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Blood Monocytes: Development, Heterogeneity, and Relationship with Dendritic Cells

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Abstract

Monocytes are circulating blood leukocytes that play important roles in the inflammatory response, which is essential for the innate response to pathogens. But inflammation and monocytes are also involved in the pathogenesis of inflammatory diseases, including atherosclerosis. In adult mice, monocytes originate in the bone marrow in a Csf-1R (MCSF-R, CD115)-dependent manner from a hematopoietic precursor common for monocytes and several subsets of macrophages and dendritic cells (DCs). Monocyte heterogeneity has long been recognized but in recent years investigators have identified three functional subsets of human monocytes and two subsets of mouse monocytes that exert specific roles in homeostatis and inflammation in vivo, reminiscent of those previously described classically and alternatively activated macrophages. Functional characterization of monocytes is in progress in humans and rodents and will provide a better understanding of the pathophysiology of inflammation.

INTRODUCTION

DCs: dendritic cells

The innate immune system has been shaped by evolution to allow multicellular organisms to live together with microorganisms and parasites. In most species, including invertebrates such as *Drosophila* and vertebrates such as zebrafish, mice, and humans, the innate immune system is composed of a humoral arm, which consists of antimicrobial peptides and opsonins, and a cellular arm, which mainly involves specialized cells known as phagocytes. Phagocytes are cells able to internalize and digest bacteria and other cells; to scavenge toxic compounds produced by the metabolism; and to produce inflammatory mediators that can kill bacteria, parasites, and viruses and contribute to the activation of other cell types and to the walling off of parasites. The cellular innate immune system thus contributes to keeping the growth of microbes under more or less tight control. However, its activation has side effects—collectively known as inflammation—mainly owing to tissue damage to the host. In the long term, inflammation contributes to the development of inflammatory diseases, including atherosclerosis. Inflammatory diseases, which are leading causes of morbidity and mortality in developed countries, could thus be seen as long-term natural side effects of innate immunity in the context of individual genetic susceptibility. This implies that tuning down but not turning off innate surveillance may delay the aging of tissues and prevent or attenuate inflammatory disorders. In this context, understanding the cellular basis and the molecular mechanisms of the innate surveillance of tissues is a worthy goal.

Monocytes represent 10% of leukocytes in human blood and 4% of leukocytes in mouse blood. They are distinct from polymorphonuclear (PMNs) and natural killer (NK) cells, which also belong to the innate arm of the immune system, as well as from lymphoid T and B cells, which represent the adaptive arm of the immune system. Monocytes are present in mammals, birds, amphibians, and fish (1–3), and a related population of hemocytes (called plasmatocytes) is present in the fly (4, 5), which does not have lymphocytes. Monocytes play an important role in development and homeostasy, in part via the removal of apoptotic cells and scavenging of toxic compounds (4, 6). Strikingly, monocyte/macrophage specialization can already be observed among unicellular eukaryotic organisms, as phagocytes able to scavenge toxic compounds and kill bacteria differentiate inside colonies of social amoeba (*Dictyostelium discoideum*) (7).

In mammals, monocytes also represent accessory cells, which can link inflammation and the innate defense against microorganisms to adaptive immune responses. Indeed, the best known function of monocytes is as a considerable systemic reservoir of myeloid precursors for the renewal of some tissue macrophages and antigen-presenting dendritic cells (DCs) (8–11). However, differentiation of monocytes into DCs is mostly observed in inflammatory conditions, e.g., during an active infection, and evidence indicates that the renewal of tissue macrophages and DCs does not rely solely on blood monocytes (12–14).

As discussed above, blood monocytes also represent a large pool of scavenger and potential effector cells inside blood vessels in homeostasis as well as during inflammatory processes (15). Monocytes are equipped with a large array of scavenger receptors that recognize microorganisms but also of lipids and dying cells, and stimulated monocytes can produce large quantities of effector molecules involved in the defense against pathogen, as reviewed recently (16–18), and in the pathogenesis of several inflammatory diseases, including arthritis and atherosclerosis (19). The role of monocytes in the control of microorganisms is likely to be under evolutionary pressure, although their detrimental effects may not be under such pressure given that, in most cases, these detrimental effects are only apparent after several decades of life. Studying the biology of monocytes is therefore useful for the understanding of susceptibility to infection, but it may be even more important for providing ideas and tools to control, delay, or alleviate the long-term detrimental side effects of the inflammatory response.

Studying the functions of human monocytes in biological processes is still a difficult task, however, because monocytes permanently survey their environment and rapidly react to its modification or alteration. Isolating them, purifying them on gradients, and culturing them in vitro notably affect their phenotype and behavior. A better understanding of the functions of human monocytes comes from whole-genome array analysis on sorted prospective subsets and from a careful comparison with the results obtained from studies in mice and maybe other model systems in which an in vivo analysis of the effector functions of prospective subsets is possible by combining genetic engineering and intravital studies (15).

MOLECULAR CONTROL OF MONOCYTE DEVELOPMENT FROM HEMATOPOIETIC PRECURSORS

CSF1-R Controls Monocyte Development

The development of blood monocytes is dependent on the growth factor Csf-1 (also known as M-CSF and CD115). In mice deficient in Csf-1R (c-fms, M-CSFR, CD115) and its ligand Csf-1, the number of blood monocytes is dramatically reduced (20–22), and expression of an M-CSF transgene rescues the differentiation of monocytes (22a). Csf-1R is a hematopoietic growth factor receptor expressed in monocytes, macrophages, and DCs and their precursors (23, 24), a population of cells sometimes referred to as the mononuclear phagocyte system (MPS). The two known ligands of Csf-1R, Csf-1/M-CSF (25) and the more recently described IL-34 (26), are both important for the development of this lineage, as M-CSF-deficient mice (*op/op* and *csf1*$^{-/-}$) have a milder phenotype than do Csf-1R-deficient mice (20). Other cytokines, such as GM-CSF, Flt3, and lymphotoxin $\alpha 1\beta 2$ (12, 27, 28), control the development and homeostasis of the macrophage and DC networks but appear to be dispensable for monocyte development.

MDP: macrophage and DC precursor

Transcription Factors that Control Monocyte Development from Hematopoietic Stem Cells

Monocytes develop from hematopoietic stem cells in the bone marrow via several commitment steps and intermediate progenitor stages that, in the prevalent model, pass through the common myeloid progenitor (CMP), the granulocyte/macrophage progenitor (GMP), and the macrophage/DC progenitor (MDP) stages (14, 29) (see below). Each of these differentiation steps involves cell fate decisions that successively restrict developmental potential. In several of these steps, the Ets family transcription factor PU.1 plays an important role. PU.1 can induce myeloid commitment in immature multipotent progenitor cells (30) and is required for the generation of CMP in early myelopoiesis (31, 32). Besides PU.1's role in early commitment, we also know from gain-of-function and retroviral reconstitution experiments of PU.1-deficient cells that PU.1 controls several cell fate decisions along the myelo-monocytic pathway by engaging in antagonistic interactions with different transcription factors. Initially, inhibitory interactions with GATA-1 shut down the megakaryocytic/erythroid pathway, and repression of GATA-2 blocks mast cell development (33). At the later bipotent GMP stage, PU.1 is critical for driving monocytic differentiation, at the expense of granulocytic differentiation (31), by antagonizing C/EBPα (34), a transcription factor required for granulocytic development (35). During myelopoiesis, PU.1 thus appears to successively close development options by overruling key regulators of other pathways. The antagonism with these factors, however, is not absolute but exquisitely dependent on relative expression levels and the balance of both factors. Whereas PU.1 expression over a certain threshold of antagonist can block the associated cell fate, low or equal levels may actually result in cooperative readouts

(33, 34, 36). For example, the combination of C/EBPα and PU.1 is thus required ectopically to induce macrophage fate in B cells (37), T cells (38), and fibroblasts (39), underscoring the importance of partner molecules in defining transcription factor function (40).

Such behavior could be explained by a quantitative model in which metastable cooperation of C/EBPα and PU.1 on both granulocytic and macrophage target genes is converted into a stable antagonism and fixed granulocyte or macrophage fate by the activation of the cross-inhibitory Gfi-1 and Egr/Nab downstream regulators (36). C/EBPα thus activates Gfi-1, which is required for granulocytic but not monocytic differentiation (41, 42), whereas PU.1 activates the monocyte/macrophage-determining Egr transcription factors and their cofactor Nab. Egr1 can selectively induce macrophage differentiation (43, 44), and although egr1 deficiency by itself does not prevent macrophage development (45), composite $egr1^{-/-}egr2^{+/-}$ bone marrow progenitors show a defect in Csf-1-dependent macrophage differentiation (36).

Although compelling, this model is certainly not complete and may involve additional transcription factors to determine monocyte fate. For example, ICSBP/IRF-8 (IFN consensus sequence binding protein/IFN regulatory factor 8) can also drive monocytic differentiation, at the expense of granulocytic differentiation, in ICSBP/IRF-8-deficient progenitors (46). It is tempting to speculate that this may involve its direct interaction with PU.1 (47). Similarly, the Krueppel-like factor KLF4 can induce macrophage fate, and, as a downstream target of PU.1, KLF4 can selectively rescue monocyte differentiation of $PU.1^{-/-}$ progenitors, whereas KLF4 deficiency biases myeloid progenitor differentiation toward the granulocytic fate (48).

Finally, the MafB and c-Maf transcription factors are highly expressed in monocytes and macrophages (49–52) and can selectively drive monocyte fate in myeloid progenitors (2, 52, 53). Similar to the cell fate choices described above involving an expression level–dependent switch from cooperative to antagonistic interactions, moderate expression of PU.1 is compatible with MafB and macrophage fate, whereas higher levels antagonize MafB in the macrophage-to-DC choice. In addition, MafB plays a critical role in integrating monocytic differentiation and cell cycle arrest (see below).

Integration of Cytokine Signaling and Transcription Factor Activity

As indicated above, the growth factor Csf-1 and its receptor Csf-1R/c-fms are critical for monocyte differentiation from bone marrow progenitors. Dissection of the human and mouse c-fms proximal promoters has revealed that c-ets-1, c-ets-2, and PU.1 *trans*-activate the c-fms proximal promoter (54). Consistent with this, PU.1-deficient myeloid progenitors do not express c-fms (55). Already in early progenitor cells with low c-fms levels, PU.1 is assembled in a primed chromatin conformation on both the proximal promoter and an *fms* intronic regulatory element (FIRE) enhancer (56), which is required for recruitment of Egr transcription factors that then mediate high-level expression in more mature cells. These observations have led to the suggestion that cell intrinsic commitment events induce the upregulation of the c-fms receptor, which allows proliferation and survival of monocytic cells (57). However, c-fms expression cannot rescue macrophage differentiation in PU.1-deficient cell (55), indicating that, in the absence of PU.1, c-fms signaling is not sufficient to drive macrophage differentiation. Furthermore, c-fms is already expressed at low levels in early multipotent stem and progenitor cells (58). Understanding commitment to the monocytic lineage requires determining which transcriptional events control the sensitivity of c-fms signaling in these cells.

MONOCYTES AND THE MONONUCLEAR PHAGOCYTE SYSTEM (MPS)

Macrophages and DCs form networks of phagocytic cells throughout most

tissues—sometimes referred to as the MPS (23)—and play major roles in development, scavenging, inflammation, and antipathogen defenses (59, 60). The MPS was initially defined as a population of cells, derived from a bone marrow progenitor, that differentiate and enter the blood as monocytes and then enter tissues to become resident tissue macrophages and antigen-presenting cells (61). However, it was soon recognized that DCs and macrophages have a remarkable heterogeneity related to their origin, phenotype, tissue localization, proliferative potential, and function (59, 62).

Macrophages and DCs can be divided into three main groups according to their half-life, to their replacement after bone marrow graft, and to whether their differentiation is elicited by inflammation or not. The potential mechanisms for the renewal of individual subsets include (*a*) self-renewal of resident postmitotic cells; (*b*) migration, homing, and limited proliferation of adult bone marrow–derived progenitor cells in peripheral tissues (13, 63); and (*c*) the extravasation and differentiation of circulating precursors such as blood monocytes. These mechanisms are not mutually exclusive—they could operate in parallel or sequentially during the life of the animal—and they are likely to depend on environmental cues such as inflammation.

Langerhans cells (LC) of the epidermis and microglia are macrophages of the central nervous system. Most remain host-derived after syngeneic bone marrow transplantation but can be replaced by bone marrow–derived cells, possibly blood monocytes, in circumstances in which the resident cells are depleted by UV or gamma irradiation (64, 65).

A second group of cells is exemplified by conventional DCs (cDCs). The work of Ralph Steinman and colleagues established that DCs represent a distinct family of cells that regulate the immune responses (59). DCs were originally described as the population of cells enriched from mouse spleens that are responsible for so-called mixed lymphocyte reaction activity (66). These splenic cells, now known as cDCs, are present in all lymphoid organs and can be divided into subsets according to phenotype, location, and function (67). cDCs, such as $CD8a^+$ and $CD8a^-$ DCs of the spleen and lymph nodes, have a short half-life and renew in the steady state from a bone marrow precursor without a monocytic intermediate (12, 14, 63) (see below and **Figure 1**).

cDCs: conventional dendritic cells of the lymphoid organs

Tip-DC: TNF-α and iNOS-producing DC

A third group of cells represents short-lived cells that differentiate from blood monocytes in response to inflammation or infection such as monocyte-derived DCs or TNF-α- and iNOS-producing (Tip)-DCs (9, 10, 68, 69).

Of note, the work of Massberg et al. (13) has shown that hematopoietic stem and progenitor cells (HSPCs) can circulate and proliferate within extramedullary tissues and give rise to tissue-resident myeloid cells, preferentially DCs. HSPC differentiation is amplified upon exposure to Toll-like receptor (TLR) agonists such as LPS (13). Therefore, the MPS cannot be considered as a simple family of monocyte-derived cells, but must be considered as a more complex cellular system involved in the scavenging of dying cells, pathogens, and molecules via a variety of cellular processes, such as phagocytosis and endocytosis using, for example, membrane pattern-recognition receptors (60). And the contribution of monocytes to this complex cellular system is an area of active research.

MDP, A COMMON PROGENITOR FOR MONOCYTE MACROPHAGES AND DENDRITIC CELLS

Today, investigators accept, on the basis of transplantation studies, the prevalent model that monocytes, many macrophage subsets, most of the cDCs in the secondary lymphoid organs of mice, and at least a fraction of the DCs in the mouse thymus probably originate from a myeloid progenitor (14, 70–72).

Among myeloid precursors, the MDP was identified as a subset of bone marrow–proliferating cells that share the

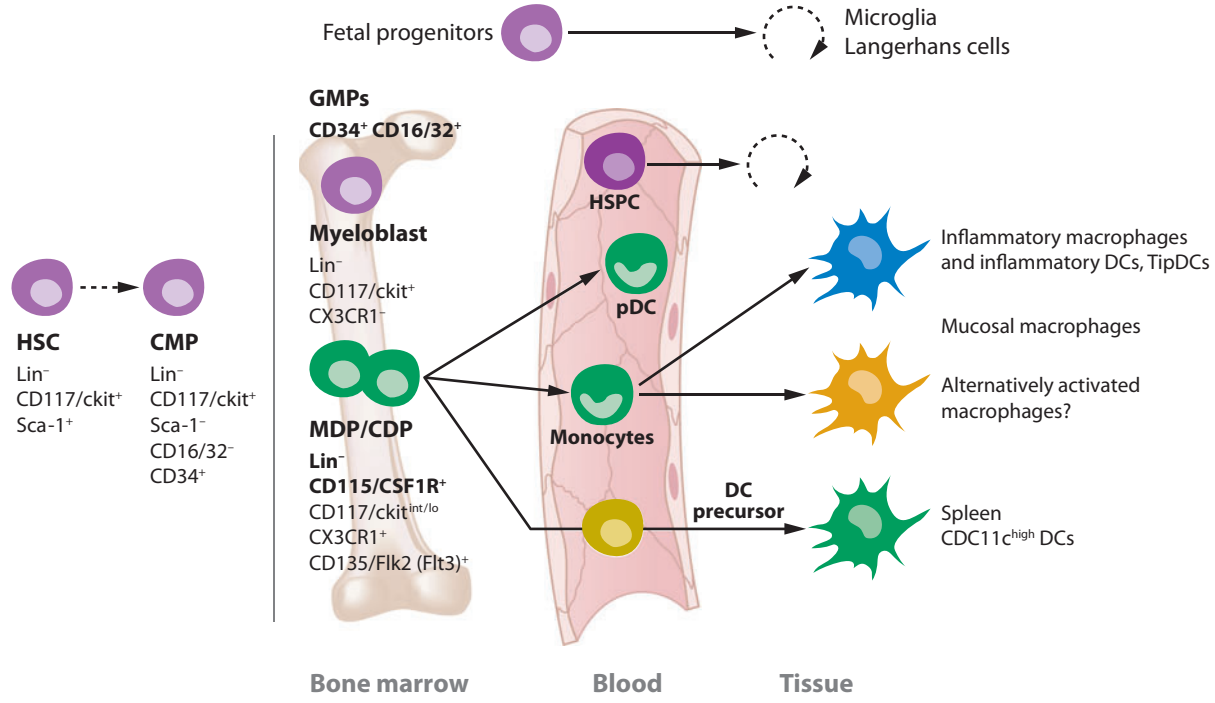

Figure 1

Differentiation of the macrophage/DC progenitor and origin of macrophage and DC subsets.

phenotype of GMPs (73), Lin$^-$Sca1$^-$IL-7Rα$^-$CD117(cKit)^{low}CD34$^+$CD16$^+$, and that specifically express the Csf-1R (CD115) and the chemokine receptor CX3CR1 (**Figure 1**) (11, 14; C. Auffray, D.K. Fogg, E. Narni-Mancinelli, B. Senechal, C. Touillet, et al., manuscript submitted). The MDP gives rise to monocytes, several macrophage subsets, and spleen cDCs (11, 12, 14). Of note, the MDP generates cDCs directly, without a monocytic intermediate (11, 12, 14, 63), whereas monocytes themselves generate other types of DCs, including inflammatory DCs or mucosal DCs (**Figure 1**) (9–11, 16).

The MDP has no significant granulocytic potential (11, 12, 14). These initial studies did not detect plasmacytoid DC (pDC) potential cells (11, 12, 14), but our recent data indicate that MDPs actually give rise to pDCs in vivo (C. Auffray, D.K. Fogg, E. Narni-Mancinelli, B. Senechal, C. Touillet, et al., manuscript submitted). Therefore, the MDP is a common precursor that gives rise in vivo to monocytes, macrophages, and the two main subsets of DCs: cDC and pDCs.

The chemokine receptor and adhesion molecule CX3CR1 is not expressed on early hematopoietic progenitors, including CMPs and GMPs, but it is first detected on MDPs. CX3CR1 is therefore associated with the commitment of myeloid progenitors to the monocyte/macrophage/DC lineage (12, 14), although its role in the development and homeostasis of cells of the MPS remains unknown.

Common Dendritic Cell Precursor (CDP), MDP, and Monocytes

However, the controversy on the origin of monocytes and DCs is not completely resolved, and another precursor—the CDP, for common DC precursor—was recently reported to generate cDCs and pDCs, but not monocytes (74, 75). Importantly, the CDP did not respond to

CDP: common DC precursor

CSF-1 (74, 75). This result was interpreted as indicating the existence of two pathways for cDC generation: the CDP pathway involved in homeostasy and the MDP pathway involved in inflammation.

However, the authors did not compare the potential of CDP with that of MDP in their experimental system, and thus another explanation for the discrepancy in the literature is that the differences in differentiation potential may reflect differences in experimental protocols rather than in intrinsic properties of the cells.

We favor the latter explanation because, as expected from the original papers (14, 75), the MDP and CDP share the same surface phenotype by flow cytometry ($Lin^{-}IL\text{-}7Ra^{-}CD117^{int/low}CD135^{+}CD115^{+}CX3CR1^{+}$) (C. Auffray, D.K. Fogg, E. Narni-Mancinelli, B. Senechal, C. Touillet, et al., manuscript submitted). Furthermore, CDP was reportedly purified from mouse bone marrow using an antibody against CD115 (AFS98) that very efficiently blocks CSF-1 binding to its receptor and CSF-1-dependent proliferation in vitro (76–79). In our laboratory, MDPs purified in the presence of AFS98 antibody failed to respond to subsequent culture with M-CSF (C. Auffray, D.K. Fogg, E. Narni-Mancinelli, B. Senechal, C. Touillet, et al., manuscript submitted). Therefore, as proposed recently (12), MDP and CDP may represent overlapping populations with a similar differentiation potential, and the purification process of CDP likely explains at least in part, its impaired response to M-CSF and its poor macrophage potential.

Homeostasis of cDCs of the Lymphoid Organs Is Independent of Monocytes

Although monocytes can generate several subsets of DCs in inflammatory conditions, the homeostasis of cDCs of the lymphoid organs is independent of blood monocytes and dependent on the rate of DC progenitor (MDP) input from blood and its proliferation within the spleen (12, 63). Flt3 (Fms-like tyrosine kinase 3, Flk2, CD135) is closely related to cFms/Csf1-R and is broadly expressed on early hematopoietic precursors (28). At physiological levels, MDPs do not require Flt3-mediated signals for their generation, but precursors that have entered the spleen undergo cell division locally under the control of Flt3 while they differentiate into cDCs, and thus Flt3 controls homeostatic cDC division in the periphery in vivo (12). Another study identified lymphotoxin-α as a critical mechanism in maintaining the size of the $CD8\alpha^{-}$ cDC pool in the spleen via local homeostatic expansion (27). Flt3 and lymphotoxin-α therefore control the local homeostatic replenishment of cDCs of the peripheral lymphoid organ.

HOMEOSTATIC CONTROL OF THE MONOCYTE POOL AND RELEASE FROM THE BONE MARROW

Control of Monocyte Proliferation

In general, besides being associated with the suppression of alternative developmental pathways, macrophage differentiation is also tightly associated with cell cycle withdrawal, and it is believed that monocytes do not proliferate. Whereas myeloid progenitor cells both differentiate and proliferate in response to Csf-1, terminally differentiated cells become refractory to proliferative signals (80), despite their continued ability to sense Csf-1 (81). Proliferative Csf-1 signaling involves the activation of c-*myb* and c-*myc* target genes via Ets-1/2 transcription factors (82, 83). Consistent with this, constitutively active alleles of c-*myb* and c-*myc* or their overexpression induce continued cycling of myelo-monocytic progenitor cells and macrophages, respectively (84, 85).

MafB not only induces macrophage differentiation but also inhibits progenitor proliferation (86). It is tempting to speculate that this may involve MafB's ability to repress Ets-1 (49),

particularly as inhibitory Ets factor complexes participate in cell cycle arrest during terminal macrophage differentiation (80). Furthermore, MafB directly engages in SUMO modification–dependent physical cross-inhibitory interactions with Myb proteins (86), indicating that relative c-Myb and MafB activities can shift the homeostatic balance between progenitor proliferation and terminal differentiation. As c-Maf can also inhibit Ets-1 and c-Myb transactivation (87) and MafB deficiency causes compensatory c-Maf upregulation (88), MafB and c-Maf may cooperate in this process.

However, investigators have suggested that a fraction of blood monocytes can be induced to proliferate in vitro after exposure to M-CSF and GM-CSF, and recent evidence shows that M-CSF-driven monocyte-to-macrophage differentiation is associated in vitro with transcription of positive regulators of cell proliferation, such as cell cycle–associated cyclin A2, B1 and B2, D1 and D3, and E2 genes (89). Our own results suggest that a similar phenomenon occurs in vivo in monocytes that extravasate during infection with *Listeria monocytogenes* (*Lm*) (C. Auffray and F. Geissmann, unpublished results). However, we could not directly detect proliferation of monocytes in vivo so far. This may suggest that postmitotic blood monocytes that extravasate and enter tissues and differentiate into macrophages or DCs could be induced to proliferate in response to homeostatic or inflammatory stimuli within their microenvironment. Local homeostatic control of monocyte, macrophage, and DC proliferation is in fact an area of active investigation. Local proliferation appears to be sufficient for the renewal of microglia (65) and LCs (64) throughout life in the steady state as well as during the course of a variety of diseases. Only under defined conditions are both microglia and LCs replaced with bone marrow–derived cells. However, the molecular mechanisms that control LC and microglial homeostasis remain to be understood. The molecular basis for this is unknown, and it would be interesting to determine in these cases whether the mechanisms that assure cell cycle withdrawal at terminal differentiation can be temporarily suspended.

Roles of $p21^{cip}$ and Fas

Although the homeostatic control of the monocyte pool in the periphery is relatively poorly understood beyond the role of Csf1-R, PU.1, and Maf family transcription factors, studies of gene-targeted mice have revealed some new aspects of monocyte biology. Mice deficient in the cyclin-dependent kinase inhibitor $p21^{cip}$ have a decreased number of blood monocytes and are resistant to serum transfer–induced arthritis, a phenotype reversed by the transfer of wild-type monocytes, suggesting a role for $p21^{cip}$ in regulating the development and/or differentiation of monocytic populations (90). The Fas pathway is also likely to play a role in vivo in governing monocyte/macrophage homeostasis, perhaps via the control of monocyte survival. Compared with congenic control C57BL/6 mice, Fas-deficient mice display increased numbers of circulating monocytes in the steady state and in a model of systemic inflammatory arthritis (91).

Control of Monocyte Emigration from the Bone Marrow by Inflammation and Chemokine Receptors

The mechanisms controlling monocyte emigration from the bone marrow niche where they are generated is an area of active investigation and were recently reviewed (16). Inflammation, owing either to infection or to a high-fat diet, clearly has a profound effect on the number of blood monocytes, most likely by increasing their egress from the bone marrow (16, 92, 93). Important studies have demonstrated that the chemokine receptor CCR2 and its ligands CCL7 and CCL2 are required for the emigration of the inflammatory $Ly6c^{+}$ ($Gr1^{+}$) subset of monocytes (see below) from the bone marrow and determine their frequency in the circulation (8, 92, 94, 95). The role of CCR2 and its ligands has recently been reviewed elsewhere (16).

Interestingly, the simultaneous inactivation of CCR2 (or its ligands) and of CX3CR1 and CCR5, two other chemokine receptors expressed on monocytes, has a synergistic effect in decreasing monocyte numbers in the blood, monocytosis induced by a high-fat diet, and atherosclerosis (93, 95). This suggests either that each of these chemokine receptors has additive effects on the same monocytes and/or that different monocyte subsets are dependent on distinct chemokine receptors for their egress from bone marrow and their recruitment into tissues. The mechanisms by which CCR5 and CX3CR1 contribute to controlling the number of monocytes in the periphery are not known.

HETEROGENEITY OF MONOCYTIC CELLS

Over the past 25 years, numerous lines of evidence have indicated that the roles of monocytes, both in the control of pathogens and the pathophysiology of inflammation, may be attributable to discrete functional subsets. Therefore, as our understanding of monocyte biology improves and these cells appear more and more important in the general field of inflammation, the issue of monocyte heterogeneity becomes more relevant to human health. It is now recognized that mouse and human, but also rat and pig, blood monocytes can be divided into phenotypic and functional subsets (10, 96–98); however, in the present review we only consider human and mouse cells.

General Features of Monocytes

In humans and mice, monocytes have some typical morphological features such as irregular cell shape, oval- or kidney-shaped nucleus, cytoplasmic vesicles, and high cytoplasm-to-nucleus ratio. However, they are still very heterogeneous in size and shape and are difficult to distinguish by morphology or by light scatter analysis alone from blood DCs, activated lymphocytes, and NK cells.

Human and mouse blood monocytes can be defined by the expression of the Csf-1 receptor (MCSF-R, CD115) and the chemokine receptor CX3CR1. They are distinct from PMNs, NK cells, and lymphoid T and B cells and do not express Nkp-46, CD3, CD19, or CD15. Monocytes are equipped with a large array of scavenger receptors that recognize lipids and various microorganisms, and stimulated monocytes can produce large concentrations of ROS; complement factors; prostaglandins; nitric oxide (NO) (in mice); cytokines such as TNF-α, IL-1β, CXCL8, IL-6, and IL-10; vascular endothelial growth factor; and proteolytic enzymes; and they have been involved in the defense against pathogen, as reviewed recently (16–18, 99). Antigen presentation has been described as a classical feature of monocytes, but since the identification of discrete subsets of DCs among monocyte cells, bona fide monocytes have been found in most cases to be far less efficient than DCs for antigen presentation (59). Initial work, performed when separation techniques that allow the investigator to distinguish monocytes from NK cells were less efficient, reported that monocytes have a cytotoxic potential. However, more recent studies have not conclusively addressed this issue.

In recent years, investigators have identified several distinct populations of blood monocytic cells. Two of these populations have been characterized as DCs and are briefly discussed below. In humans, three populations defined by the expression of CD14 and CD16 ($CD14^+CD16^-$, $CD14^+CD16^+$, and $CD14^{dim}CD16^+$) (97, 99–101) have retained the name monocytes and are discussed below. In mice, two main subsets have been characterized (8, 10, 15, 69, 102–104), although additional subsets have been proposed (105). At present, direct comparison of the functions of mouse monocyte subsets with their putative human orthologs is relatively difficult, at least in part because the experimental systems used in human and mouse studies are different.

Blood Dendritic Cells

The myeloid blood DC population represents 5% of monocytic-like cells (~0.5% of

peripheral blood mononuclear cells) in human (106). These blood DCs stimulate T cell proliferation in vitro, express class II antigens and CD11c, and in human—for most authors—are negative for the monocyte markers CD14 and CD16. A second DC subset corresponds to pDCs and also represents ~0.5% of peripheral blood mononuclear cells in human. pDCs are the most potent IFN-α-producing cells in response to viral pathogens (107, 108).

Bona Fide Monocytes

The remaining 95% of monocytic cells are presently considered by most authors as bona fide monocytes (10, 62, 69, 101, 109, 110). Distinction between monocytes and circulating DCs is easy in the mouse because mouse monocytes do not express MHC class II antigens or the integrin CD11c. In contrast, although they are poor antigen-presenting cells, human monocytes express MHC class II antigens (106) and the integrin CD11c.

THREE SUBSETS OF HUMAN MONOCYTES AS DEFINED BY THEIR PHENOTYPE AND CYTOKINE PRODUCTION

Almost 30 years ago, it was shown that human peripheral blood monocytes were not a homogeneous population but rather differ in their phenotype and functions (reviewed in 99). In the early 1980s, Yasaka et al. (112), Weiner et al. (115), Figdor et al. (111), Akiyama et al. (113–114), and Elias et al. (116) described the existence of two functional subsets of monocytes in human. On the basis of size and density, these authors distinguished a major population of regular or large monocytes with higher phagocytic and myeloperoxydase activity and higher superoxide release, and a minor population of intermediate or small monocytes with low peroxydase activity but with a higher capacity to elaborate and release IL-1 and to mediate antibody-dependent cytotoxicity.

Although they were limited by the purification of monocytes using density gradient alone, and the likely presence of contaminating NK cells and DCs, many results from these early studies were confirmed in more recent studies, where prospective monocyte subsets have been defined on the basis of their difference in surface marker expression.

The work by the group of Ziegler-Heitbrock (97) has revealed that the small monocytes could be identified by the expression of CD16 (FcγR-III). Accordingly, the major subset of monocytes that express CD14 but lack CD16 has higher phagocytic activity but lower cytokine production than does the minor subset of small monocytes that express CD16 (101).

The $CD14^+CD16^-$ monocytes represent 80% to 90% of blood monocytes, express high levels of the chemokine receptor CCR2 and low levels of CX3CR1, and produce IL-10 rather than TNF and IL-1 in response to LPS in vitro (117, 118) (10, 109). Their phenotype resembles that of mouse $Ly6c^+$ ($Gr1^+$) monocytes, although the latter are very efficient at producing inflammatory cytokines (16, 104).

In contrast to this major subset, human $CD16^+$ monocytes express high levels of CX3CR1 and low levels of CCR2 (10, 109, 118), are responsible for the production of TNF-α in response to LPS stimulation, and were called proinflammatory (101, 119). Several studies have reported that $CD16^+$ monocytes are found in larger numbers in the blood of patients with acute inflammation (120) and infectious diseases (121, 122). Of interest, $CD16^+$ monocyte numbers are reduced in the blood after treatment with glucocorticoids (121).

However, work by the group of Grage-Griebenow has shown that $CD16^+$ monocytes are composed of at least two populations with strikingly distinct functions (99). Monocytes that express CD16 and CD14 ($CD14^+CD16^+$) also express the Fc receptors CD64 and CD32, have phagocytic activity, and are entirely responsible for the production of TNF-α and IL-1 in response to LPS (123). In contrast, monocytes that express CD16 but very low levels of CD14 ($CD14^{dim}CD16^+$) lack the expression of other Fc receptors, are poorly phagocytic

and do not produce TNF-α or IL-1 in response to LPS (124). The actual function of the $CD14^{dim}CD16^{+}$ monocytes remains elusive, but they may be expanded in the blood of septic patients (121).

MONOCYTE SUBSETS IN MICE

During the past few years, several teams have developed strategies for studying in vivo the differentiation, recruitment, and functions of blood monocytes using adoptive transfer and intravital studies (15, 23). They have generated mouse models by inserting green fluorescent protein (GFP) into the *Lysozyme-M* gene (125), into the *Cx3cr1* gene (126), or as a transgene driven by the *c-fms* promoter (23). Monocytes have also been labeled using latex bead injection (102, 127) and ^{111}Indium (104).

Mouse monocytes are identified in blood based on the expression of CD115; based on their FSC SSC profile; based on the expression of F4/80, CD11b, Dectin-1 (the beta glucan receptor); and based on the variable expression of the Gr1/Ly6C antigen and 7/4 antigen (10, 18, 62). All circulating monocytes express the GFP reporter in $Cx3cr1^{gfp/+}$ mice (126), and several teams, including us, have studied blood monocytes in some detail in this model (10, 15, 128). The Ly6C antigen is a glycosylphosphatidylinositol-anchored molecule also expressed by granulocytes, 40% of NK cells, and pDCs. Ly6C is recognized by several antibodies, including AL-21 (which is specific for Ly6C) and RB6-8C5 antibody (Gr1) (which also recognizes Ly6G). Ly6G is only expressed by granulocytes. Therefore, the Gr1 antibody and Ly6C-specific antibodies label the same cells in the mouse blood, i.e., a subset of monocytes, granulocytes, pDCs, and NK cells, whereas pDCs and NK cells do not express CD115, and granulocytes express CD115 at low level (18, 129).

MURINE $CD115^{+}$ $Ly6C^{+}$ ($Gr1^{+}$) INFLAMMATORY MONOCYTES

The main subset of $CD115^{+}$ monocytes expresses Ly6C ($Gr1^{+}$), the chemokine receptor CCR2, the adhesion molecule L-selectin (CD62L), and a low level of the chemokine receptor CX3CR1. As discussed above, they are a phenotypic equivalent to human $CD14^{+}$ monocytes (10). Murine $Ly6C^{+}$ ($Gr1^{+}$) monocytes are selectively recruited to inflamed tissues and lymph nodes in vivo, produce high levels of TNF-α and IL-1 during infection or tissue damage, and were termed inflammatory monocytes (8, 10, 16, 69, 104, 128). A number of studies using either adoptive transfer of monocytes or latex bead–labeled monocytes strongly support the conclusion that at least a proportion of TNF-α-producing inflammatory DCs are the progeny of $Ly6C^{+}$ ($Gr1^{+}$) monocytes (9, 10, 16). These inflammatory DCs either uptake antigen in peripheral tissues and then migrate into lymphoid organs or, in the case of Tip-DCs, may migrate from the red pulp to the white pulp of the spleen (8, 68, 130).

$Ly6C^{+}$ ($Gr1^{+}$) monocytes can also replenish macrophages and DC resident cell compartments in the skin (e.g., LCs) (131), digestive tract (mucosal DCs) (11), and lung (127, 132, 133) (see **Figure 1**).

Roles of $CD115^{+}$ $Gr1^{+}$ Monocytes During Microbial Infection In Vivo

The role of monocytes during microbial infection has been reviewed recently (16, 17). Seminal studies from the groups of Pamer

(8, 68, 130) and of Drevets and Leenen (69) have characterized the role of these cells in vivo following microbial infection, using mice infected with the intracellular bacteria *Lm*. Following infection, Ly6C$^+$ (Gr1$^+$) blood monocytes egress massively from bone marrow to the bloodstream in a CCR2-dependent fashion and differentiate via a MyD88-dependent mechanism into cells that produce TNF-α and NO and that upregulate MHC class II antigens, CD80, CD86, and CD11c (8, 94, 130). These cells were therefore termed Tip-DCs (for TNF-α/iNOS-producing DCs) (68) (**Figure 2**). The severe reduction of Tip-DCs in CCR2-deficient mice was associated with a reduced control and clearance of *Lm* following primary infection (68).

Inside infected spleens, Tip-DCs secrete high levels of TNF-α and rapidly migrate to T cell zones of splenic follicles, where they also express high levels of the inducible nitric oxid synthase (iNOS) that generates NO radicals. However, although Tip-DCs efficiently stimulate a mixed lymphocyte reaction in vitro, their marked reduction in the spleen of CCR2-deficient mice did not lead to a defective T cell priming and proliferative response or to an impaired differentiation into IFN-γ/TNF-α-secreting T cell effectors (68). This suggests that the main function of these

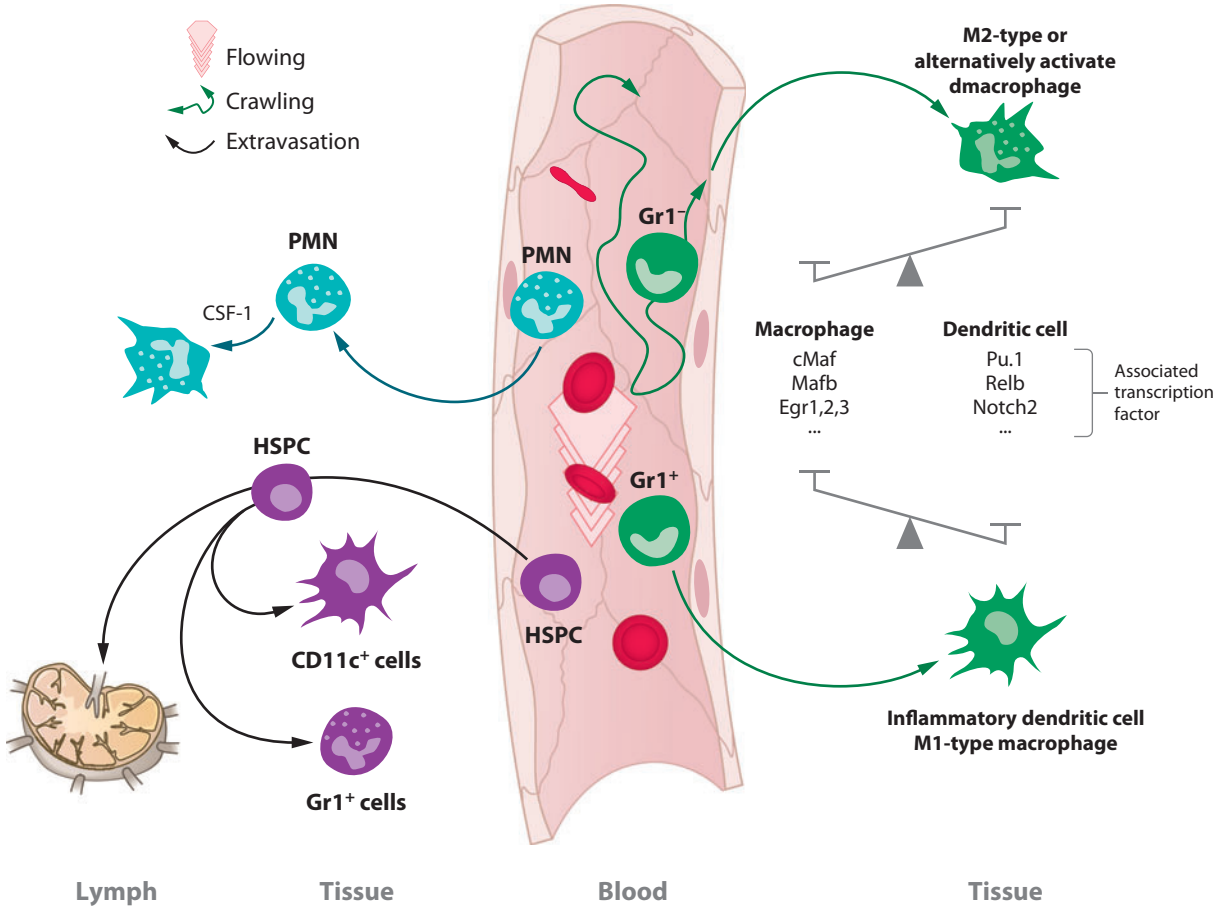

Figure 2

During inflammatory conditions, tissue macrophages and DCs can arise from Ly6C$^+$ (Gr1$^+$) monocytes and Ly6C$^-$ (Gr1$^-$) monocytes, but also from HPSCs (13) and granulocytes (129).

monocyte-derived inflammatory DCs is to kill bacteria rather than to regulate T cell functions. However, these data do not rule out such a role for these cells. They are found in very close proximity with *Lm*-specific cells and participate in the inflammatory environment by releasing high levels of inflammatory mediators such as TNF-α (130).

Ly6C$^+$ (Gr1$^+$) monocytes have also been reported in other infectious models in vivo and contribute to the control of pathogen growth during infections with other bacteria, such as *Brucella melitensis* (134), or with parasites, such as *Toxoplasma* (135).

A recent study by the Dubois group (136) indicates that CD115$^+$ Gr1$^+$ monocytes are recruited to the inflamed dermis via the chemokine receptor CCR6 and its ligand CCL20 and that depletion of monocytes prevents in vivo priming of CD8$^+$ cytotoxic T lymphocytes against the model protein antigen ovalbumin administered with adjuvant. Transfer of CCR6-sufficient Gr1$^+$ monocytes was enough to restore CD8$^+$ T cell priming in CCR6$^{-/-}$ mice via a direct antigen presentation mechanism (136). This work identified a mechanism for the recruitment of CD115$^+$ Gr1$^+$ monocytes to the skin and suggested that these monocytes are required for efficient cross-priming of CD8$^+$ cytotoxic T lymphocytes after mucosal or skin immunization in this model. Lauvau and colleagues (137) have recently described cells similar to the Tip-DCs that can play a critical role in protection against secondary *Lm* infection, suggesting that these cells may be important for primary and secondary protective immunity.

CD115$^+$ Ly6C$^+$ (Gr1$^+$) Monocytes in Tumor-Bearing Mice In Vivo

It is remarkable and intriguing that cells with the very same phenotype, expressing CD115, Gr1, and CD11b, also expand in the spleen of a tumor-bearing host (138, 139). These cells have been characterized as part of the myeloid-derived suppressor cell (MDSC) population that mediates the development of tumor-induced T regulatory cells and T cell anergy. Tumors induce the expansion of these MDSCs, in both animal models and human patients. MDSCs impair antigen-specific T cell responses and, particularly, CD8$^+$ T cell responses via molecular mechanisms that involve NO and/or reactive oxygen intermediate production (140–143). This suggests that CD115$^+$ Ly6C$^+$ (Gr1$^+$) monocytes could be expanded and polarized toward MDSCs that inhibit T cell–mediated immunity and toward Tip-DCs that strengthen T cell immunity by signals associated with tumor and infection, respectively, or that the CD115$^+$ Gr1$^+$ monocyte population contains two distinct functional subsets that can be expanded by signals associated with tumor and infection. Investigating the relationship between MDSCs and Tip-DCs may be of interest both for basic understanding of monocyte biology and for the potential clinical applications.

MDSCs: myeloid-derived suppressor cells

MURINE CD115$^+$ Ly6C$^-$ (Gr1$^-$) MONOCYTES

The second subset of monocytes is characterized by a smaller size; high expression of the chemokine receptor CX3CR1, of LFA-1 (lymphocyte-function associated antigen 1), and of CD43; and by the lack of expression of Ly6c (Gr1$^-$), CCR2, or L-selectin (10, 69). This subset has been initially termed resident in mice because these monocytes have a longer half-life in vivo and are found in both resting and inflamed tissues after adoptive transfer (10). We and others initially proposed that they may be involved in the renewal of resident macrophage and DC populations (10, 62). However, there is not yet strong supporting evidence for this hypothesis. Investigators had suggested—by analogy with human CD16$^+$ monocytes—that Ly6C$^-$ (Gr1$^-$) monocytes are the main producers of TNF-α (144), but, as discussed above, this is not the case because Ly6C$^+$ (Gr1$^+$) monocytes are clearly the main producers of TNF-α during infection.

Progress came from the use of adoptive transfer and of intravital microscopy, a powerful

method to monitor in vivo dynamic parameters of innate or adaptive immune responses (145).

Murine Ly6C$^-$ (Gr1$^-$) Monocytes Patrol Blood Vessels in the Steady State

Intravital microscopy observation, using *Cx3cr1*$^{gfp/+}$ mice as reporters, revealed that Ly6C$^-$ (Gr1$^-$) monocytes exhibited a constitutive long-range crawling on the luminal side of the endothelium, in steady-state condition, within most blood vessels in the dermis and within branches of the mesenteric vein and the mesenteric artery (15). Ly6C$^-$ (Gr1$^-$) monocytes crawl with an average velocity of 12 μm/min. Crawling requires firm binding to the endothelium mediated by the β_2 integrin LFA-1 (CD11a/CD18, $\alpha_L\beta_2$) and by the chemokine receptor CX3CR1.

Surprisingly, most crawling monocytes stay within blood vessels in the steady state and appear to patrol the endothelium, independently of the direction of the blood flow, for extended periods of time (i.e., 30 min to several hours). In the absence of overt inflammation, extravasation is a rare event observed in less that 1% of crawling cells (15). Therefore, we hypothesize that murine Ly6C$^-$ (Gr1$^-$) monocytes constitutively patrol blood vessels and may play important functions in scavenging oxidized lipids, dead cells, and potential pathogens. Patrolling Ly6C$^-$ (Gr1$^-$) monocytes are ideally located to survey endothelial cells and surrounding tissues. CX3CR1, as well as TNF-α and LFA-1, have been implicated in the pathogenesis of atherosclerosis (146–148). It will be important to investigate whether Ly6C$^-$ (Gr1$^-$)-patrolling monocytes may contribute to the pathogenesis of inflammatory disorders and could represent a target for their treatment. Besides the involvement of LFA-1 and CX3CR1, the molecular mechanisms that control the apparently random crawling of Ly6C$^-$ (Gr1$^-$) monocytes and their potential roles as scavenger are yet to be characterized.

Patrolling Ly6C$^-$ Gr1$^-$ Monocytes Extravasate and Are Responsible for a Very Early Inflammatory Response During Infection with *Listeria monocytogenes*

However, in response to tissue damage (irritants, aseptic wounding, and peritoneal infection with *Lm*), Ly6C$^-$ (Gr1$^-$) monocytes extravasate rapidly within 1 h and invade the surrounding tissues (**Figures 2** and **3**). Global gene expression of Ly6C$^-$ (Gr1$^-$) monocytes purified from the peritoneum of mice at an early time (2 h) after infection with *Lm* revealed that, when exposed to this pathogen in vivo, Ly6C$^-$ (Gr1$^-$) develop a very early but transient inflammatory response that includes the transcription of genes coding for cytokines, lysozyme, defensins, and complement and includes *Lm*-associated pattern-recognition receptors and phagocytosis such as TLRs (TLR1, TLR2), scavenger receptors (Sr-A, Cd36, dectin-2, and MDL1), IgFc receptors, and genes associated with antigen presentation (15) (see also **Figure 3**). This early response also included numerous chemokines involved in the recruitment and activation of other effector cells such as granulocytes, Ly6C$^-$ (Gr1$^-$) monocytes, NK cells, and T cells. At this time, 1 and 2 h after infection, Ly6C$^-$ (Gr1$^-$) monocytes are the main blood cell type extravasated into the peritoneum and are the only producers of TNF-α, a cytokine central to macrophage-mediated inflammation and the innate immune response (15). However, this inflammatory response is only transient, and at 8 h after infection, Ly6C$^+$ (Gr1$^+$) monocytes are the main producers of inflammatory cytokines (**Figure 3**).

Patrolling Ly6C$^-$ Gr1$^-$ Monocytes Differentiate into Alternatively Activated Macrophages During Infection with *Listeria monocytogenes* and in the Healing Myocardium

Interestingly, following this transient production of inflammatory mediators, the balance of

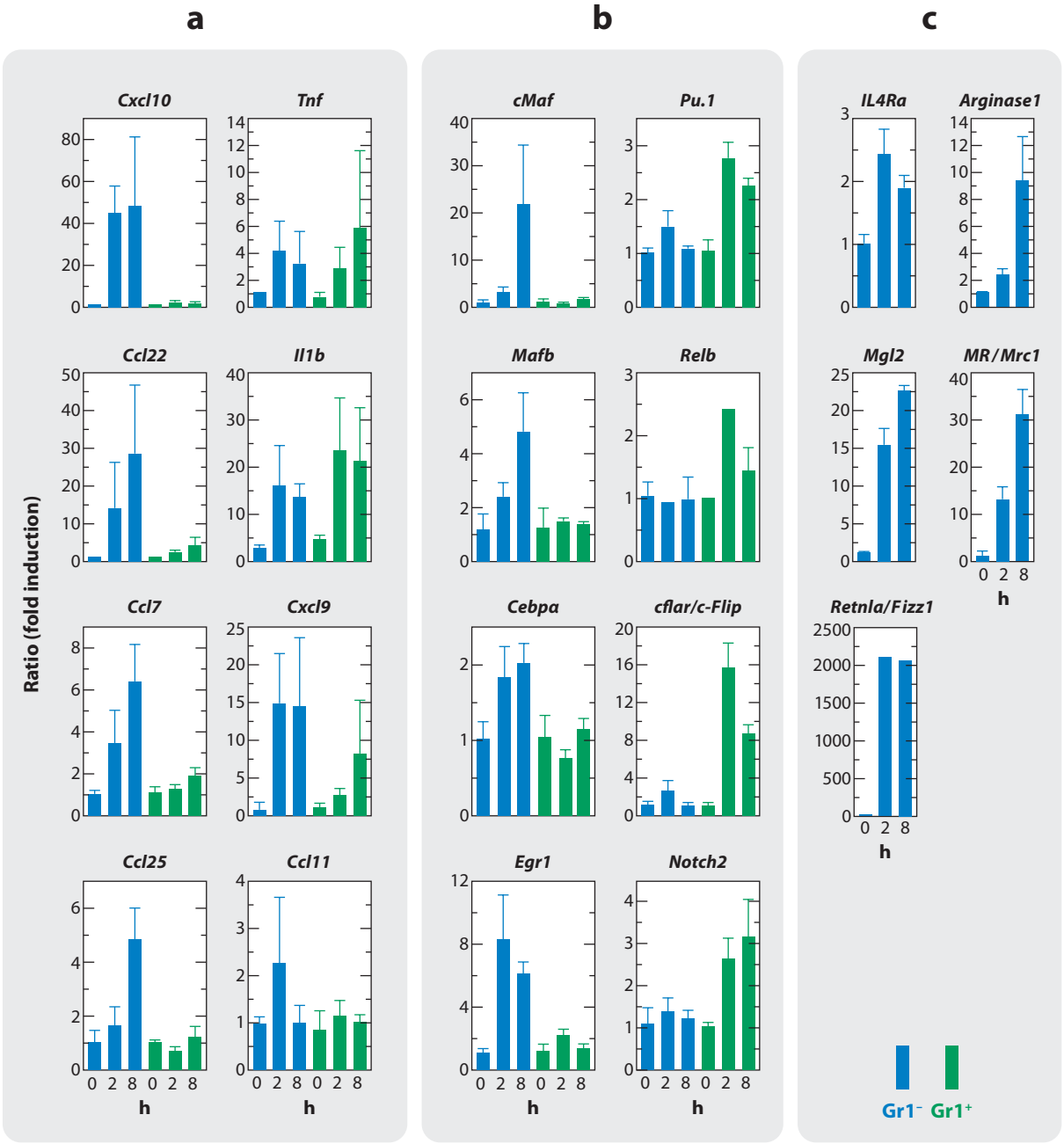

Figure 3

Gene expression analysis of monocyte subsets purified from the peritoneum of mice 2 and 8 h after infection with *Listeria monocytogenes*: (*a*) cytokines, (*b*) transcription factor, and (*c*) M2 type–associated genes (15).

transcription factors that specify the alternative macrophage or DC fate of monocytes (51, 149) indicates that extravasated Ly6C$^-$ (Gr1$^-$) monocytes initiate a typical macrophage differentiation program, characterized by upregulation of cMaf, MafB, egr1, egr2, and egr3 and of genes such as Arginase, Fizz1, MR, Mgl2, and IL-4Rα, markers of alternatively activated, also termed M2-like, macrophages (89, 150) (**Figures 2** and **3**). This finding is in contrast with the differentiation of the conventional Ly6C$^+$ (Gr1$^+$) monocytes that, when they enter the peritoneum in response to the same *Lm* infection, initiate a DC differentiation program characterized for example by the upregulation of RelB and PU.1, but not of cMaf and MafB (15) (**Figures 2** and **3**). Therefore, in the presence of the same pathogen in vivo, the two subsets of monocytes differentiate into distinct cell types: Ly6C$^-$ (Gr1$^-$)-patrolling monocytes initiate a macrophage differentiation program that resembles that of M2

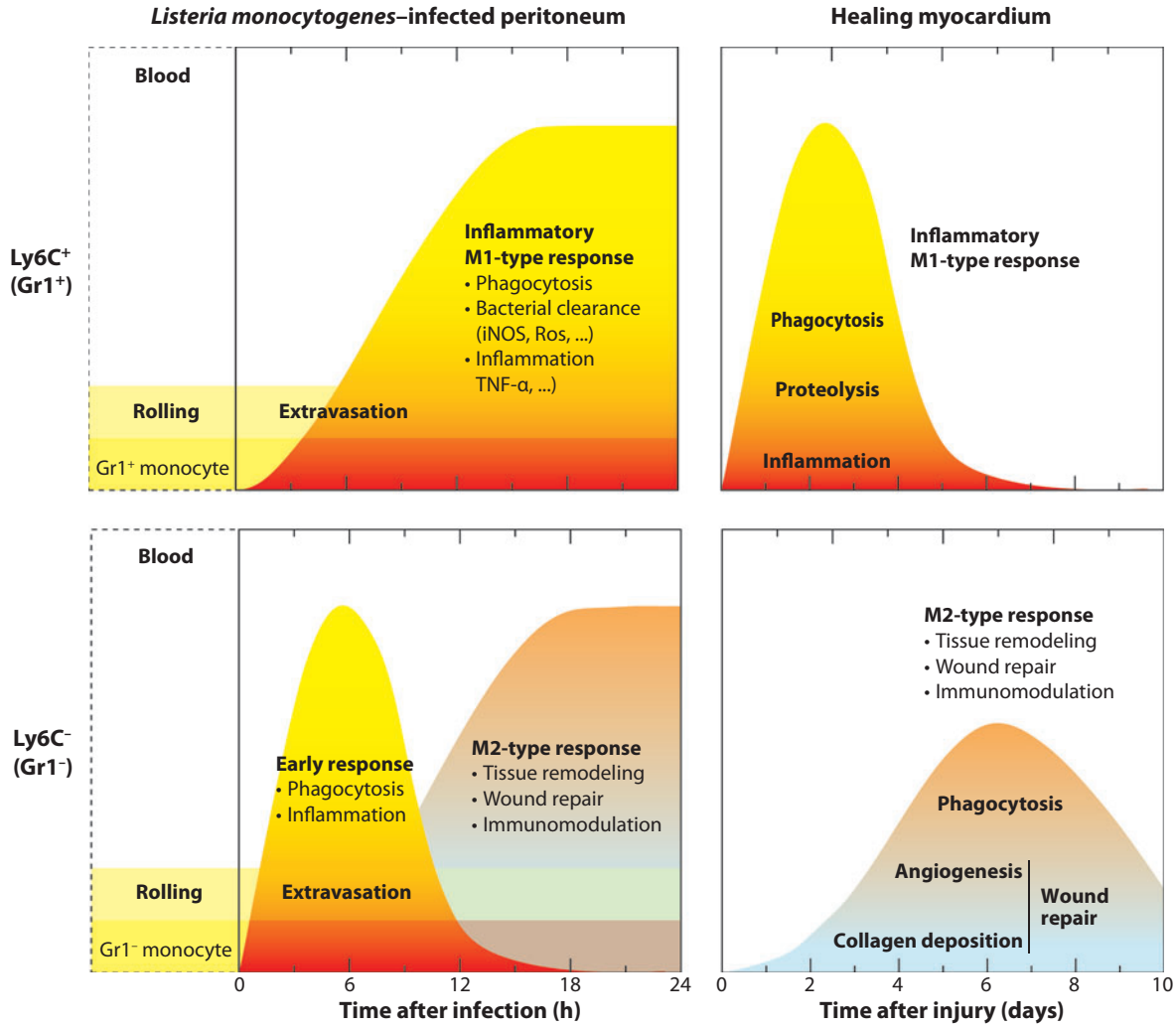

Figure 4

Differentiation potential and effector functions of blood monocyte subsets during *Listeria monocytogenes* infection and myocardial infarction.

macrophages, while Ly6C$^+$ (Gr1$^+$) monocytes differentiate into DC-like cells that resemble Tip-DCs (15).

These findings are consistent with three other recent studies. Nahrendorf et al. (104) have shown that the healing myocardium sequentially mobilizes Ly6C$^+$ (Gr1$^+$) monocytes, with phagocytic, proteolytic, inflammatory functions that can digest damaged tissue, and Ly6C$^-$ (Gr1$^-$) monocytes that have attenuated inflammatory properties, express vascular endothelial growth factor, and may promote healing via myofibroblast accumulation, angiogenesis, and deposition of collagen. Landsman et al. (133) have reported that adoptively transferred CD115$^+$ Gr1$^-$ monocytes, but not CD115$^+$ Gr1$^+$ monocytes, were able to generate macrophages in the lung of recipient mice, whereas both monocyte subsets could generate pulmonary DCs. Arnold et al. (151) studied the phenotype of monocytes in the tibialis anterior muscles after local injection of a drug that induces muscle necrosis followed by a regeneration process. Using latex bead labeling of circulating monocyte subsets, they concluded that during the first days after injury, the muscle recruited only F4/80^{low}CX3CR1^{low}Ly6C$^+$ (Gr1$^+$) nondividing monocytes that express mRNA for TNF-α and IL-1, whereas after day 4 their numbers decreased, and CX3CR1^{high}Ly6C$^-$ (Gr1$^-$) cells increased in numbers and exhibited features of antiinflammatory macrophages. Arnold et al. (151) concluded that Ly6C$^+$ (Gr1$^+$) monocytes had differentiated into Ly6C$^-$ (Gr1$^-$) monocytes, whereas the studies by Auffray et al. (15), Nahrendorf et al. (104), and Landsman et al. (133) concluded that distinct populations of monocytes are recruited from the blood.

Together, these observations reveal an unsuspected dichotomy (depicted in **Figure 4**) of the differentiation potential and functions of blood monocyte subsets during *Lm* infection and myocardial infarction.

CONCLUDING REMARKS

As our understanding of monocyte biology improves, monocytes appear more and more important in the general field of inflammation and inflammatory diseases, including atherosclerosis, and the issues of monocyte differentiation and heterogeneity become relevant. There is accumulative evidence that blood monocytes actually consist in several functional subsets. Open questions remain: the numbers of these subsets, the similarities between human and mouse subsets, and whether the differentiation of monocyte subsets represents an early commitment, at the stage of the MDP for example, akin to the division of lymphocytes into B, T CD4$^+$, and T CD8$^+$ cells, or whether it involves a potentially reversible polarization of more mature cells akin to the polarization of mature T cells. These questions are being actively investigated by a growing number of laboratories. The putative roles of Ly6C$^+$ (Gr1$^+$) monocytes in the regulation of lymphocyte-mediated responses and their relationship with MDSCs are of particular interest for tumor biology. Finally, the analysis of the functions of Ly6C$^-$ (Gr1$^-$)-patrolling monocytes and of their potential involvement in vascular inflammation should foster new investigations of the role of monocytes inside blood vessels—an already very active area of investigation in the field of atherosclerosis—and the mechanisms that control patrolling.

DISCLOSURE STATEMENT

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