**:1611–1622.
37. Swirski FK, Libby P, Aikawa E, Alcaide P, Lusinskas FW, Weissleder R, Pittet MJ: **Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atherosclerotic plaques.** *J Clin Invest* 2007, **117**:195–205.
38. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, Gorbatorov R, Sukhova GK, Gerhardt LM, Smyth D, Zavitz CC, Shikata EA, Parsons M, van Rooijen N, Lin HY, Husain M, Libby P, Nahrendorf M, Weissleder R, Swirski FK: **Local proliferation dominates lesional macrophage accumulation in atherosclerosis.** *Nat Med* 2013, **19**:1166–1172.
39. Swirski FK, Hilgendorf I, Robbins CS: **From proliferation to proliferation: monocyte lineage comes full circle.** *Semin Immunopathol* 2014, **36**:137–148.
40. Libby P, Nahrendorf M, Swirski FK: **Monocyte heterogeneity in cardiovascular disease.** *Semin Immunopathol* 2013, **35**:553–562.
41. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, Lang R, Haniffa M, Collin M, Tacke F, Habenicht AJ, Ziegler-Heitbrock L, Randolph GJ: **Comparison of gene expression profiles between human and mouse monocyte subsets.** *Blood* 2010, **115**:e10–e19.
42. Sunderkotter C, Nikolic T, Dillon MJ, van Rooijen N, Stehling M, Drevets DA, Leenen PJ: **Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response.** *J Immunol* 2004, **172**:4410–4417.
43. Landsman L, Varol C, Jung S: **Distinct differentiation potential of blood monocyte subsets in the lung.** *J Immunol* 2007, **178**:2000–2007.
44. Leuschner F, Panizzi P, Chico-Calero I, Lee WW, Ueno T, Cortez-Retamozo V, Waterman P, Gorbatorov R, Marinelli B, Iwamoto Y, Chudnovskiy A, Figueiredo JL, Sosnovik DE, Pittet MJ, Swirski FK, Weissleder R, Nahrendorf M: **Angiotensin-converting enzyme inhibition prevents the release of monocytes from their splenic reservoir in mice with myocardial infarction.** *Circ Res* 2010, **107**:1364–1373.
45. Swirski FK, Nahrendorf M, Eitzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, Figueiredo JL, Kohler RH, Chudnovskiy A, Waterman P, Aikawa E, Mempel TR, Libby P, Weissleder R, Pittet MJ: **Identification of splenic reservoir monocytes and their deployment to inflammatory sites.** *Science* 2009, **325**:612–616.
46. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, Kalchenko V, Geissmann F, Jung S: **Monocytes give rise to mucosal, but not splenic, conventional dendritic cells.** *J Exp Med* 2007, **204**:171–180.
47. Pfeffer MA, Braunwald E: **Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications.** *Circulation* 1990, **81**:1161–1172.
48. Tsujioka H, Imanishi T, Ikejima H, Kuroi A, Takarada S, Tanimoto T, Kitabata K, Okochi K, Arita Y, Ishibashi K, Komukai K, Kataiwa H, Nakamura N, Hirata K,

- Tanaka A, Akasaka T: **Impact of heterogeneity of human peripheral blood monocyte subsets on myocardial salvage in patients with primary acute myocardial infarction.** *J Am Coll Cardiol* 2009, **54**:130–138.
49. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, Grosse-Dunker G, Heisel I, Hornof F, Jeken J, Rebling NM, Ulrich C, Scheller B, Bohm M, Fliser D, Heine GH: **CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography.** *J Am Coll Cardiol* 2012, **60**:1512–1520.
 50. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, Pittet MJ: **The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions.** *J Exp Med* 2007, **204**:3037–3047.
 51. Saxena A, Chen W, Su Y, Rai V, Uche OU, Li N, Frangogiannis NG: **IL-1 induces proinflammatory leukocyte infiltration and regulates fibroblast phenotype in the infarcted myocardium.** *J Immunol* 2013, **191**:4838–4848.
 52. van der Laan AM, Hirsch A, Robbers LF, Nijveldt R, Lommerse I, Delewi R, van der Vleuten PA, Biemond BJ, Zwaginga JJ, van der Giessen WJ, Zijlstra F, van Rossum AC, Voermans C, van der Schoot CE, Piek JJ: **A proinflammatory monocyte response is associated with myocardial injury and impaired functional outcome in patients with ST-segment elevation myocardial infarction: monocytes and myocardial infarction.** *Am Heart J* 2012, **163**:57–65. e2.
 53. Tsujioka H, Imanishi T, Ikejima H, Tanimoto T, Kuroi A, Kashiwagi M, Okochi K, Ishibashi K, Komukai K, Ino Y, Kitabata H, Akasaka T: **Post-reperfusion enhancement of CD14(+)CD16(-) monocytes and microvascular obstruction in ST-segment elevation acute myocardial infarction.** *Circ J* 2010, **74**:1175–1182.
 54. Panizzi P, Swirski FK, Figueiredo JL, Waterman P, Sosnovik DE, Aikawa E, Libby P, Pittet M, Weissleder R, Nahrendorf M: **Impaired infarct healing in atherosclerotic mice with Ly-6C(hi) monocytes.** *J Am Coll Cardiol* 2010, **55**:1629–1638.
 55. Tapp LD, Shantsila E, Wrigley BJ, Pamukcu B, Lip GY: **The CD14++CD16+ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction.** *J Thromb Haemost* 2012, **10**:1231–1241.
 56. Urra X, Villamor N, Amaro S, Gomez-Choco M, Obach V, Oleaga L, Planas AM, Chamorro A: **Monocyte subtypes predict clinical course and prognosis in human stroke.** *J Cereb Blood Flow Metab* 2009, **29**:994–1002.
 57. Urra X, Cervera A, Obach V, Climent N, Planas AM, Chamorro A: **Monocytes are major players in the prognosis and risk of infection after acute stroke.** *Stroke* 2009, **40**:1262–1268.
 58. Kaito M, Araya S, Gondo Y, Fujita M, Minato N, Nakanishi M, Matsui M: **Relevance of distinct monocyte subsets to clinical course of ischemic stroke patients.** *PLoS one* 2013, **8**:e69409.
 59. Combadiere C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z: **Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytes and almost abolishes atherosclerosis in hypercholesterolemic mice.** *Circulation* 2008, **117**:1649–1657.
 60. Robbins CS, Chudnovskiy A, Rauch PJ, Figueiredo JL, Iwamoto Y, Gorbatov R, Eitzrodt M, Weber GF, Ueno T, van Rooijen N, Mulligan-Kehoe MJ, Libby P, Nahrendorf M, Pittet MJ, Weissleder R, Swirski FK: **Extramedullary hematopoiesis generates Ly-6C(high) monocytes that infiltrate atherosclerotic lesions.** *Circulation* 2012, **125**:364–374.
 61. Saederup N, Chan L, Lira SA, Charo IF: **Fractalkine deficiency markedly reduces macrophage accumulation and atherosclerotic lesion formation in CCR2-/- mice: evidence for independent chemokine functions in atherogenesis.** *Circulation* 2008, **117**:1642–1648.
 62. Rothe G, Gabriel H, Kovacs E, Klucken J, Stohr J, Kindermann W, Schmitz G: **Peripheral blood mononuclear phagocyte subpopulations as cellular markers in hypercholesterolemia.** *Arterioscler Thromb Vasc Biol* 1996, **16**:1437–1447.
 63. Rothe G, Herr AS, Stohr J, Abletshaus C, Weidinger G, Schmitz G: **A more mature phenotype of blood mononuclear phagocytes is induced by fluvastatin treatment in hypercholesterolemic patients with coronary heart disease.** *Atherosclerosis* 1999, **144**:251–261.
 64. Poitou C, Dalmas E, Renovato M, Benhamo V, Hajduch F, Abdennour M, Kahn JF, Veyrie N, Rizkalla S, Fridman WH, Sautes-Fridman C, Clement K, Cremer I: **CD14dimCD16+ and CD14 + CD16+ monocytes in obesity and during weight loss: relationships with fat mass and subclinical atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2011, **31**:2322–2330.
 65. Jaiparsad AS, Shantsila A, Lip GY and Shantsila E: **Expression of monocyte subsets and angiogenic markers in relation to carotid plaque neovascularization in patients with pre-existing coronary artery disease and carotid stenosis.** *Annals Med* 2014, **46**(7):530–538. doi: 10.3109/07853890.2014.931101.
 66. Zeng S, Zhou X, Ge L, Ji WJ, Shi R, Lu RY, Sun HY, Guo ZZ, Zhao JH, Jiang TM, Li YM: **Monocyte subsets and monocyte-platelet aggregates in patients with unstable angina.** *J Thromb Thrombolysis* 2014, **38**:439–446.
 67. Imanishi T, Ikejima H, Tsujioka H, Kuroi A, Ishibashi K, Komukai K, Tanimoto T, Ino Y, Takeshita T, Akasaka T: **Association of monocyte subset counts with coronary fibrous cap thickness in patients with unstable angina pectoris.** *Atherosclerosis* 2010, **212**:628–635.
 68. Coen PM, Flynn MG, Markofski MM, Pence BD, Hannemann RE: **Adding exercise to rosuvastatin treatment: influence on C-reactive protein, monocyte toll-like receptor 4 expression, and inflammatory monocyte (CD14 + CD16+) population.** *Metab Clin Exp* 2010, **59**:1775–1783.
 69. Rogacev KS, Zawada AM, Emrich I, Seiler S, Bohm M, Fliser D, Woollard KJ, Heine GH: **Lower Apo a-I and lower HDL-C levels are associated with higher intermediate CD14++CD16+ monocyte counts that predict cardiovascular events in chronic kidney disease.** *Arterioscler Thromb Vasc Biol* 2014, **34**:2120–2127.
 70. Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, Ulrich C, Fliser D, Heine GH: **CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease.** *Eur Heart J* 2011, **32**:84–92.
 71. Boring L, Gosling J, Cleary M, Charo IF: **Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis.** *Nature* 1998, **394**:894–897.
 72. Brauersreuther V, Steffens S, Arnaud C, Pelli G, Burger F, Proudfoot A, Mach F: **A novel RANTES antagonist prevents progression of established atherosclerotic lesions in mice.** *Arterioscler Thromb Vasc Biol* 2008, **28**:1090–1096.
 73. Brauersreuther V, Zerneck A, Arnaud C, Liehn EA, Steffens S, Shagdarsuren E, Bidzhekov K, Burger F, Pelli G, Luckow B, Mach F, Weber C: **Ccr5 but not Ccr1 deficiency reduces development of diet-induced atherosclerosis in mice.** *Arterioscler Thromb Vasc Biol* 2007, **27**:373–379.
 74. Ali ZA, Bursill CA, Douglas G, McNeill E, Pappaspyridonos M, Tatham AL, Bendall JK, Akhtar AM, Alp NJ, Greaves DR, Channon KM: **CCR2-mediated antiinflammatory effects of endothelial tetrahydrobiopterin inhibit vascular injury-induced accelerated atherosclerosis.** *Circulation* 2008, **118**:571–577.
 75. Heine GH, Ortiz A, Massy ZA, Lindholm B, Wiecek A, Martinez-Castelao A, Covic A, Goldsmith D, Suleymanlar G, London GM, Parati G, Sicari R, Zoccali C, Fliser D, European R: **Cardiovascular medicine working group of the European renal association-European D and transplant a. Monocyte subpopul Cardiovasc Dis Nat Rev Nephrol** 2012, **8**:362–369.
 76. Suk SH, Sacco RL, Boden-Albala B, Cheun JF, Pittman JG, Elkind MS, Paik MC, and Northern Manhattan Stroke S: **Abdominal obesity and risk of ischemic stroke: The northern Manhattan stroke study.** *Stroke* 2003, **34**:1586–1592.
 77. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P Jr, Razak F, Sharma AM, Anand SS, Investigators IS: **Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study.** *Lancet* 2005, **366**:1640–1649.
 78. Rogacev KS, Ulrich C, Blomer L, Hornof F, Oster K, Ziegelin M, Cremers B, Grenner Y, Geisel J, Schlitt A, Kohler H, Fliser D, Girndt M, Heine GH: **Monocyte heterogeneity in obesity and subclinical atherosclerosis.** *Eur Heart J* 2010, **31**:369–376.
 79. Cottam DR, Schaefer PA, Fahmy D, Shaftan GW, Angus LD: **The effect of obesity on neutrophil Fc receptors and adhesion molecules (CD16, CD11b, CD62L).** *Obes Surg* 2002, **12**:230–235.
 80. Cottam DR, Schaefer PA, Shaftan GW, Velcu L, Angus LD: **Effect of surgically-induced weight loss on leukocyte indicators of chronic inflammation in morbid obesity.** *Obes Surg* 2002, **12**:335–342.
 81. Murphy AJ, Akhtari M, Tolani S, Pagler T, Bijl N, Kuo CL, Wang M, Sanson M, Abramowicz S, Welch C, Bochem AE, Kuivenhoven JA, Yvan-Charvet L, Tall AR: **ApoE regulates hematopoietic stem cell proliferation, monocytes, and monocyte accumulation in atherosclerotic lesions in mice.** *J Clin Invest* 2011, **121**:4138–4149.
 82. Huang LH, Gui J, Artinger E, Craig R, Berwin BL, Ernst PA, Chang CC, Chang TY: **Acat1 gene ablation in mice increases hematopoietic progenitor cell proliferation in bone marrow and causes leukocytosis.** *Arterioscler Thromb Vasc Biol* 2013, **33**:2081–2087.
 83. Murphy AJ, Funt S, Gorman D, Tall AR, Wang N: **Pegylation of high-density lipoprotein decreases plasma clearance and enhances antiatherogenic activity.** *Circ Res* 2013, **113**:e1–e9.
 84. Brown NK, Zhou Z, Zhang J, Zeng R, Wu J, Eitzman DT, Chen YE, Chang L: **Perivascular adipose tissue in vascular function and disease: a review of**

- current research and animal models. *Arterioscler Thromb Vasc Biol* 2014, **34**:1621–1630.
85. Manka D, Chatterjee TK, Stoll LL, Basford JE, Konaniah ES, Srinivasan R, Bogdanov VY, Tang Y, Blomkalns AL, Hui DY, Weintraub NL: **Transplanted perivascular adipose tissue accelerates injury-induced neointimal hyperplasia: role of monocyte chemoattractant protein-1.** *Arterioscler Thromb Vasc Biol* 2014, **34**:1723–1730.
 86. Omar A, Chatterjee TK, Tang Y, Hui DY, Weintraub NL: **Proinflammatory phenotype of perivascular adipocytes.** *Arterioscler Thromb Vasc Biol* 2014, **34**:1631–1636.
 87. Owen MK, Noblet JN, Sassoon DJ, Conteh AM, Goodwill AG, Tune JD: **Perivascular adipose tissue and coronary vascular disease.** *Arterioscler Thromb Vasc Biol* 2014, **34**:1643–1649.
 88. Withers SB, Bussey CE, Saxton SN, Melrose HM, Watkins AE, Heagerty AM: **Mechanisms of adiponectin-associated perivascular function in vascular disease.** *Arterioscler Thromb Vasc Biol* 2014, **34**:1637–1642.
 89. Henrichot E, Juge-Aubry CE, Pernin A, Pache JC, Velebit V, Dayer JM, Meda P, Chizzolini C, Meier CA: **Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis?** *Arterioscler Thromb Vasc Biol* 2005, **25**:2594–2599.
 90. Jabs A, Okamoto E, Vinten-Johansen J, Bauriedel G, Wilcox JN: **Sequential patterns of chemokine- and chemokine receptor-synthesis following vessel wall injury in porcine coronary arteries.** *Atherosclerosis* 2007, **192**:75–84.
 91. Alonso A, Lopez FL, Matsushita K, Loehr LR, Agarwal SK, Chen LY, Soliman EZ, Astor BC, Coresh J: **Chronic kidney disease is associated with the incidence of atrial fibrillation: the atherosclerosis risk in communities (ARIC) study.** *Circulation* 2011, **123**:2946–2953.
 92. Druke TB, Massy ZA: **Atherosclerosis in CKD: differences from the general population.** *Nat Rev Nephrol* 2010, **6**:723–735.
 93. Heine GH, Ulrich C, Seibert E, Seiler S, Marell J, Reichart B, Krause M, Schlitt A, Kohler H, Girndt M: **CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients.** *Kidney Int* 2008, **73**:622–629.
 94. Ulrich C, Heine GH, Seibert E, Fliser D, Girndt M: **Circulating monocyte subpopulations with high expression of angiotensin-converting enzyme predict mortality in patients with end-stage renal disease.** *Nephrol Dial Transplant* 2010, **25**:2265–2272.
 95. Ulrich C, Heine GH, Gerhart MK, Kohler H, Girndt M: **Proinflammatory CD14 + CD16+ monocytes are associated with subclinical atherosclerosis in renal transplant patients.** *Am J Transplant* 2008, **8**:103–110.
 96. Rogacev KS, Ziegelin M, Ulrich C, Seiler S, Girndt M, Fliser D, Heine GH: **Haemodialysis-induced transient CD16+ monocytopenia and cardiovascular outcome.** *Nephrol Dial Transplant* 2009, **24**:3480–3486.
 97. Ghigliotti G, Barisione C, Garibaldi S, Brunelli C, Palmieri D, Spinella G, Pane B, Spallarossa P, Altieri P, Fabbi P, Sambucetti G, Palombo D: **CD16(+) monocyte subsets are increased in large abdominal aortic aneurysms and are differentially related with circulating and cell-associated biochemical and inflammatory biomarkers.** *Dis Markers* 2013, **34**:131–142.
 98. Tsubakimoto Y, Yamada H, Yokoi H, Kishida S, Takata H, Kawahito H, Matsui A, Urao N, Nozawa Y, Hirai H, Imanishi J, Ashihara E, Maekawa T, Takahashi T, Okigaki M, Matsubara H: **Bone marrow angiotensin AT1 receptor regulates differentiation of monocyte lineage progenitors from hematopoietic stem cells.** *Arterioscler Thromb Vasc Biol* 2009, **29**:1529–1536.
 99. Vuk-Pavlovic Z, Kreofsky TJ, Rohrbach MS: **Characteristics of monocyte angiotensin-converting enzyme (ACE) induction by dexamethasone.** *J Leukoc Biol* 1989, **45**:503–509.
 100. Merino A, Alvarez-Lara MA, Ramirez R, Carracedo J, Martin-Malo A, Aljama P: **Losartan prevents the development of the pro-inflammatory monocytes CD14 + CD16+ in haemodialysis patients.** *Nephrol Dial Transplant* 2012, **27**:2907–2912.
 101. Baeten D, Boots AM, Steenbakkers PG, Elewaut D, Bos E, Verheijden GF, Berheijden G, Miltenburg AM, Rijnders AW, Veys EM, De Keyser F: **Human cartilage gp-39+, CD16+ monocytes in peripheral blood and synovium: correlation with joint destruction in rheumatoid arthritis.** *Arthritis Rheum* 2000, **43**:1233–1243.
 102. Iwahashi M, Yamamura M, Aita T, Okamoto A, Ueno A, Ogawa N, Akashi S, Miyake K, Godowski PJ, Makino H: **Expression of Toll-like receptor 2 on CD16+ blood monocytes and synovial tissue macrophages in rheumatoid arthritis.** *Arthritis Rheum* 2004, **50**:1457–1467.
 103. Benucci M, Saviola G, Baiardi P, Manfredi M, Sarzi Puttini P, Atzeni F: **Determinants of risk infection during therapy with anti TNF-alpha blocking agents in rheumatoid arthritis.** *Rheumatol J* 2012, **6**:33–37.
 104. Abeles RD, McPhail MJ, Sowter D, Antoniadou CG, Vergis N, Vijay GK, Xystrakis E, Khamri W, Shawcross DL, Ma Y, Wendon JA, Vergani D: **CD14, CD16 and HLA-DR reliably identifies human monocytes and their subsets in the context of pathologically reduced HLA-DR expression by CD14(hi)/CD16(neg) monocytes: expansion of CD14(hi)/CD16(pos) and contraction of CD14(lo)/CD16(pos) monocytes in acute liver failure.** *J Int Soc Anal Cytol* 2012, **81**:823–834.
 105. Cooper DL, Martin SG, Robinson JI, Mackie SL, Charles CJ, Nam J, Consortium Y, Isaacs JD, Emery P, Morgan AW: **Fc gamma R11a expression on monocytes in rheumatoid arthritis: role in immune-complex stimulated TNF production and non-response to methotrexate therapy.** *PLoS ONE* 2012, **7**:e28918.
 106. Hanai H, Iida T, Takeuchi K, Watanabe F, Yamada M, Kikuyama M, Maruyama Y, Iwaoka Y, Hirayama K, Nagata S, Takai K: **Adsorptive depletion of elevated proinflammatory CD14 + CD16 + DR++ monocytes in patients with inflammatory bowel disease.** *Am J Gastroenterol* 2008, **103**:1210–1216.
 107. Koch S, Kucharzik T, Heidemann J, Nusrat A, Luegering A: **Investigating the role of proinflammatory CD16+ monocytes in the pathogenesis of inflammatory bowel disease.** *Clin Exp Immunol* 2010, **161**:332–341.
 108. Banks C, Bateman A, Payne R, Johnson P, Sheron N: **Chemokine expression in IBD. Mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn's disease.** *J Pathol* 2003, **199**:28–35.
 109. Nishimura M, Kuboi Y, Muramoto K, Kawano T, Imai T: **Chemokines as novel therapeutic targets for inflammatory bowel disease.** *Ann N Y Acad Sci* 2009, **1173**:350–356.
 110. Thiesen S, Janciauskiene S, Uronen-Hansson H, Agace W, Hogerkerp CM, Spee P, Hakansson K, Grip O: **CD14(hi)HLA-DR(dim) macrophages, with a resemblance to classical blood monocytes, dominate inflamed mucosa in Crohn's disease.** *J Leukoc Biol* 2014, **95**:531–541.
 111. Chiu YG, Shao T, Feng C, Mensah KA, Thullen M, Schwarz EM, Ritchlin CT: **CD16 (FcRgamma) as a potential marker of osteoclast precursors in psoriatic arthritis.** *Arthritis Res Therapy* 2010, **12**:R14.
 112. Appleby LJ, Nausch N, Midzi N, Mdluluzi T, Allen JE, Mutapi F: **Sources of heterogeneity in human monocyte subsets.** *Immunity Lett* 2013, **152**:32–41.

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