Monocyte subsets in atherosclerosis

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Summary
Endothelial dysfunction and chronic inflammation of the arterial wall continuously drive the development of atherosclerotic lesions. Monocytes, as cells of the innate immunity, are particularly involved in this process. In the last decade, heterogeneity of circulating monocytes has widely been acknowledged, and a recent consensus nomenclature subdivides classical, intermediate and nonclassical monocytes. Accumulating experimental and clinical data suggest a differential, subset-specific contribution of monocytes to the pathology of atherosclerosis.
This review summarizes recent key findings on human and mouse monocyte subpopulations, specifically highlighting their phenotype, functional characteristics and mechanisms of recruitment at homeostatic conditions, in atherosclerotic vascular disease, and after acute myocardial infarction.

Zusammenfassung
Endotheliale Dysfunktion und chronische Entzündung der Gefäßwand treiben die Entwicklung atherosklerotischer Läsionen kontinuierlich voran. Als Zellen der angeborenen Immunität sind Monozyten in zentraler Weise in diesen Prozess involviert. Im vergangenen Jahrzehnt erlangte die Heterogenität der Monozyten zunehmende Beachtung, wobei eine rezente Konsensus-Nomenklatur klassische, intermediäre und nicht-klassische Monozyten differenziert. Zahlreiche experimentelle und klinische Daten deuten darauf hin, dass Monozytensubpopulationen auf spezifische Weise zur Pathogenese der Atherosklerose beitragen.
Diese Übersichtsarbeit soll zentrale Ergebnisse kürzlich publizierter Studien über humane und murine Monozytensubpopulationen zusammenfassen. Sie fokussiert auf die phänotypischen und funktionellen Charakteristika von Monozytensubpopulationen und auf Mechanismen der Rekrutierung unter homöostatischen Bedingungen, bei atherosklerotischen Gefäßerkrankungen sowie nach akutem Myokardinfarkt.

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Monocytes are mononuclear cells of myeloid origin that are part of the innate immune system and represent around 6% of total leukocytes in men. After maturation and release from bone marrow they circulate for a relative short period of few days, followed by cell death or tissue infiltration with differentiation into macrophages or dendritic cells *in situ*.

> Peripheral demand of monocytes increases during inflammation or infection when monocytes are rapidly replaced by mobilization from the bone marrow and possibly by sequestration from the spleen, which result in elevated blood counts known as monocytosis (1).

Monocytes can secrete inflammatory cytokines and are characterized by specific surface molecules (e.g. integrins, TLRs, scavenger and G-protein coupled receptors) allowing locomotion towards distinct gradients but also direct immune effects and phagocytosis (2). Beside inflammation and infection, monocytes are involved in angiogenesis and myocardial healing after infarction. They play a further key role at homeostatic conditions by scavenging of toxic compounds and elimination of apoptotic cells mainly in gut and lung (2).

> Although monocyte plasticity is functionally restricted to conversion into 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 (3).

Atherosclerosis as an enduring inflammatory disease of the arterial wall is characterized by sub-endothelial lipoprotein accumulation and retention, endothelial activation with migration of immune cells to the inflamed intima, which result in chronic formation of fatty streaks and atheromas, followed by acute atherothrombotic events (4).

> Monocytes are prominently involved in the initiation, progression and complication of atherosclerotic lesions, especially when transforming into lipid-loaded macrophages or dendritic cells (4, 5).

Several recently published studies reviewed herein have addressed in detail the mechanisms of subset-specific recruitment of mouse monocytes in experimental atherosclerosis and further provided novel data

about relations of human monocyte subsets to cardiovascular risk factors and outcome.

Phenotype and function of monocyte subsets

Human monocyte subsets

Conferring to current nomenclature established in 2010, the human bloodstream monocytes are classified into three subsets following their expression patterns of the lipopolysaccharide (LPS) receptor CD14 and the FcγIII receptor CD16:

- classical $CD14^{++}CD16^{-}$,
- intermediate $CD14^{++}CD16^{+}$ and
- non-classical $CD14^{+}CD16^{++}$ monocytes (6).

These subsets differ significantly in proportion, phenotype and function.

So far, the $CD14^{++}CD16^{-}$ monocytes represent the major fraction (about 85% of total monocytes) and highly express Ccr2, FcγRI, CD62L, PSGL-1, IL-6R, scavenger receptor class-A (SR-A) and CD36 (7, 8) (▶Tab. 1).

The intermediate $CD14^{++}CD16^{+}$ monocytes (near 5% of monocytes) show an expression profile which is partly shared with classical and partly with nonclassical monocytes. However, this subset has some unique features, which distinguish it from the other two subsets. For instance, $CD14^{++}CD16^{+}$ monocytes are also enriched in bone marrow, and they display highest levels of Ccr5, TLR4, CD163, Tie2, VEGFR1/2, CD105 and HLA-DR (8–10) (▶Tab. 1).

The nonclassical $CD14^{+}CD16^{++}$ monocytes (near 10% of monocytes) are less granular and smaller in size. They show highest levels of Cx_3cr1, CD49d, sialophorin and SLAN (▶Tab. 1) but much lower amounts of Ccr2 and Ccr5 as compared to classical and intermediate subsets (8, 9, 11).

Functionally, classical monocytes are professional phagocytes giving rise to M1 macrophages and foam cells that ingest native LDL, generate reactive oxygen species and secret cytokines in response to LPS during infection or inflammation (8, 10, 12). In contrast, the nonclassical monocytes are weak phagocytes taking-up preferentially ox-LDL but substantially secreting inflammatory cytokines such as TNF-α, IL-1b and Ccl3 after TLR-dependent activation, e.g. by viruses and nucleic acids (8, 11).

> Thus, nonclassical monocytes rather serve as patrolling immune cells that selectively remove virally infected or injured cells and detoxify ox-LDL in the microcirculation at steady state (11).

Finally, the intermediate subset shows highest spontaneous generation of reactive oxygen species and had the most pronounced proangiogenic capacity (10).

Mouse monocyte subsets

Several published studies have divided mouse monocytes in two major subsets according to their expression of Gr1/Ly6C (13):

- $Gr1^{+}/Ly6C^{hi}$ and
- $Gr1^{-}/Ly6C^{low}$.

The rather dominant $Ly6C^{hi}$ subset shows high expression of Ccr2, CD62L and PSGL-1 (▶Tab. 1) together with preferential recruitment to inflamed tissue, extravasation and differentiation into inflammatory macrophages (7, 13–15). In contrast, $Ly6C^{low}$ monocytes display higher levels of Cx_3cr1, LFA-1, CD11c, CD36 and SR-A (▶Tab. 1) thus featuring more intravascular retention with endothelial crawling, removal of cellular debris or homing to non-inflamed tissue as regenerative cells (7, 13, 14, 16). Nevertheless, $Ly6C^{low}$ monocytes may also initiate inflammatory endothelial damage, e.g. by activation and recruitment of neutrophils (17).

The $Ly6C^{hi}$ subset is the possible murine equivalent of human classical monocytes, while the patrolling $Ly6C^{low}$ subset corresponds partly to nonclassical monocytes. In contrast to men, mouse monocytes represent, however, a central leukocyte fraction and the prevalence of $Ly6C^{hi}$ monocytes is less pronounced. Furthermore, $Ly6C^{low}$ monocytes show an expression pattern of scavenger receptors which is opposite to their human counterparts and very few data exist about the significance of intermediate mouse monocytes. It should be also taken into account that most of the experimental *in vivo* studies (as summarized in the next section) were performed in *Apoe* or *Ldlr* deficient mice on forced high-fat diet (HFD) to induce atherosclerosis, which is hardly comparable to human disease such as dyslipidaemia. Moreover, *Apoe* deficiency itself causes monocytosis independent of hypercholesterolaemia (18).

Monocyte recruitment during atherosclerosis

The specific mechanisms of differential mobilization, trafficking and survival of mouse monocyte subsets during atherosclerosis was investigated in detail by several experimental models of receptor knock-out, bone marrow transplantation, cell depletion and reconstitution or adoptive transfer. However, similar mechanistic research on human monocyte subsets is not possible, being thereby very restricted and mainly correlative. Up to now, only one study has provided comparative microarray data on sorted human monocyte subsets and plaque samples collected during carotid endarterectomy (19).

Tab. 1 Differential expression patterns of crucial markers on human CD14/CD16 and mouse Gr1/Ly6C monocyte subsets

monocyte subset	major markers
$CD14^{++}CD16^{-}$ (classical)	Ccr2, FcgRI, CD62L, PSGL-1, IL-6R, SR-A, CD36
$CD14^{++}CD16^{+}$ (intermediate)	Ccr5, CD163, TLR4, HLA-DR, CD105, Tie2, VEGFR1/2
$CD14^{+}CD16^{++}$ (nonclassical)	Cx3cr1, CD49d, sialophorin, SLAN
$Gr1^{+}/Ly6C^{hi}$	Ccr1, Ccr2, Ccr5, Cxcr2, CD62L, PSGL-1
$Gr1^{-}/Ly6C^{low}$	Cx3cr1, Ccr5, LFA-1, CD11c, SR-A, CD36

Referring further to the extensive data from atherosclerotic mouse models, *Apoe*$^{-/-}$ mice kept on HFD reveal monocytosis with shifts to Ly6C^{hi} monocytes that efficiently homed to plaques and depletion of these monocytes or inhibition of their plaque influx was associated with reduction in lesion size, macrophage content and apoptosis (20–24). By comparison, the Ly6C^{low} monocytes were either unaffected or equally increased, their count correlated with lesion size or they were robustly recruited to atherosclerotic plaques that was associated with plaque location, e.g. aortic arch versus sinus (21, 23, 24).

The Ly6C^{low} monocytes usually patrol the microvasculature through long-range Cx_3cr1-dependent endothelial crawling, which allows their rapid extravasation at sites of inflammation with subsequent initiation of early immune response (25). Accordingly, mice deficient in either Cx_3cr1 or Cx_3cl1 display reduced numbers of Ly6C^{low} monocytes, whereas the Ly6C^{hi} subset remains unaffected (26). However, the Ly6C^{low} monocytes do not require Cx_3cr1 for plaque recruitment but instead use Ccr5, which is overexpressed in *Apoe*$^{-/-}$ mice (14, 27). By comparison, Ly6C^{hi} monocytes are mobilized by both, the Ccl2/Ccr2 and Cxcl1/Cxcr2 axes, but require Ccr1 and Ccr5 for trafficking to atherosclerotic plaques, while the Cx_3cl1/Cx_3cr1 axis rather mediates their survival in the lesion (20, 22, 26).

The role of Ccr2 and Ccl5/Ccr5 during recruitment of both subsets is consistent with the observation of decreased monocyte counts and attenuated atherogenesis when Ccr2 and/or Ccr5 are absent or selectively blocked (27–30). Similarly, a more complex therapeutic approach of combined Ccl2, Cx_3cr1 and Ccr5 inhibition was again associated with monocytopenia and pronounced atheroprotection in *Apoe*$^{-/-}$ mice (23).

Beyond these implications of monocyte heterogeneity in the initiation and progression of atherosclerotic lesions, recent experimental evidence points towards a central role of monocyte subsets in the pathophysiology of acute myocardial infarction (AMI). Initial rodent models suggested that the induction of myocardial ischaemia is followed by a biphasic, subset-specific monocyte recruitment to the infarcted tissue:

> In the peripheral blood, an early increase in Ly6C^{hi} monocytes occurs within the first four days after AMI, whereas Ly6C^{low} monocytes counts rise to a much lesser extent (16).

Subsequently, the ratio between Ly6C^{hi} and Ly6C^{low} monocytes normalizes in the peripheral blood. Initially, it had been hypothesized that such a subset-specific monocytosis may allow Ly6C^{hi} monocytes to be preferentially recruited to myocardial tissue within the first days after infarction, where they contribute to digestion of damaged myocardial tissue and to removal of necrotic debris. After these initial days, a selective recruitment of Ly6C^{low} monocytes to the myocardial tissue was propagated which might contribute to healing and tissue repair. Notably, very recent animal data challenge the idea of a biphasic recruitment of monocytes subsets after AMI.

> In a series of elegant experimental studies, Hilgendorf et al. first confirmed that Ly6C^{hi} monocytes are rapidly recruited to infarcted myocardial tissue (31).

However, rather than being replaced by Ly6C^{low} monocytes from the peripheral circulation, Ly6C^{hi} monocytes develop within a few days into Ly6C^{low} macrophages. Other recent data in *Apoe*$^{-/-}$ mice has shown that AMI or stroke exacerbate atheroprogression that was attributable to increased plaque size, accelerated myeloid cell recruitment and activation of the Ly6C^{hi} monocytes inside the lesion (32). This may explain the higher risk for recurrent cardiovascular events in patients after an acute coronary syndrome.

Experimental AMI is further associated with sustained lymphocyte infiltration, production of proinflammatory cytokines and cardiac autoantibodies (33). The potential role of monocyte subsets during this innate autoimmune response after ischaemia/reperfusion injury is another central aspect which scientists (and clinicians) will have to face in the future. Again, such animal data should not uncritically be transferred to human pathophysiology.

Monocytes in clinical studies

Determination of monocyte subsets by flow cytometry

Epidemiological data have acknowledged leukocytosis as independent risk factor and predictor of future cardiovascular events (34). Ongoing differential flow cytometric analysis of monocyte subsets in several clinical studies reviewed below further dissects this knowledge, thus allowing more detailed risk assessment next to defining possible new therapeutic options.

> According to these studies, monocyte subsets appear to represent novel, intriguing biomarker in cardiovascular disease.

However, there is still no agreement on their flow cytometric determination, and some previous clinical studies discussed below have analysed the CD16-positive monocytes as one population, thus preventing any reliable conclusion on the individual role of intermediate and nonclassical monocytes. According to current nomenclature, the flow cytometric measurement of monocytes is always based on staining for CD14 and CD16 (6). This core panel can be further modified by including pan-haematopoietic (CD45), pan-monocytic (CD86, HLA-DR) and/or other markers such as Ccr2 at appropriate gating in order to increase assay sensitivity and specificity (35–38). The monocyte number is presented either as percentage or absolute count. Additional details concerning sample handling and storage are also important. In this regard, the staining procedure should be performed at room temperature as single platform, lyse-no-wash assay from freshly obtained, EDTA-anticoagulated venous blood to prevent any potential cell loss or activation but also to allow quick sample proceeding.

> As recently shown, monocyte counts should be analysed no later than two hours after blood sampling (38).

Further technical merits regarding monoclonal antibody clones, fluorochrome selection, fluorescence compensation, instru-

ment calibration and data acquisition need attention and standardization. Hence, the appropriate flow cytometric protocol for enumeration of monocyte subsets should be uniform, technically sound for routine epidemiological use and ideally approved by an expert committee.

Monocyte subsets and cardiovascular risk factors

The subset-specific contribution of monocytes towards the initiation and progression of atherosclerotic vascular disease, which was suggested in experimental work discussed, inspired several scientific groups to analyse human monocyte heterogeneity in the context of cardiovascular risk factors (39).

Among classical cardiovascular risk factors, obesity is most strongly associated with altered monocyte subset distribution. This link was first observed by Cottam et al. who demonstrated a shift towards CD16-positive monocytes in a small group of 26 patients with WHO obesity class III (body-mass index, BMI >40 kg/m^2) (40). These initial findings were confirmed and extended by Poitou et al. among 105 patients with WHO obesity class II and III (BMI >35 kg/m^2) who participated in a gastric surgery program, 39 patients with less advanced obesity (25–35 kg/m^2) undergoing a weight reduction program, and 32 lean healthy controls (41).

> Frequencies and cell counts of intermediate and nonclassical monocyte subsets were substantially higher in patients with obesity classes II and III, but not in less severe obesity, compared to controls.

Moreover, CD16-positive monocytes were correlated with fat mass (measured via dual-energy X-ray absorptiometry), and with insulin resistance (HOMA-IR), even though the latter associations lost significance after adjustment for BMI and other confounders.

Even beyond these selected cohorts from centres caring for people with morbid obesity, an association between BMI and counts of nonclassical monocytes could be confirmed in larger cohorts of apparently healthy individuals and patients referred for elective coronary angiography, whereas a relevant association between BMI and intermediate monocyte counts was not found in these cohorts (37, 42). A pathophysiological link between obesity and a shift towards CD16-positive monocytes is underscored by the observation that intermediate and nonclassical monocyte counts were significantly decreased in individuals undergoing RYGB (Roux-en-Y gastric bypass) for weight loss (41). A shift in monocyte subsets is also observed in nicotine abuse, which has first been reported among 80 patients with coronary artery disease (39).

While increased counts of total leukocytes and monocytes in smokers have been acknowledged for decades, current evidence suggests that this monocytosis does not affect all monocyte subsets. Instead, counts of classical and intermediate monocytes are elevated, whereas counts of nonclassical monocytes are lower in smokers compared to non-smokers.

Chronic kidney disease (CKD), which has been acknowledged as a central nontraditional cardiovascular risk factor in recent years, severely affects circulating monocyte subsets (43). Even in mild to moderate CKD, a gradual rise in cell counts of intermediate monocytes occurs. Patients with more advanced CKD have significant higher counts of both intermediate and nonclassical monocytes compared to healthy individuals, whereas counts of classical monocytes do not rise substantially (44). It is beyond the scope of this review to discuss monocyte heterogeneity in the context of renal replacement therapy in detail, and the interested reader may refer to a recent overview article from the EURECAM group (45).

Finally, preliminary reports points towards a shift in monocyte subset distribution among physically inactive individuals, with an increase in CD16-positive monocytes (46). Of note, the initiation of regular endurance and resistance exercise may normalize this altered monocyte subset distribution within three months (46, 47). Unfortunately, these studies on the implications of physical inactivity in the context of monocyte heterogeneity were performed before the consensus report on monocyte heterogeneity (6), so that data separately analysing intermediate and nonclassical monocyte counts by using state-of-the-art flow cytometry as discussed above are eagerly awaited.

> In summary, current evidence suggests that smoking status, obesity, CKD and physical inactivity may affect monocyte subset counts, even though the exact pathophysiological pathways underlying these associations remain to be resolved (▶ Tab. 2).

Monocyte subsets and adverse cardiovascular outcome

In aggregate, experimental work on a pathogenic role of specific monocyte subsets in atherosclerotic vascular disease, and clinical cross-sectional studies on associations between cardiovascular risk factors and monocyte heterogeneity, gave rise to the idea that cell counts of specific monocyte subsets may predict adverse cardiovascular outcome.

However, as monocyte subset quantification is technically demanding and necessitates the analysis of fresh blood samples, few prospective studies analysed the prognostic implications of monocyte subset biology prospectively. The first studies in this context were performed in patients with CKD, because they have a very pronounced shift in monocyte subset distribution, and as they suffer from accelerated atherosclerotic and arteriosclerotic vascular disease, so that epidemiological studies requested only small patient cohorts. Indeed, the first prospective study recruited 94 patients on chronic dialysis treatment, who were followed for 35 months. Elevated counts of intermediate monocytes predicted adverse cardiovascular outcome both in univariate and multivariate analyses, whereas counts of total, classical and nonclassical monocytes were not associated with outcome (48). These findings were confirmed in similarly sized cohorts of dialysis patients, which suggested that analysis of intradialytic monocyte kinetics or expression of angiotensin-converting enzyme yields further prognostic information, and a rather small cohort of 119 CKD patients not depending on renal replacement therapy (49–51).

These preliminary data gave rise to larger prospective cohort studies: Recent data from CARE FOR HOMe, an ongoing cohort study which follows patients with mild to moderate chronic kidney disease (CKD stage G2–G4, corresponding to an estimated glomerular filtration rate 90–15 ml/min/1.73 m^2) confirmed that, among 438 CKD patients, intermediate monocyte counts are independent predictors of adverse cardiovascular outcome (52).

Two studies analysed the prognostic implications of monocyte subsets beyond the field of manifest CKD (42, 53), namely the

- Malmö Diet and Cancer cohort and
- HOM SWEET HOMe study.

These studies yielded partly contradictory results. Within the population-based Malmö Diet and Cancer cohort, monocyte subsets were determined from 700 study participants without prevalent cardiovascular disease. 123 out of 700 individuals suffered an ischaemic cardiovascular event (AMI, ischaemic stroke or coronary death) during a median follow-up of 15.2 years. In univariate analyses, patients who suffered an ischaemic cardiovascular events had more classical ($p = 0.003$) and intermediate ($p = 0.051$) monocytes. The authors reported that after adjustment for confounders, relative cell counts of classical monocytes predicted adverse cardiovascular outcome, while CD16-positive monocytes did not. Interestingly, separate multivariate regression analysis data for intermediate monocytes are not presented. One major limitation of these data from the Malmö Diet and Cancer cohort is the retrospective analysis of monocyte subsets. Blood samples had been collected in 1991–1994, and monocytes were analysed from frozen samples stored for several years, which definitely affects monocyte phenotypes. This is exemplarily shown by an altered pattern of chemokine receptors (e.g. overexpression of Ccr5 on nonclassical monocytes) and the appearance of an ill-defined $CD14^{dim}CD16^{dim}$ monocyte population, which is not found within fresh samples. Next, no pan-monocyte marker was used, which is mandatory for proper separation of CD16-positive monocytes from CD16-expressing natural killer cells and granulocytes, and thus for proper

Tab. 2 Univariate Spearman correlation coefficients between cell count of monocyte subsets and cardiovascular risk factors
HDL-C: high density lipoprotein-cholesterol; eGFR: estimated glomerular filtration rate (estimated from CKD-EPIcreat equation [CARE FOR HOMe / HOM SWEET HOMe] or MDRD equation [I LIKE HOMe]). In aggregate, intermediate ($CD14^{++}CD16^{+}$) and nonclassical ($CD14^{+}CD16^{++}$) monocytes are more strongly associated with cardiovascular risk factors than classical ($CD14^{++}CD16^{-}$) monocytes.

program (ref.)	patients (n)	monocyte subset	body mass index	age	systolic blood pressure	total cholesterol	HDL-C	triglycerides	eGFR
CARE FOR HOME (52)	with chronic kidney disease G2–G4 (438)	$CD14^{++}CD16^{-}$ (classical)	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$
		$CD14^{++}CD16^{+}$ (intermediate)	$-0.10 \le r < 0.10$	$0.10 \le r < 0.15$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.15 \le r < -0.10$	$-0.10 \le r < 0.10$	$-0.20 \le r < -0.15$
		$CD14^{+}CD16^{++}$ (nonclassical)	$0.15 \le r < 0.20$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.15 \le r < -0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$
HOM SWEET HOMe (42)	before elective coronary angiography (951)	$CD14^{++}CD16^{-}$ (classical)	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.15 \le r < -0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$
		$CD14^{++}CD16^{+}$ (intermediate)	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.15 \le r < -0.10$	$-0.10 \le r < 0.10$	$-0.15 \le r < -0.10$
		$CD14^{+}CD16^{++}$ (nonclassical)	$0.15 \le r < 0.20$	$-0.10 \le r < 0.10$	$0.10 \le r < 0.15$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$0.10 \le r < 0.15$	$-0.10 \le r < 0.10$
I LIKE HOMe (37)	healthy individuals (569)	$CD14^{++}CD16^{-}$ (classical)	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$
		$CD14^{++}CD16^{+}$ (intermediate)	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$
		$CD14^{+}CD16^{++}$ (nonclassical)	$0.20 \le r < 0.25$	$0.10 \le r < 0.15$	$0.15 \le r < 0.20$	$-0.10 \le r < 0.10$	$-0.10 \le r < 0.10$	$0.15 \le r < 0.20$	$-0.10 \le r < 0.10$

quantification of intermediate and nonclassical monocytes. Data on participant outcomes were taken from registry data by ICD codes, and loss to follow-up was not reported. Finally, the concern was raised that the study sample may have been underpowered, with similar effect sizes for classical (hazard ratio, HR 3rd vs. 1st tertile: 1.66; 95% CI, 1.02–2.72), CD16-positive (HR 3rd vs. 1st tertile: 1.44; 0.87–2.39), and total monocyte counts (HR 3rd vs. 1st tertile: 1.61, 0.98–2.66) (54).

The second large prospective cohort is the HOM SWEET HOMe, which was specifically designed to address the prognostic implications of monocyte heterogeneity among patients referred for elective coronary angiography (42). Monocyte subpopulations were analysed from fresh blood samples on the day of each individual's study inclusion, and patients were followed for a mean of 2.6 years. 93 patients suffered the primary end-point, which was nearly identical to the Malmö Diet and Cancer cohort, namely the first occurrence of an AMI, a non-haemorrhagic stroke, or a fatal cardiovascular event. Event status was assessed by regular follow-up interviews with study participants, or their next of kin, and subsequent verification from treating physicians' medical records. Similar to the Malmö Diet and Cancer cohort, classical and intermediate monocytes predicted adverse outcome at univariate analysis. After adjustment for classical cardiovascular risk factors, both monocyte subsets remained independent outcome predictors. However, after further adjustment for renal function, prevalent cardiovascular disease, and other markers of inflammation, classical monocyte counts were no longer associated with future cardiovascular events. In contrast, patients with higher counts of intermediate monocytes had a threefold elevated risk compared to patients with lowest cell counts even in the fully adjusted model.

Limitations of HOM SWEET HOMe are the short follow-up period. As follow-up is ongoing until 2014, long-term outcome data are scheduled to be presented in 2015.

In summary, the Malmö Diet and Cancer cohort and HOM SWEET HOMe suggest a subset-specific contribution of monocytes to the development of future cardiovascular events. They further agree in that classical and intermediate, rather than nonclassical, monocytes predict adverse outcome. Differences exists in the study population, and in the applied immunological methods, which may explain their partly divergent results on the independent contribution of classical vs. intermediate monocytes to the occurrence of future cardiovascular events. Moreover, both studies were conducted in Northern/Central Europe and comprise mainly Caucasians, and their findings should not uncritically be transferred to other ethnic groups.

Despite these limitations, we are positive that the general acknowledgement of monocyte heterogeneity in the context of atherosclerotic cardiovascular disease will stimulate pharmacologists to design novel treatment strategies, which may interfere with subset-specific monocytic functions.

Currently, no class of routinely applied immunosuppressive drugs has modulating effects on monocyte heterogeneity that could be potentially beneficial in cardiovascular medicine. For example, therapy with low-dose steroids was associated with lower nonclassical monocytes but higher classical and intermediate monocyte counts (36, 55). Thus, from today's standpoint, inhibitors of chemokine receptors – which are differentially expressed by monocyte subpopulations – appear most promising, as such agents have already been introduced in other fields of internal medicine (5, 56).

Monocyte heterogeneity in acute myocardial infarction

Data on a subset-specific contribution of monocytes to AMI in humans are scarce and less comprehensive as (unlike in animal studies) it is virtually impossible to have baseline data before the onset of AMI. Nonetheless, first data from cohort studies were seemingly in line with animal data. They suggested a sequential mobilization of monocyte subsets in human AMI with an initial rise in circulating $CD14^{++}CD16^{-}$ monocytes, followed by a subsequent rise in CD16-positive monocytes (57). Moreover, patients with highest $CD14^{++}CD16^{-}$ monocyte counts had poor short-term and intermediate-term outcome, as reflected by lower myocardial salvage at day seven, and worse ejection fraction at month six post infarction (57).

Admittedly such cohort data cannot prove a direct detrimental effect of $CD14^{++}CD16^{-}$ monocytes in human AMI, as high $CD14^{++}CD16^{-}$ monocytes counts might merely reflect more severe ischaemic injury at baseline. Moreover, subsequent studies, inspired by the consensus nomenclature for monocyte subpopulations yielded partly divergent results and suggested a prominent increase in intermediate $CD14^{++}CD16^{+}$ monocytes (but not in $CD14^{+}CD16^{++}$ monocytes) within the first 24 hours after AMI (58, 59).

AMI was further associated with significant and persistent increase in monocyte-platelet complexes despite antiplatelet therapy and the intermediate subset had highest proportion of platelet aggregates (58). These findings imply that the monocyte-platelet interaction during the onset of AMI obviously associate with other mechanisms different from platelet aggregation. Beyond haemostasis and thrombosis, platelets are considered as versatile regulators of inflammation and immune response (60). Especially under coronary flow conditions, the P-selectin-mediated adhesion of platelets to monocytes results in circulating complexes with increased adhesion to endothelial cells and immigration into the arterial wall (61, 62).

Vice versa, monocytes are the major source of blood tissue factor and may accelerate thrombosis (59, 63). As shown in one previous study, patients after AMI have significantly higher monocytic procoagulant activity as compared to age-matched controls (64). Thus, the detailed, subset-specific role of monocytes in the activation of platelets during AMI denotes a further central aspect of clinical research.

Conclusions

To allow a better understanding of the hitherto partly contradictory findings from

single-centre epidemiologic studies, an even larger, multicentre approach would be required, which prospectively analyses monocyte heterogeneity in the context of cardiovascular morbidity, preferentially across different ethnical groups. As a prerequisite for such a multicentre approach, a consensus on methodical standards for enumeration of monocyte subset counts across different laboratories is mandatory. Once such a multicentre epidemiologic approach suggests the specific contribution of a single monocyte subset to the onset and progression of cardiovascular disease, future experimental and clinical studies should design therapeutic strategies to target this specific monocyte subset with the aim to reduce cardiovascular events in individuals at elevated risk.

> Until such data are available, individuals should be encouraged to follow general life style recommendations for primary and secondary disease prevention, such as cessation of smoking and weight control, both of which will centrally contribute to cardiovascular health, and may (as a side effect) beneficially affect monocyte subset distribution as well.

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Conflict of interest

The authors declare that they have no conflict of interest.

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