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Sprangers, Sara; de Vries, T.J.; Everts, V.

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Review Article

Monocyte Heterogeneity: Consequences for Monocyte-Derived Immune Cells

Sara Sprangers, 1 Teun J. de Vries, 2 and Vincent Everts 1

1Department of Oral Cell Biology and Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, MOVE Research Institute Amsterdam, Gustav Mahlerlaan 3004, 1081 LA Amsterdam, Netherlands
2Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, MOVE Research Institute Amsterdam, Gustav Mahlerlaan 3004, 1081 LA Amsterdam, Netherlands

Correspondence should be addressed to Sara Sprangers; s.l.sprangers@acta.nl

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Blood monocytes are precursors of dendritic cells, macrophages, and osteoclasts. They are a heterogeneous cell population with differences in size, phenotype, and function. Although monocytes maintain several tissue-specific populations of immune cells in homeostasis, their contribution to populations of dendritic cells, macrophages, and osteoclasts is significantly increased in inflammation. Identification of a growing number of functionally different subsets of cells within populations of monocyte-derived immune cells has recently put monocyte heterogeneity in sharp focus. Here, we summarize recent findings in monocyte heterogeneity and their differentiation into dendritic cells, macrophages, and osteoclasts. We also discuss these advances in the context of the formation of functionally different monocyte-derived subsets of dendritic cells, macrophages, and osteoclasts.

1. Monocyte Phenotypical and Functional Heterogeneity

Monocytes are circulating leukocytes that are key players in tissue homeostasis and immunity. They are formed in the bone marrow and continuously enter the blood circulation, where they constitute 4% of the total leukocyte population in mice and 10% in humans [1]. In human peripheral blood, three functionally different subsets of monocytes have been identified and characterized based on their expression of surface markers CD14 and CD16 [2]. The major monocyte subset, accounting for approximately 90% of the total monocyte population, expresses high levels of CD14 and no CD16 (CD14++CD16−), and these cells are referred to as classical monocytes. Monocytes expressing CD16 can be further divided into two distinct subpopulations: intermediate monocytes that express relatively high levels of CD14 and some CD16 (CD14+CD16++) and nonclassical monocytes that express low levels of CD14 and high levels of CD16 (CD14−CD16++) [3]. Analogously, mouse monocytes can be separated into two functionally different subsets based on their expression of Ly6C, CCR2, and CX3CR1. The Ly6C+CCR2highCX3CR1low subset is equivalent to human classical and intermediate monocytes, whereas the Ly6C−CCR2lowCX3CR1high subset is represented by nonclassical monocytes in humans [4, 5] (Table 1). Considering the strong evidence for comparable systems, murine monocyte subsets will be referred to as their human classical/intermediate or nonclassical counterparts from now on in this review.

Monocytes represent accessory cells that can link inflammatory conditions to the adaptive immune response. Although the monocyte subsets share several common features, distinct functions have been attributed to the classical, intermediate, and nonclassical monocytes. During injury or inflammation, classical monocytes are rapidly recruited to invade the inflamed tissue and contribute to immunological responses, such as recognizing and removing microorganisms and dying cells [6]. Intermediate monocytes...
2. Recruitment and Differentiation of Monocyte Subsets during Distinct Stages of Inflammation

Monocytes are recruited sequentially to sites of inflammation as part of the host-protective immune response. In response to natural killer (NK) cell-produced interferon (IFN-γ), the monocytes locally differentiate into inflammatory macrophages and DCs [20] and efficiently replace the resident mononuclear phagocytes [21]. Trafficking of the monocyte subset is controlled by different mechanisms and at least two sequential phases of monocyte recruitment to sites of inflammation have been identified [22] (Figure 3). Following a myocardial infarction, classical monocytes are recruited within the first few hours, and their egression from the bone marrow is in principle controlled by the chemokine receptor CCR2 and its ligands CCL2 (or MCP-1) and CCL7 (or MCP-3) [23]. The recruited classical monocytes arrive in a highly inflammatory milieu where they exert an immediate and potent immune response by producing high levels of proinflammatory cytokines, such as interleukin IL-1β and TNF-α. In addition, they locally digest extracellular matrix and dead cells [24] and produce IL-18 to activate NK cells [25], thereby playing an important role in the progression of the immune response. A prolonged immune response from classical monocytes can contribute to tissue damage and initiate inflammatory cascades, as well as drive autoimmunity [26, 27].

Some days later, when the acute inflammation resolves into a cardiac wound, the presence of classical monocytes diminishes and they are subsequently replaced by intermediate and nonclassical monocytes. In contrast to classical monocytes, CD16-expressing monocytes express low levels of CCR2 and rely on migration signals mediated by chemokine
The origin and differentiation of peripheral blood monocytes. Monocytes are generated from hematopoietic stem cells in the bone marrow (left) and enter the blood stream (middle) in response to different microenvironmental cues. In homeostasis, classical monocytes are continuously recruited to populate DC and macrophage levels in the intestine and dermis and bone-degrading osteoclasts at the bone surface. It remains unknown whether intermediate monocytes contribute to populations of monocyte-derived immune cells in homeostasis. Nonclassical monocytes patrol the endothelium and do not contribute to the maintenance of populations of mature immune cells in physiology.

The distinct recruitment of the three monocyte subsets was recently also observed when studying an infected kidney mouse model [16], where classical monocytes/macrophages were observed to appear rapidly after infection. Here, they expressed genes associated with immune response and monocyte/macrophage differentiation. Intermediate monocytes/macrophages arrived later and expressed genes associated with wound healing and released vascular endothelial growth factor and TGF-β, supporting angiogenesis and collagen production. The nonclassical monocyte/macrophage population peaked 10 days after the kidney infection and expressed genes associated with fibrosis [16]. Similar observations have been made in patients with chronic inflammatory and fibrotic liver diseases, where intermediate monocytes accumulated in the inflamed liver as a consequence of enhanced recruitment of these monocytes from the circulation and local differentiation of classical monocytes in response to inflammatory factors [17]. The same study concluded that these intermediate monocytes expressed both early macrophage and DC markers and were associated with increased phagocytic activity, antigen presentation, and secretion of proinflammatory cytokines (such as tumor necrosis factor TNF-α, IL-6, and IL-1β) and different growth factors consistent with a role in wound healing [17, 32]. Thus, observations in both murine disease models and human patients suggest that the delayed recruitment of intermediate and nonclassical monocyte subsets and their subsequent differentiation into macrophages and DCs is a conserved mechanism that reflects a host-driven response to limit possible tissue damage caused by strong immune responses from classical monocytes/macrophages. It should be noted, however, that the fate of differentiated monocytes after resolution of inflammation remains as a subject of debate, although it has been suggested that they are able to undergo in situ phenotype conversation to become tissue-resident macrophages [9].

3. Inflammation Enhances Monocyte Contribution to the Tissue-Resident Cell Populations

Monocytes can function as precursors of DCs, macrophages, and osteoclasts. However, the fact that monocytes are the immediate upstream precursors of these specialized cell
Figure 2: Proposed increased recruitment and differentiation of monocytes during inflammation. The contribution of monocytes to populations of mature immune cells is dramatically increased in various inflammatory conditions. Populations of immune cells normally not maintained by monocyte influx, such as populations of DCs and macrophages in the lungs, CNS, heart, liver, and kidney, are being provided by monocyte-derived counterparts during inflammation. Intermediate and nonclassical monocytes differentiate into immune cells with features distinctly different from the ones generated from classical monocytes during inflammation.

populations is a dogma that only recently was refined with the usage of sophisticated fate-mapping techniques and different in vivo disease models [9, 33]. Instead of depending on monocyte recruitment, several tissue-resident macrophage and DC populations rather appear to be maintained through longevity and local proliferation of precursors seeded during the embryonic development [13]. Yet, depletion of tissue-resident cell populations has demonstrated that circulating precursors in the blood can replenish numerous populations of specialized macrophages and DCs [34, 35], supporting the idea of blood monocytes as a circulating precursor reservoir that can be exploited on demand. Although classical
monocytes are contributing to some populations of tissue-resident DCs, macrophages, and osteoclasts in the steady-state, monocyte recruitment is strongly increased during inflammation and the affected distribution of monocytes, favoring an expansion of CD16-expressing monocytes [36], has great impact on the formation of monocyte-derived immune cells during inflammation.

3.1. Monocyte-Derived Dendritic Cells. Dendritic cells (DCs) are professional antigen-presenting cells and key regulators of innate and adaptive immune responses. A number of positive DC lineage markers have been identified that separates DCs into either “classical” or “plasmacytoid” DCs [37]. The latter are not derived from circulating monocytes and are therefore not discussed further in this review. Monocytes cultured in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4 generate immature DC that differentiate further into mature DCs by TNF-α stimulus [38, 39]. Within the total population of classical DCs, several distinct subpopulations have been identified, each possessing distinct phenotypical and functional features [40–42]. Although DCs primarily are generated from pre-DCs in the circulation [43], selected DC populations in the dermis and the intestine are continuously repopulated by recruited classical monocytes [34, 44]. Evidence supporting a role for CD16-expressing monocytes in the replenishment of DC populations in steady-state is lacking, and it is possible that the patrolling nonclassical monocytes leave the blood vessels and function as DC precursors exclusively in response to inflammatory stimuli [45]. During inflammation, monocyte differentiation into DCs is not restricted to the skin or intestine but includes peripheral tissues normally not maintained by monocyte input [46] (Figure 2).

The monocyte-derived DCs during inflammation have unique features, distinctly different from tissue-resident DCs generated during steady-state conditions. In vivo transfer experiments have shown that injected monocytes migrate to inflammatory sites and differentiate into DCs in various models of inflammation, including rheumatoid arthritis [47] and Dengue virus infection [48]. As part of the innate immune system, monocyte-derived DCs during inflammation secrete high amounts of the anti-inflammatory cytokine IL-10 and

**Figure 3:** Time course of human monocyte subsets recruitment and their differentiation into macrophages and DCs during inflammation. The monocyte subsets are sequentially recruited to a site of inflammation. Their subsequent differentiation into distinct different macrophages and DCs is taking place locally and is schematically depicted above, together with their specific contributions to the resolution of the inflammation.
ing engulf apoptotic erythroid cells. Accordingly, blocking differ-
entiation of monocytes into DCs results in tissue damage due
to severe and prolonged inflammation, cytotoxic T cell activ-
ity, and shortened host survival expectancy [49]. Monocyte-
derived DCs have been suggested to contribute to the regu-
lar control of immune responses [50], and they produce large
amounts of proinflammatory cytokines and enhance Th2 cell-mediated immunity in the lungs [51]. The specific
contributions of classical, intermediate, and nonclassical
monocytes to DC populations during inflammation were
recently investigated in patients suffering from end stage
renal disease, where chronic inflammation and dramatically
increased numbers of circulating nonclassical monocytes
were associated with an increased generation of DCs [52].
The specific contribution of the monocyte subsets to populations
of DCs during inflammation has long been a topic of
debate, and it has been reported that functional differences
exist between DCs generated from the different monocyte
subsets [53]. These differences include more potent immune
responses from DCs derived from classical monocytes and
better immune tolerance from DCs generated from non-
classical monocytes [54]. Similarly, it has been reported
that classical monocytes selectively repopulate populations
of CD103⁺ DCs, whereas nonclassical monocytes differentiate
into populations of CD11b⁺ DCs in the lungs [55]. More
recently, these findings were supported by similar observa-
tions reported in patients with tuberculosis. Patients with
tuberculosis have increased numbers of both intermediate
and nonclassical monocytes in the circulation [56], and the
CD16-expressing monocytes in these patients differentiate
into DCs with poor mycobacterial antigen-presenting capac-
ity [18]. This is explained by the observation that stimulated
CD16-expressing monocytes differentiate into alternative
DCs with poor antigen-presenting function, expressing no
CD1a and low levels of DC-SIGN on their plasma membrane
[18]. After LPS stimulation, these inflammatory DCs produce
large amounts of IL-2 and IFN-γ, further driving the differ-
entiation of monocytes into inflammatory mature immune
cells [57]. Classical monocytes, on the other hand, generate
functional CD1a⁺ DC-SIGN⁺ DCs that efficiently stimulate
T cell proliferation and secrete high amounts of IL-12, IL-1β,
IL-10, and TNF-α upon Mycobacterium tuberculosis infection
or LPS stimulation [18].

The increased presence of circulating CD16-expressing
monocytes during inflammation appears to play a critical
role in the development of DCs also in other pathological
conditions. For example, in sepsis—a systemic inflammatory
response syndrome that occurs during infection—an expan-
sion of intermediate monocytes has been detected in the
blood circulation [6]. Monocytes derived from sepsis patients
preferably differentiate into alternative CD1a⁺ DCs (similar
to the DCs derived from CD16-expressing monocytes
in patients with tuberculosis discussed above) [58]. These
alternative DCs have an increased capacity to induce regu-
latory Foxp3⁺ T cells, as compared with monocytes derived
from healthy controls with a higher distribution of classical
monocytes [58]. Thus, a growing body of circumstantial
evidence suggests that the monocyte subsets give rise to
functionally distinct DCs during inflammation and that the
enhanced presence of circulating intermediate and nonclassi-
cal monocytes shapes populations of DCs during pathologi-
cal conditions.

3.2. Monocyte-Derived Macrophages. Macrophages are exqui-
sitely adapted to their local environment and acquire organ-
specific functionalities as part of their role in the maintenance
to tissue homeostasis. Development, differentiation, prolif-
eration, and function of macrophages are regulated by the
growth factor colony stimulating factor CSF-1 and IL-34 [59].
Macrophages belong to a heterogeneous cell population, with
several phenotypically and functionally distinct subsets [58].
Most macrophage populations are established prior to birth
and maintain themselves by longevity and local proliferation,
rather than monocyte recruitment. These macrophage popu-
lations include microglia in the central nervous system,
Kupffer cells in the liver, peritoneal macrophages, and splenic
macrophages [9, 15]. Microglia was early shown to originate
from embryonic progenitors [60], and more recent research
has identified the microglia precursors as primitive macroph-
ages in the yolk sac [61].

Yet, in other tissues, including the intestine [62] and the
dermis [63], classical monocytes are continuously recruited
to maintain the local macrophage populations in home-
ostasis. In addition, monocyte-derived cardiac macrophages
appear to replace macrophages seeded during the embryonic
development throughout the life span of an individual [64]. It
has been reported that monocyte-derived macrophages, simi-
lar to monocyte-derived DCs, are functionally different from
their tissue-resident counterparts. Monocyte-derived macro-
phages in the intestine express higher levels of CXC, R1 [65],
induce differentiation of Foxp3⁺ T cells from naïve CD4⁺ T
cells [66], and are required for induction of Th17 cells and
antigen-specific responses [65]. Whether CD16-expressing
monocytes also contribute to macrophage populations in
steady-state is unknown, and although early reports indi-
cated that nonclassical monocytes differentiate into alveolar
macrophages in homeostasis [67, 68], more recent research
indicates that alveolar macrophages are in fact derived from
fetal monocytes with minimal contribution of circulating
blood monocytes [69].

Although classical monocytes appear to be the primary
precursors of selected populations of macrophage during
steady-state, the recruitment of all monocyte subsets during
inflammation is strongly increased. Inflammatory insults
result in recruitment of monocytes to populations of tissue-
residential macrophages that normally are maintained indepen-
dently of monocyte influx, such as macrophages in the heart
[70], in the ischemia brain tissue [71], and in the inflamed
liver tissue [31] (Figure 2). The monocyte heterogeneity plays
an important role in the generation of functionally distinct
macrophages and the monocyte subsets appear to function as
macrophage precursors in different pathological conditions.
For example, infection with helminth parasites Schistosoma
mansoni and Heligmosomoides polygyrus results in rapid inva-
sion of classical monocytes into the adult murine heart, where
they drive inflammation and generate oxidative stress [72].
These classical monocytes subsequently differentiate into
macrophages with limited capacity to promote tissue repair [73]. However, in the absence of parasite challenge, such as during cardiac pressure overload, preferential recruitment and accumulation of nonclassical monocytes/macrophages in the cardiac tissue have been observed [15]. Similar to the selective recruitment of monocytes discussed above in Section 2, the sequential differentiation of the monocyte subsets into macrophages in response to myocardial challenges is likely due to the individual features of the different monocytes/macrophages.

Macrophages derived from the different monocyte subsets have been shown to maintain some of the properties of their progenitors. For example, macrophages derived from classical monocytes express higher levels of CD14 on their surface compared to macrophages derived from nonclassical monocytes when cultured in vitro [74]. While macrophages from classical monocytes exhibit phagocytic, proteolytic, and inflammatory functions, macrophages derived from CD16-expressing monocytes promote healing of the cardiac tissue by angiogenesis and deposition of collagen [75]. The functional differences between macrophages derived from classical and CD16-expressing monocytes have given rise to the idea that classical monocytes differentiate into cardiac M1 macrophages, whereas CD16-expressing monocytes become M2 macrophages [76]. This, however, still needs to be confirmed. In either way, the selective recruitment of specific monocyte subsets is context dependent and based on the nature of the challenge. Thus, the sequentially recruited monocyte subsets during inflammation differentiate locally into macrophages with distinct capacities to drive inflammatory responses or promote tissue repair.

3.3. Monocyte-Derived Osteoclasts. Osteoclasts comprise a subset of specialized macrophages that arise from fusion of monocytes in the presence of the cytokines macrophage-colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). These cells are uniquely capable of resorbing mineralized tissue, like bone, by binding tightly to the surface and by degrading the different matrix components by secreting acid followed by a cocktail of different proteolytic enzymes. Although not considered traditional immune cells, a growing body of evidence suggests that osteoclasts contribute to inflammation and immune responses via the release of cytokines and via antigen presentation [77]. Functional differences between subsets of osteoclasts have been reported in homeostasis and include differences in size and proteolytic enzymes used for bone matrix digestion [78]. Although it was long assumed that monocytes are an important source of osteoclast precursors, this was not proven in situ until recently when fluorescently labeled monocytes were recruited from the circulation to the bone surface and differentiated locally into osteoclasts [79]. Accordingly, depletion of blood monocytes decreases osteoclastic bone degradation by limiting the homing of precursors to the bone surface [80].

Bone degradation by osteoclasts is crucial for skeletal maintenance, but increased and uncontrolled bone degradation during inflammation results in a severe pathological phenotype [81]. Due to their roles as osteoclast precursors, circulating monocytes form an excellent tool to study the early onset of inflammatory bone loss [82]. In healthy individuals, it is the classical monocytes that harbor the highest propensity to differentiate into osteoclasts [83]. Interestingly, however, Chiu et al. in 2010 reported a major shift in osteoclast precursors, from classical monocytes in healthy individuals towards an increased osteoclast formation from intermediate and nonclassical monocytes in patients with psoriatic arthritis (a chronic inflammatory arthritis characterized by severe bone erosion) [84]. The influence of an affected distribution of circulating monocytes on osteoclast formation during inflammation has also been observed when osteoclastogenesis of monocytes from patients with inflammatory bone loss has been studied in detail. Monocytes isolated from patients with Gaucher’s disease form osteoclasts faster than monocytes isolated from healthy controls and the generated osteoclasts display an increased bone-resorptive capacity when compared with osteoclast derived from monocytes isolated from healthy controls [85]. Similar observations were reported for patients with rheumatoid arthritis, where osteoclasts with increased bone-resorptive capacity were generated from monocytes derived from patients with rheumatoid arthritis [86]. This indicates that it is in fact intrinsic properties of the isolated monocytes that cause the generation of distinct different osteoclasts and not altered cytokine levels in an inflammatory environment. Interestingly, in all above-mentioned conditions (psoriatic arthritis, Gaucher’s disease, and rheumatoid arthritis), a selective expansion of the intermediate monocyte subset has been reported, suggesting that in particular this subset is involved in the formation of functionally distinct osteoclasts in these inflammatory conditions [6, 87, 88]. Accordingly, we recently demonstrated that in particular osteoclasts generated from intermediate monocytes expressed an increased capacity to resorb bone when they are treated with the inflammatory cytokine IL-17A [14]. Taken together, the increased numbers of CD16-expressing monocytes and in particular intermediate monocytes appear to play a critical role in the generation of osteoclasts during inflammation and can possibly serve as an explanation for the increased osteoclast-associated bone loss observed in several inflammatory disorders.

4. Concluding Remarks and Future Perspectives

The role of monocytes as precursors for various mature immune cells has been well established. As our understanding of monocyte heterogeneity improves, their intriguing role as precursor cells is becoming increasingly important, and targeting of specific monocyte subsets to control differentiation and function of monocyte-derived immune cells emerges as an appealing therapeutic approach. The putative role of classical, intermediate, and nonclassical monocytes as distinct precursor cells during inflammation is of particular interest for immunological research, but our knowledge is limited and several important aspects are still unknown. This is partly due to the fact that the data collected so far mainly consists of in vitro observations and, unfortunately, few studies have investigated the correlation between an affected precursor
population and the development of unconventional downstream immune cells during inflammation. Defining the distinct differentiation fates of the monocyte subsets in different inflammatory conditions will enable more precise targeting of immune cells and provide a better understanding of the pathophysiology of inflammation.

Competing Interests

The authors declare that they have no competing interests.

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