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Monocyte-mediated Defense against Bacteria, Fungi, and Parasites

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Abstract

Circulating blood monocytes are a heterogeneous leukocyte population that contributes critical antimicrobial and regulatory functions during systemic and tissue-specific infections. These include patrolling vascular tissue for evidence of microbial invasion, infiltrating peripheral tissues and directly killing microbial invaders, conditioning the inflammatory milieu at sites of microbial tissue invasion, and orchestrating the activation of innate and adaptive immune effector cells. The central focus of this review is the *in vivo* mechanisms by which monocytes and their derivative cells promote microbial clearance and immune regulation. We include an overview of murine models to examine monocyte functions during microbial challenges and review our understanding of the functional roles of monocytes and their derivative cells in host defense against bacteria, fungi, and parasites.

Keywords

Monocyte; Bacterium; Fungus; Parasite; Innate Immunity; Inflammation

1. Introduction

Circulating monocytes arise from pluripotent hematopoietic stem cells through a series of progressively committed oligopotent progenitors in the bone marrow [1, 2]. The common monocyte progenitor (CMoP) represents a committed proliferative progenitor that is restricted to monocytes and monocyte-derived macrophages under homeostatic conditions [3]. Beyond their steady-state function in seeding mononuclear phagocytes in peripheral tissues during post-natal life [4], monocytes are increasingly recognized as critical cellular constituents of innate and adaptive immune responses against a wide range of microbes [5].

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This review summarizes the role of monocytes against major bacterial, fungal, and parasitic organisms and associated human clinical syndromes (Table I).

Human and murine monocytes express the receptor for monocyte colony-stimulating factor (M-CSF receptor; CD115) and the integrin CD11b. Monocytes are classified into two major subsets on the basis of chemokine, adhesion, pattern recognition, and Fc receptor expression [5–7]. In mice, Ly6C^{hi} monocytes, often termed inflammatory or CCR2⁺ monocytes, express high levels of the chemokine receptor CCR2 and low levels of CX3CR1. Conversely, murine Ly6C^{lo} monocytes, often termed resident monocytes, express low levels of CCR2 and high levels of CX3CR1. Murine Ly6C^{hi} and Ly6C^{lo} monocytes are present in similar numbers in the blood under steady state conditions. Murine Ly6C^{hi} monocytes seem functionally equivalent to human CD14^{hi}CD16[–] monocytes, often termed CD14⁺ monocytes, while Ly6C^{lo} monocytes seem functionally equivalent to human CD14^{lo}CD16⁺ monocytes, often termed CD16⁺ monocytes. While the similarities between murine and human subsets were initially established on the basis of a limited set of surface antigens, in-depth transcriptional analyses support the notion that murine Ly6C^{hi} and human CD14⁺ monocytes as well as murine Ly6C^{lo} and human CD16⁺ monocytes form broadly conserved counterparts [8]. Several excellent reviews cover important species-specific differences between murine and human monocyte subsets in more detail and discuss minor monocyte subsets as well [2, 5].

During microbial tissue invasion, Ly6C^{hi} monocytes mobilize in the bone marrow, enter the circulation, and extravasate into the periphery at portals of infection. Ly6C^{hi} monocytes and their derivative cells condition the local inflammatory milieu, activate innate effector cells through cellular crosstalk, engulf and kill microbes, and play critical roles in naïve T cell activation and in directing CD4 T cell differentiation. Recent work indicates that murine Ly6C^{hi} and human CD14⁺ monocytes can acquire features of “trained immunity”, a functional attribute with memory-like features that may facilitate pathogen clearance in subsequent encounters. In addition to these antimicrobial activities, murine Ly6C^{lo} and human CD16⁺ monocytes exhibit exquisite vascular and endothelial surveillance functions and can detect microbial nucleic acids and viruses via Toll-like receptor (TLR) 7 and 8 signaling pathways [9, 10].

Our understanding of the contribution of monocytes and their derivative cells to vascular and endothelial surveillance, pathogen clearance in peripheral tissues, and to resolution of tissue damage following infectious challenges has extended to a wide range of microbes. The aim of this review is to summarize recent literature and highlight new insights on the role of monocytes and their derivative cells in innate and adaptive host defense against prokaryotic and eukaryotic pathogens. While the primary emphasis focuses on *in vivo* studies in murine experimental infection models, with an overview of genetically engineered mouse models to investigate monocyte functions *in vivo*, we discuss some relevant human examples as well. Monocyte development and monocyte trafficking during homeostasis, inflammatory states, and antimicrobial immunity has been reviewed extensively elsewhere [2, 11, 12].

2. Murine models to study monocyte function during microbial challenges

Researchers have developed a number of experimental strategies to trace and manipulate murine monocyte subsets and functions, primarily based on their expression of specific chemokine or adhesion receptors and transcription factors as well as susceptibility to toxin-loaded liposomes (Table II).

First among these was the development of CCR2^(-/-) mice in which the frequency of circulating Ly6C^{hi} monocytes is ~50–80% lower than in CCR2^(+/+) mice [13]. Ccr2^{-/-} Ly6C^{hi} monocytes fail to emigrate from bone marrow stores during experimental infections, notably *Listeria monocytogenes*. CCR2^(-/-) mice are highly susceptible to systemic listeriosis due to a deficiency of Ly6C^{hi} monocyte-derived effector cells that produce TNF and express inducible nitric oxide synthase, best known as TNF/iNOS-producing dendritic cells (Tip-DCs), at sites of listerial tissue invasion [14]. This mouse strain is particularly well suited to study CCR2-dependent monocyte functions during microbial challenges and its role in mobilizing monocytes to portals of pathogen infection. Gene knockout and fluorescent reporter mouse strains for CCR2 ligands (i.e. CCL2/MCP-1, CCL7/MCP-3, and CCL12/MCP-5) are available [15–17] and have been utilized in pathogenesis studies, primarily to decipher the relative contribution of individual CCR2 ligands to monocyte trafficking [18] and to identify their cellular sources [17].

The development of CX3CR1 and CCR2 fluorescent reporter mice enabled the identification of circulating murine monocyte subsets and bone marrow progenitors on the basis of fluorescent transgene expression (Table I) [1, 7]. These strains have facilitated numerous adoptive transfer studies of highly purified bone marrow or circulating monocytes to examine monocyte differentiation and effector functions during infectious challenges. The promoters for these chemokine receptors have been harnessed to drive a human or simian diphtheria toxin receptor (DTR) transgene to enable conditional cell ablation upon DT administration (Table II). An important limitation of these strategies is that both CCR2 and CX3CR1 are expressed in non-monocytic leukocytes that include subsets of NK cells and T cells. Thus, it is imperative to account for this ectopic expression in interpreting experimental results. For example, researchers have adoptively transferred purified DT-resistant Ly6C^{hi} monocytes to reverse infectious phenotypes in DT-treated CCR2-DTR mice [19–21]. To improve the specificity of monocyte ablation strategies, scientists have generated intersectional approaches in which restricted Cre recombinase expression (in LysM⁺ or CX3CR1⁺ cells) can activate DTR expression in M-CSF receptor (CD115)-expressing leukocytes. The latter approach targets bone marrow and circulating monocytes and M-CSF signaling-dependent tissue macrophages, while leaving splenic lymphoid and conventional DC populations intact in the steady state [22, 23].

The development of murine models to enable both specific and efficient Cre-lox-mediated recombination in circulating monocytes has not been fully achieved. A recent study compared the specificity and efficiency of constitutive or inducible Cre recombinase transgenes under control by promoters expressed predominately in myeloid cells [24]. Constitutive CX3CR1-Cre mice did not achieve efficient gene targeting when this strain was crossed to *ROSA26*-flox-stop-flox-EYFP reporter mice and progeny were analyzed for YFP

expression in circulating Ly6C^{hi} and Ly6C^{lo} monocytes [24]. Similarly, LysM-Cre and F4/80-Cre mice were inefficient in targeting circulating monocyte subsets, as judged by the same criteria [24]. Greater recombination efficiency in circulating monocytes has been reported for mice that encode an inducible CCR2-Cre-ERT2 transgene in a homozygous manner [25], though the issue of specificity remains important for the interpretation of experimental results achieved with this strain.

In sum, the specificity of promoters that drive DTR and Cre transgene expression remains a limitation in contemporary studies of monocytes and their derivative cells in antimicrobial immunity. The lack of a well-characterized marker that is restricted to the common monocyte progenitor or to one or both major monocyte subset(s) remains a barrier to developing more accurate tools than the current approaches. The development of intersectional Cre recombinase driver lines provides a potential solution to this problem [26]. The fact that no contemporary gene targeting approach discriminates between the major circulating monocyte subsets may in part reflect the developmental relationship between murine Ly6C^{hi} and Ly6C^{lo} monocytes, since Ly6C^{hi} monocytes have the capacity to give rise to Ly6C^{lo} monocytes *in vivo* [4, 27, 28].

3. Monocyte-mediated defense against bacteria

3.1 Early responders during bacterial infections

Ly6C^{hi} monocytes rapidly exit the bone marrow and traffic to sites of bacterial infection. Because they express high levels of the chemotactic receptor CCR2 [14], bone marrow Ly6C^{hi} monocytes enter venous sinusoids en route to injured peripheral tissues in response to CCR2 ligands, i.e. CCL2/MCP-1, CCL7/MCP-3 and CCL12/MCP-5 [13]. Circulating inflammatory signals, e.g. bacterial LPS, induce bone marrow mesenchymal stem cells and their progeny, including CXC chemokine ligand (CXCL)12-abundant reticular cells [17], to secrete CCL2 and to mediate Ly6C^{hi} monocyte bone marrow egress. Hematopoietic cells can participate in CCL2 production through TLR- and type I interferon-dependent pathways [49]. Triggering of cytosolic Nod2 via bacterial-derived MDP can also induce the secretion of type I IFN and CCL2 and induce Ly6C^{hi} monocyte mobilization from the bone marrow [50, 51].

Once in the blood, activated Ly6C^{hi} monocytes utilize adhesion mechanisms to access infected tissues from peripheral blood. For instance, during *L. monocytogenes* infection, the integrins CD11b and CD44 as well as ICAM-1 mediate Ly6C^{hi} monocyte egress from the circulation and entry into infected hepatic foci [52]. This trafficking step occurs largely independent of G-protein mediated chemotaxis, since pertussis toxin treatment of adoptively transferred purified Ly6C^{hi} monocytes does not block their influx into the infected liver parenchyma from the vasculature, as compared to untreated monocytes. Other mechanisms may be involved in the context of various bacterial infections, in the liver as well as in other organs. CX3CR1 in particular, contributes to splenic access of Ly6C^{hi} monocytes during *L. monocytogenes* infection [53]. Fractalkine/CX3CL1, the CX3CR1 ligand, is found in marginal/T cell zones of the spleen, consistent with a function in monocyte trafficking into this tissue. Ly6C^{hi} monocytes also express low levels of CCR1 and CCR5, a finding observed in human CD14⁺CD16⁻ monocytes as well [31, 54]. While several groups have

found evidence of CCR1 and CCR5 [55–58] cell surface up-regulation during monocyte activation, the precise contribution of these receptors in monocyte trafficking and in regulating monocyte activation during antimicrobial responses requires further study.

Ly6C^{hi} monocytes express the pattern recognition receptors (PRRs) Nod1 and Nod2 [59] that detect bacterial invasion of the cytosol, and in particular bacterial cell wall peptidoglycan (PGNs) constituents such as d-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP) [60]. Interestingly, Nod2(–/–) mice exhibit increased susceptibility to oral challenge with *L. monocytogenes* and with other intestinal bacteria such as *Citrobacter rodentium*, but not to intravenous challenge with *L. monocytogenes* [50, 61]. This observation may be accounted for by impaired detection of invading bacteria by non-hematopoietic cells and by altered intestinal myeloid cell responsiveness and homeostasis. Altogether these data suggest that, at least *in vivo*, the importance of cell-intrinsic Nod1 and Nod2 sensing for Ly6C^{hi} monocyte activation is complex and requires further investigation.

3.2. Microbicidal functions during bacterial infections

Pioneering studies in the systemic listeriosis mouse model established Ly6C^{hi} monocytes as key players involved in the clearance of this intracellular Gram-positive bacterium through their ability to rapidly produce bactericidal nitric oxide (NO) and to secrete the potent inflammatory mediator TNF [14, 62] (Figure 1A). During listeriosis, signaling through the common TLR and IL-1 receptor superfamily adaptor protein MyD88 [62] and by NK cell-dependent IFN- γ [63] regulates the production of these effector molecules. Recently, Ly6C^{hi} monocytes have been shown to be required for effective pulmonary clearance of distinct clinical isolates of the Gram-negative bacterium *Klebsiella pneumoniae*, though the effector mechanisms were not addressed in this study [64]. Mice infected with the Gram-negative bacterium *Brucella melitensis*, the causative agent of human brucellosis, added further support to the importance of MyD88 (but not TRIF) signaling in promoting Ly6C^{hi} monocyte differentiation into iNOS⁺ cells [65]. This study invoked TLR4 as the initiating sensor, yet TLR4 only partially accounted for the MyD88(–/–) phenotype, suggesting that other MyD88-coupled receptors are relevant for this process. Although these studies demonstrate a role for specific TLRs and other activation pathways in Ly6C^{hi} monocyte differentiation into iNOS⁺ and/or TNF⁺ cells, none formally established the cell-intrinsic requirements for this process. A recent report elegantly demonstrated that restricting functional MyD88 expression to CD11c⁺ DCs is sufficient to orchestrate Ly6C^{hi} monocytes differentiation into Tip-DCs during *L. monocytogenes* infection [66], consistent with the notion that cell-intrinsic MyD88 expression in Ly6C^{hi} monocytes is not required for Tip-DC development.

Ly6C^{hi} monocytes are equipped with the enzymatic machinery to produce high levels of reactive oxygen species (ROS), in addition to nitric oxide (NO), during *L. monocytogenes* infection [14, 55]. While both functions appear essential for microbial killing during primary and secondary infection [14, 55, 67], the studies suggest a distinct, sequential role for each effector function during successive bacterial challenges, with NO production required predominately during primary infection and ROS activity observed primarily during the

secondary infection; the latter is a result of TNF induction at this stage [55]. ROS production subsequently leads to antimicrobial autophagy during *L. monocytogenes* infection [67]. Interestingly, during recall infection in vaccinated hosts, massive IFN- γ secretion by memory T cells, and to some extent NK cells promotes Ly6C^{hi} monocyte activation. This results in the expression of guanylate binding proteins (Gbp), Gbp1–11 [57], which belong to the IFN- γ -inducible GTPase superfamily. These genes are implicated in cell-autonomous host defense against intracellular bacteria through the activation of phagocyte NADPH oxidase, antimicrobial peptides, and autophagy effectors [68, 69].

3.3. Antigen-presenting cell functions during bacterial infections

In the systemic *L. monocytogenes* infection model, Ly6C^{hi} monocytes do not appear to contribute to T cell priming [14], although maturing Ly6C^{hi} monocytes up-regulate functional molecules involved in T cell priming such as MHC II, CD80, CD86, and CD40. Other molecules such as OX40L, CD70 or the co-inhibitory receptors PD-L1 and PD-L2 are also expressed within ~24 hours post-infection (G. Lauvau and colleagues, unpublished data). In more recent work, researchers found that injection of purified LPS or *Escherichia coli* induced Ly6C^{hi} monocytes to rapidly differentiate into antigen-presenting cells that exhibit comparable potency as conventional dendritic cells (cDCs) through TLR4- and TRIF- but not MyD88-dependent signals [70].

However, the relative contribution of Ly6C^{hi} monocytes and derivative cells versus that of conventional DCs (cDCs) to co-stimulatory or inhibitory signals to CD4 T cells during the course of bacterial infection remains unresolved. In pulmonary tuberculosis, Ly6C^{hi} monocytes play a major role in the efficient priming of *Mycobacterium tuberculosis*-specific CD4⁺ T cells by lymph node-resident cDCs by transporting mycobacteria to draining LNs [71]. Similarly, *Yersinia pestis* establishes a systemic infection by propagating from LN to LN through lymphatic channels inside Ly6C^{hi} monocytes, using sphingosine-1-phosphate mediated LN egress [72].

Other reports indicate that Ly6C^{hi} monocytes can provide the third signal to T cells [73] following infection with *L. monocytogenes* [62, 74] or *Citrobacter rodentium* [23, 50] through IL-12 and type I IFN production. While several studies suggest that MyD88 and IFN- γ signaling control Ly6C^{hi} monocyte IL-12 production, none of them have formally established which monocyte-intrinsic sensing pathways is involved [62, 63]. During systemic listeriosis, Ly6C^{hi} monocytes represent the major source of type I IFN, another T cell polarizing cytokine, as shown in IFN- β reporter or in cell-specific IFN- β knockout mice [75, 76]. Multiple *in vitro* and *in vivo* studies have provided compelling evidence that cyclic di-nucleotides, cyclic-di-AMP and cyclic-di-GMP, secreted by *L. monocytogenes* represents a major trigger for type I IFN production. This signal occurs when *L. monocytogenes* escapes from early phagosomes into the cytoplasm [77, 78].

A number of cytosolic sensors (e.g., the helicase DDX41) are associated with type I IFN production during systemic listeriosis [79, 80]. However, recent work indicates that mammalian cyclic GMP-AMP synthase (cGAS) synthesizes cyclic dinucleotides in response to bacterial double-stranded DNA [81, 82]. Whether bacteria- or host-derived, cyclic dinucleotides activate STING, resulting in TBK1/IRF3-mediated type I IFN production

remains to be investigated [79, 80]. The role of STING in inducing type I IFN production during listeriosis was further characterized by using *L. monocytogenes* mutants that secrete variable amounts of cyclic-di-AMP and by co-immunization with synthetic cyclic di-AMPs as an adjuvant [83]. While not yet formally proven, these data collectively suggest that Ly6C^{hi} monocytes possess intrinsic pathways of activation that involve specific sets of cytosolic nucleic acid sensors that subsequently lead to type I IFN production during bacterial (and likely other [84]) infections.

3.4 “Alarmin” function during bacterial infections

In addition to providing well-established polarizing cytokines to the T cells, Ly6C^{hi} monocytes can produce significant amounts of other inflammatory cytokines (IL-1, IL-15, IL-18) and chemokines (CXCL9). Specifically, and in response to both type I IFN and IFN- γ , Ly6C^{hi} monocytes *trans*-present bioactive IL-15 [85] or turn off IL-1 secretion [86] during murine *listeriosis* or *tuberculosis*, respectively. IL-15 *trans*-presentation and IL-18 secretion by Ly6C^{hi} monocytes drives both NK and memory CD8⁺ T cell activation and their expression of cytolytic effector molecules (e.g., granzyme B) and IFN- γ [85]. The secretion of bioactive IL-18 and IL-1 depends, in most instances, on the assembly of multicomponent inflammasome complexes that incorporate either AIM2, NLRP3, NLRP4 and combinations thereof as a sensor component, Asc as a structural component, and a caspase component to cleave target cytokines, both in mice challenged with bacteria (*L. monocytogenes*, *Francisella tularensis*, *Salmonella* sp.) [85, 87] and on freshly isolated human blood monocytes [88, 89]. For example, in a model of intestinal injury, Ly6C^{hi} monocytes were shown to secrete IL-1 β via the NLRP3 inflammasome in response to the commensal bacterium *Proteus mirabilis* [90]. The same group also reported that non-canonical inflammasome activation via caspase 11 in Ly6C^{hi} monocytes drives IL-1 β secretion and type 3 innate lymphoid cell (ILC3) activation, leading to intestinal *Citrobacter rodentium* elimination [91]. During murine tuberculosis, a population of pulmonary Ly6C⁺ mononuclear cells, the likely derivative cells of Ly6C^{hi} monocytes, produce important amounts of IL-1 α and IL-1 β , two essential cytokines for controlling tuberculosis [86]. Unexpectedly, the production of IL-1 β in this model did not require a functional inflammasome cytosolic sensing pathway [92], and was negatively regulated by type I interferon, and to some extent CD4⁺ T cell-derived IFN- γ .

Massive secretion of CXCL9 by Ly6C^{hi} monocytes, in response to direct IFN- γ signaling contributes to their capacity to orchestrate sustained recruitment of multiple cellular effectors of the immune system, ultimately leading to bacterial clearance [57]. Whether the action of Ly6C^{hi} monocyte-derived CXCL9 requires CXCR3 signaling to induce protective responses and CXCL9-dependent protective mechanism remains unexplored. The role of other Ly6C^{hi} monocyte-derived chemokines in antibacterial host defense remains an active focus of investigation.

3.5 Regulatory functions in the context of commensal bacteria

Recent studies reveal that Ly6C^{hi} monocytes can acquire a tissue-specific regulatory phenotype in the gut in response to commensal bacteria. Following commensal bacterial exposure due to intestinal parasitic infection with *Toxoplasma gondii*, recruited Ly6C^{hi}

monocytes secrete the lipid mediator, prostaglandin E2 (PGE₂) that dampens neutrophil-mediated tissue damage, and interleukin-10 [93] (Figure 1B). This regulatory phenotype occurs even as activated Ly6C^{hi} monocytes contribute to parasite killing during acute toxoplasmosis [94, 95]. Similarly, human CD14⁺ monocytes also produce PGE₂ and IL-10 following stimulation with commensal bacteria *in vitro* [93].

In a follow-up study, the same group demonstrated that Ly6C^{hi} monocytes are functionally primed in the bone marrow prior to egress into the bloodstream and recruitment to the infected gut during murine *Toxoplasma gondii* and *Yersinia pseudotuberculosis* infections [96]. Acquisition of PGE₂/IL-10 regulatory capacity by Ly6C^{hi} monocytes required IFN- γ signals from NK cells in the bone marrow and IFN- γ -dependent programming was observed as early as in cMoPs. The production of IL-12 by Batf3⁺ mucosal-associated DCs during either infection promoted NK cell production of IFN- γ . Though in different experimental setting, steady-state Ly6C^{hi} CD64⁺ monocyte-derived intestinal macrophages can control the local induction of mucosal T helper 17 CD4 T cells after intestinal colonization with segmented filamentous commensal bacteria [19].

4. Monocyte-mediated defense against fungi

Fungi are saprophytic eukaryotic organisms that can be divided into yeast or molds. Yeasts (e.g., *Cryptococcus neoformans*, agent of cryptococcosis) are round or oval cells that divide by budding or fission. Molds (e.g., *Aspergillus fumigatus*, agent of aspergillosis) form branching, tubular hyphae (filaments) that propagate via small vegetative spores, termed conidia. Dimorphic fungi (e.g. *Blastomyces dermatiditis*, agent of blastomycosis) have the capacity to transition between filamentous and yeast cell growth. For example, *B. dermatiditis* grows as a mold in the environment and exists as yeast cells in human tissue.

Humans acquire most invasive mycoses by inhalation of airborne conidia (i.e. vegetative spores) or yeast cells. Animal models of pulmonary *Aspergillus fumigatus* (the major causative agent of aspergillosis) [32, 97], *Blastomyces dermatidites* (blastomycosis) [98], *Histoplasma capsulatum* (histoplasmosis) [98, 99], *Cryptococcus neoformans* (cryptococcosis) [100–102] demonstrate critical functional roles for Ly6C^{hi} monocytes and derivative cells in innate and adaptive antifungal immunity. In addition, monocytes and their derivatives play essential roles in clearing systemic infections with *Candida albicans* (candidiasis) [20], a commensal dimorphic fungus that resides in human mucosal tissues, particularly within the alimentary tract.

4.1. Early responders during fungal infections

Ly6C^{hi} monocytes rapidly accumulate in the lungs of mice challenged with *A. fumigatus*, *B. dermatiditis*, *H. capsulatum*, and *C. neoformans* via the respiratory route, via CCR2-dependent bone marrow egress. In addition to Toll-like receptors and cytosolic bacterial sensors described in the preceding section, Ly6C^{hi} monocytes express pattern recognition receptors that bind fungal polysaccharides, including Dectin-1/Clec7a, Dectin-2/CLEC4n, macrophage- C-type lectin (MCL, also called Dectin-3, Clec3f8, Clec4d), and macrophage-inducible C-type lectin (Mincle; also called CLEC4e) [103]. All of these receptors encode an intracellular signaling domain with an immunotyrosine-based activation motif (ITAM;

e.g. Dectin-1) or pair with an ITAM-containing adaptor protein (e.g., Fc receptor γ subunit, FcR γ). These ITAM modules can undergo tyrosine phosphorylation to activate a canonical signaling pathway via spleen tyrosine kinase (Syk) and a trimeric complex that consists of CARD9/Malt1/Bcl10 [103]. In this manner, Syk can direct NF- κ B activation through the IKK complex. In addition, Syk-dependent phospholipase C- γ activation results in Ca²⁺/calcineurin phosphatase-dependent activation of nuclear factor of activated T cells (NFAT) family members. This receptor repertoire enables Ly6C^{hi} monocytes to respond to fungal β -(1,3) glucan and to fungal α -mannan moieties via Dectin-1/Clec7a and Dectin-2/Dectin-3 complexes, respectively [104]. In addition, lipid moieties from the causative agent of tinea versicolor (*Malassezia* species) activate Mincle/Clec4e [105]. Thus, a diverse and growing number of conserved fungal antigens can activate monocyte responses.

4.2. Microbicidal Functions during fungal infections

Following respiratory fungal challenge with *A. fumigatus* conidia, Ly6C^{hi} monocytes are essential for host defense, since DT-mediated cellular ablation in the CCR2 deleter mouse model leads to invasive aspergillosis and murine mortality [97]. Adoptive transfer and transcriptional profiling experiments indicate that lung-infiltrating Ly6C^{hi} monocytes differentiate into monocyte-derived dendritic cells (Mo-DCs), as judged by up-regulation of the dendritic cell transcription factor Zbtb46, the integrin CD11c, and MHC class II, and down-regulation of Ly6C expression [32, 97]. Similarly, during murine cryptococcosis, the formation of Ly6C^{hi} monocyte-derived CD11b^{hi} MHC II^{hi} exudate macrophages coincides with inducible nitric oxide synthase expression in the lung, TNF production, and cryptococcal clearance. In a systemic candidiasis model, Ly6C^{hi} monocytes infiltrate the kidneys and the central nervous system, the major target organs in this model, and contribute to protection at both sites [20].

At the portal of fungal infection, Ly6C^{hi} monocytes and their derivative cells condition the inflammatory environment, e.g., by producing TNF, products of inducible nitric oxide synthase, and chemokines that include IL-12, CXCL1, CXCL2, CXCL9, CXCL10 [97]. Ly6C^{hi} monocyte-intrinsic CARD9 signaling is essential for CXCL2 and TNF production following *A. fumigatus* infection, linking fungal recognition at portals of fungal infection to the cellular activation, as judged by cytokine and chemokine production [106]. Furthermore, Ly6C^{hi} monocytes and their Mo-DC derivatives enhance neutrophil conidial killing in the lung, though the precise molecular mechanisms of this innate immune crosstalk have yet to be elucidated [97].

Ly6C^{hi} monocytes and their Mo-DC derivatives rapidly engulf and directly kill inhaled conidia, as revealed by fluorescent *A. fumigatus* reporter (FLARE) conidia that alter their fluorescence signal upon loss of viability during cellular encounters in the lung [107] (Figure 1C). Sorted human CD14⁺ monocytes display fungistatic activity when challenged with viable conidia *in vitro*, while CD16⁺ monocytes are more potent TNF-secreting cells, yet display little fungistatic activity [108]. Both murine and human studies support a cell-intrinsic role for NADPH oxidase in monocyte-dependent conidiacidal activity [97, 109]. Dectin-1 signaling in response to conidia recruits the autophagy protein LC3 to phagosomal membranes, a process that acts to limit intracellular fungal growth in human monocytes

[110]. The activation of LC3-associated phagocytosis depends on NADPH oxidase activity and contributes to murine defense against aspergillosis, as shown by Atg5 deletion in hematopoietic cells in a pulmonary challenge model [111]. In a neutropenic murine model of aspergillosis, CCR7-mediated Mo-DC egress from the *A. fumigatus*-infected lung diminishes pulmonary fungal clearance [112]. This finding suggests that lung Mo-DC retention promotes fungal killing at the portal of infection.

4.3. Induction of CD4 T cell responses during fungal infections

During pulmonary challenge with a *Blastomyces* vaccine strain CCR2-dependent Ly6C^{hi} monocyte recruitment is subverted by the induction of host matrix metalloprotease-2 (MMP2) in the respiratory mucosa. MMP2 cleaves the CCR2 ligand CCL7 and results in bone marrow retention of Ly6C^{hi} monocytes and failure to prime vaccine-induced protective CD4 T cell responses [98]. During pulmonary aspergillosis, blastomycosis vaccine challenge, and oropharyngeal candidiasis, Ly6C^{hi} monocyte-derived Mo-DCs play an essential role in transporting fungal antigens from the lung or oral cavity to draining lymph nodes [32, 113, 114] (Figure 1C). However, Mo-DCs do not appear essential for CD4 T cell priming beyond their role in antigen transport to draining LNs, since fungal antigen is transferred among a subset of LN-resident DCs that prime antigen-specific CD4 T cells *in vivo* [113, 114]. Consistent with this model, MHC class II expression on Ly6C^{hi} monocytes and their derivative cells is dispensable for the development of antigen-specific CD4 T cells against *M. tuberculosis* in the lung [71].

Beyond their role in antigen trafficking, Ly6C^{hi} monocytes and their Mo-DC derivatives control T-bet expression in and IFN- γ production by fungus-specific CD4 T cells as these cells migrate to *A. fumigatus*-infected airways and establish an immune response that is dominated by a T helper 1 cytokine response [115] (Figure 1C). Transient depletion of CCR2⁺ cells after they fulfill their antigen trafficking function results in a reduction in fungus-specific T helper 1, and an increase in fungus-specific T helper 17 CD4 T cells. In murine models of pulmonary histoplasmosis and cryptococcosis, CCR2 deficiency results in the development of a T helper 2 cytokine-dominated response and in impaired pulmonary fungal clearance that, in the setting of experimental histoplasmosis, can be reversed by exogenous transfer of bone marrow-derived DCs into the lung [99, 100, 116, 117].

4.4. Trained Immunity during fungal infections

During repetitive encounters with fungal antigens, murine Ly6C^{hi} monocytes and human CD14⁺ monocytes can acquire functional attributes that resemble specific features observed in immunologic memory [118]. Although cellular expansion has not been demonstrated in the context of secondary challenges, it has been known that macrophages can undergo stable epigenetic modifications *in vitro* following stimulation with LPS [119]. These include enhanced secondary responses following re-stimulation with microbial ligands and cytokines *in vitro* or upon *in vivo* challenges with fungal or non-fungal pathogens [118, 120]. Murine and human monocytes undergo epigenetic changes, characterized in part by stable changes in histone 3 Lys⁴ trimethylation and histone 3 Lys²⁷ acetylation patterns, following exposure to *C. albicans*-derived β -glucan. The metabolic basis for β -glucan-induced trained immunity involves activation of a Dectin-1/Akt/mammalian target of rapacin (mTOR)/hypoxia-

inducible factor 1 α (HIF1 α) signaling pathway that shifts monocyte metabolism from oxidative phosphorylation to glycolysis [120]. Genetic or pharmacologic disruption of this pathway during the initial β -glucan-dependent priming step interferes with trained immunity against secondary microbial challenges in an antigen-independent manner. In humans with chronic mucocutaneous candidiasis (CMC), defects in STAT1 signaling are linked to impaired trained immunity in monocytes via IFN- γ signaling *in vitro* [121]. Beyond the well-characterized impairment in mucosal IL-17-dependent antifungal host defense mechanisms in CMC patients, the extent to which the observed defect in trained immunity contributes to the clinical phenotype remains to be elucidated.

5. Monocyte-mediated defense against parasites

Recent studies have highlighted functional roles of monocytes in host defense against helminths and protozoa, the two major classes of endoparasites that typically reside within the body of mammalian hosts. Helminths, commonly referred to as intestinal worms, are large multicellular organisms that include tapeworms, known as cestodes (e.g. *Taenia* species, *Taenia solium* is the agent of cysticercosis), roundworms, known as nematodes (e.g. *Trichuris* and *Ascaris* species, agents of trichuriasis and ascariasis) and flatworms, known as flukes (e.g., *Shistosoma* species, agents of shistosomiasis). In contrast, protozoa are unicellular organisms, many of which have the capacity for mobility and predation. Protozoa include ciliates, amoeba, sporozoans (apicomplexa, e.g. *Plasmodium* and *Toxoplasma* species, agents of malaria and toxoplasmosis) and flagellates (e.g. *Giardia* species, agents of giardiasis). In contrast to the various classes of endoparasites mentioned above, this review does not cover monocyte responses to ectoparasites (e.g., human body louse) that can live on human skin.

5.1. Helminths

5.1.1. T helper 2 responses during helminth infections—In contrast to the other pathogens described above, helminths induce a T helper type 2 cytokine response. This type 2 response is characterized by the production of IL-4 and IL-13 by either CD4⁺ T cells or various innate immune cells. Hence, macrophage activation in the context of helminth infections is characterized by the alternatively activated M2 macrophage phenotype, instead of classically activated M1 monocytic cells. These M2 macrophages have been shown to perform important roles in parasite killing, organization of granuloma formation, prevention of tissue damage. Difficulties in pinpointing the specific function of alternatively activated M2 macrophages under different conditions could be attributed to the broad heterogeneity of phenotypes that are induced by stimulation with type 2 cytokines. Recent studies have begun to dissect the various phenotypes of M2 macrophages and different types of helminth infections serve as excellent models to understand M2 macrophage heterogeneity. Here, we will primarily focus on M2 macrophages derived from circulating monocytes.

The general assumption from the pioneering work of Zanvil Cohn was that macrophages were predominantly derived from monocytes; hence it was also assumed that M2 macrophages are derived from monocytes that originate in the bone marrow and circulate in the blood. As noted above, when blood monocytes were further sub-categorized into Ly6C^{hi}

and Ly6C^{lo} populations, the question then raised was whether M2 macrophages during helminth infections are derived from Ly6C^{hi} or Ly6C^{lo} populations during inflammatory responses.

The Ly6C^{lo} population was shown to patrol blood vessels through a distinctive long-range crawling behavior [9]. It was suggested based on the transcriptional profile of these patrolling monocytes responding to *L. monocytogenes* infection that they may subsequently differentiate into M2 macrophages [122]. A model was proposed that Ly6C^{hi} monocytes would extravasate into tissues during an inflammatory response and differentiate into Tip-DCs or M1 macrophages, whereas the Ly6C^{lo} population would patrol the vasculature under steady state conditions but extravasate into the tissue to differentiate into M2 macrophages in response to inflammation [123].

To test this model in the context of helminth infection, researchers examined whether M2 macrophages in the liver granulomas of *Schistosoma mansoni*-infected mice are derived from Ly6C^{hi} or Ly6C^{lo} monocytes [124], using adoptive transfer of highly purified populations of Ly6C^{hi} or Ly6C^{lo} splenic monocytes into infected recipient mice. Only transferred Ly6C^{hi} monocytes could extravasate from the liver sinusoids into the tissues and adopt the phenotype of M2 activation, i.e., PD-L2 expression on the cell surface [124]. Concurrently, it was also shown that the accumulation of the granuloma macrophages was dependent on CCR2 signaling [125] and that depletion of monocyte populations led to exacerbated disease associated with reduced granuloma size. Together, these data clearly indicated that recruitment of Ly6C^{hi} monocytes is the dominant process for accumulation of M2 macrophages in the liver granulomas of infected mice. Results from an allergic skin inflammation model [126] were also consistent with Ly6C^{hi} monocytes adopting an M2 phenotype, although basophils were the primary source of IL-4 in this context. In contrast, during *S. mansoni* infection, CD4⁺ Th2 cells are the primary source of IL-4 for M2 activation [124]. More recently, alternatively activated intestinal M2 macrophages that accumulate in the colon during infection with *Trichuris muris* infection were also found to be derived from CCR2-dependent blood monocytes [127]. Hence, recent studies over the last few years have clearly demonstrated through a number of different helminth infection models that Ly6C^{hi} monocytes (and not Ly6C^{lo} monocytes) are the primary source of inflammatory M2 macrophages during a type 2 response.

Although Ly6C^{lo} monocytes are thought to be uniquely able to patrol the vascular endothelium, intravital microscopy of the livers of *Sm*-infected mice revealed that Ly6C^{hi} monocytes adopt patrolling behavior in liver sinusoids near the granulomas [124]. The behavior of these patrolling Ly6C^{hi} monocytes was indistinguishable from that of Ly6C^{lo} monocytes. Notably Ly6C^{hi} monocytes down-regulate the expression of Ly6C in the inflamed liver. These results are consistent with a recent report that utilizes spinning-disk confocal to demonstrate that CCR2^{hi}CX3CR1^{lo} monocytes transition to a CX3CR1^{hi}CCR2^{lo} phenotype as they entered a site of sterile injury in the liver [128]. Sterile injury also induces M2 macrophage accumulation [129], which are likely derived from the same monocyte populations.

6.1.2. Monocyte-derived M2 macrophages versus tissue-resident M2

macrophages—In the midst of the studies described above, a new paradigm emerged in macrophage biology. Tissue-resident macrophages in a variety of organ systems were found to be derived from non-hematopoietic-derived precursors that are seeded during embryonic development [130]. These embryonically derived precursor cells undergo proliferative self-renewal in their respective tissues. Concurrently, IL-4 was found to drive proliferative expansion of tissue-resident macrophages and the adoption of an M2 phenotype [131]. During murine infection with the filarial nematode *Litomosoides sigmodontis*, M2 macrophage accumulation in the thoracic cavity did not depend on a monocytic intermediate, but instead on resident macrophages that derive from the embryonic precursor lineage [131]. Similarly, infection with the nematode *Heligmosomoides polygyrus* leads to the expansion of peritoneal M2 macrophages that are derived from tissue-resident macrophages, and not from circulating monocytes [132].

M2 macrophages can thus be derived from either Ly6C^{hi} monocytes or from tissue-resident macrophages. But do the type 2 cytokines IL-4 and IL-13 activate Ly6C^{hi} monocyte derived macrophages differently from tissue-resident macrophages? Are these dual macrophage populations functionally distinct during parasitic infections?

By comparing thioglycollate-elicited M2 macrophages that are derived entirely from circulating blood Ly6C^{hi} monocytes and M2 macrophages that accumulate through IL-4-driven proliferative expansion of resident macrophages, researchers clearly demonstrated that the cellular lineage of M2 macrophages affects the phenotype and function of these cells. Although both monocyte-derived M2 macrophages (M2^{mono}) and tissue M2 macrophages (M2^{tiss}) express the canonical M2 genes arginase 1, Chi3l3/Ym1 and Relma/Fizz1, their transcriptional profiles are otherwise almost completely different. M2^{mono} and M2^{tiss} also differ in terms of their cell surface phenotype. M2^{mono} are F4/80^{int}, CX3CR1⁺, MHCII⁺, CD206⁺, PD-L2⁺, whereas M2^{tiss} are F4/80^{high}, CX3CR1[−], MHCII[−], CD206[−], and PD-L2[−]. Hence, it is now possible to distinguish between M2 macrophages that originate from recruited blood monocytes or via proliferation of tissue-resident macrophages.

Under conditions where there is a non-inflammatory increase in circulating IL-4 (or IL-13), e.g., in the thoracic cavity during *L. sigmodontis* infection or in the peritoneal cavity during *H. polygyrus* infection, CCR2-dependent Ly6C^{hi} monocyte recruitment does not occur and M2^{tiss}s accumulate. Under conditions where bacteria are present (e.g., in the gastrointestinal tract) during *T. muris* infection, or where there is a strong irritant (e.g., in the liver around *S. mansoni* eggs), circulating Ly6C^{hi} monocyte recruitment predominantly drives the accumulation of M2^{mono}. Therefore, the anatomic location and type of helminth infection determines whether the accumulation of M2 macrophages results from the recruitment of circulating Ly6C^{hi} monocytes or from the proliferation of tissue-resident macrophages.

5.1.3. Monocytes and immune regulatory functions during helminth infections

—Two important features of monocyte-derived M2 macrophages that are absent in tissue-resident M2 macrophages include the expression of RALDH2 and PD-L2. Both of these molecules are important for immune regulation. RALDH2 (or ALDH1A2) encodes an

aldehyde dehydrogenase that can catalyze the synthesis of retinoic acid (RA) from retinaldehyde [133]. Intestinal CD103⁺ DCs express RALDH2 and may be an important source of RA, which promotes the generation of Foxp3⁺ regulatory T cells (Tregs) in combination with TGF- β [134, 135]. RALDH2 is highly expressed during helminth infection, particularly in alternatively activated M2 macrophages [136], and further experiments demonstrated that only monocyte-derived M2 macrophages could promote the Treg differentiation via RALDH2 expression and RA synthesis [137]. Hence, as Ly6C^{hi} monocytes differentiate into M2 macrophages during inflammatory responses, they may activate a regulatory feedback loop by promoting the generation of Tregs. Whether this M2^{mono}-Treg interaction occurs in the tissue itself or requires trafficking of RALDH2⁺ macrophages to the lymph nodes remains to be established.

PD-L2 (or B7-DC) was initially identified as the second ligand for PD-1 (Programmed death 1) but was also described to be a co-stimulatory molecule expressed on DCs. Hence, there was considerable debate regarding whether this was a stimulatory or inhibitory ligand for T cells. PD-L2 is expressed on thioglycollate-elicited peritoneal macrophages when stimulated with IL-4 [138]. Hence, PD-L2 is an excellent cell surface marker for monocyte-derived M2 macrophages and is not expressed in M2 macrophages derived from replicating tissue-resident progenitors, even when these cells are treated with IL-4 [137]. This observation was initially confusing, since M2 macrophages elicited by *Brugia malayi* in the peritoneal cavity did not express PD-L2 (P. Loke and colleagues, unpublished data). Further studies revealed that *B. malayi* was most likely inducing the proliferation of tissue-resident peritoneal macrophages derived from embryonic precursors that do not up-regulate PD-L2 in response to IL-4. Indeed, *Litomosoides sigmodontis*-induced M2 macrophages in the thoracic cavity and *H. polygyrus*-induced M2 macrophages in the peritoneal cavity also do not express PD-L2. Hence, the accumulation of M2 macrophages that express canonical markers (arginase, Chi3l3/Ym1, Relma/Fizz1) but not PD-L2 are likely to be derived from tissue-resident macrophages. PD-L2 may inhibit T cell responses by engaging PD-1 during helminth infections [139, 140], promoting T_H2 hyporesponsiveness [141].

In summary, during helminth infections M2 macrophages accumulate as a result of the elevated levels of the cytokines IL-4 and IL-13, but these macrophages can be derived from either blood Ly6C^{hi} monocytes or tissue-resident macrophages. Recruited M2 macrophages are derived primarily from the Ly6C^{hi} monocytes and not from the patrolling Ly6C^{lo} monocytes. However, Ly6C^{hi} monocytes can adopt patrolling behavior and transition through a Ly6C^{lo} state. These monocyte-derived M2 macrophages are characterized by the expression of RALDH2 and PD-L2, which have important roles in immune regulation.

5.2. Protozoa

The importance of Ly6C^{hi} monocytes in the defense against parasitic infections has been investigated in a number of studies. Results from these reports reveal comparable roles for this subset of blood monocyte in parasitic as in bacterial infections, namely as microbial killer cells, orchestrators of innate immune activation, and as antigen presenting and polarizing cells.

5.2.1. Monocytes and parasite killing—In the course of non-lethal blood stage murine *Plasmodium chabaudi* infection, which exhibits features reminiscent of the chronic human infection with *Plasmodium falciparum*, Ly6C^{hi} monocytes contribute to elimination of blood parasites during the later stage of the disease, in which parasitemia can rebound over ~20–30 days [142]. Consistent with these results, patients with acute uncomplicated malaria exhibited higher numbers of CD14⁺ monocytes in the peripheral blood compared to malaria-exposed uninfected controls [143]. This monocyte subset was expanded and exhibited a strongly activated phenotype (CD40⁺, ICAM⁺, BST2⁺) in patients with severe cerebral malaria, as well as in a lethal *Plasmodium yoelii* mouse model of severe malaria (G. Lauvau and colleagues, unpublished data).

C57BL/6 mice are resistant to leishmaniasis due to the formation of an inflammatory response that is dominated by T helper 1 cytokines, a process that is controlled by the formation of Ly6C^{hi} monocyte-derived DCs at the site of infection and migration to draining LNs [144]. The formation of these iNOS⁺ effector cells that represent the major infected cell population in cutaneous lesions and infected LNs underlies control by MyD88 and TNF signaling. In the absence of TNF signaling, Ly6C^{lo} macrophages accumulate within infected LNs and display a defect in killing parasites [145]. During visceral leishmaniasis, IL-17A production by $\gamma\delta$ T cells suppresses Ly6C^{hi} monocyte-dependent parasite killing in the liver [146]. In humans, both reactive oxygen and nitrogen species contribute to monocyte-dependent killing of *Leishmania braziliensis* [147].

Ly6C^{hi} monocytes represent heavily infected cells during murine intestinal *Toxoplasma gondii* infection. Defects in the CCL2-CCR2 signaling axis lead to failure in intestinal parasite control [94, 95]. Toxoplasmosis can spread to the central nervous system (CNS) and cause a chronic infection in the brain in mice and humans. CNS-infiltrating Ly6C^{hi} monocytes are essential contributors to parasite control and elimination at this site. A variety of effector mechanisms, such as the production of IL-1, IL-6, TNF, reactive nitric and oxygen species, phagocytosis and T cell activation [148], all contribute to this process.

5.2.2. Monocytes and collateral damage during parasitic infections—Although intestinal tissue-infiltrating Ly6C^{hi} monocytes contribute to early parasite killing, acute toxoplasmosis is associated with severe intestinal ileitis. IL-18-activated NK cells in the bone marrow stimulate production of the chemokine CCL3 that promotes intestinal recruitment of Ly6C^{hi} monocytes via CCR1 [149]. This signaling pathway involves Ly6C^{hi} monocytes and may amplify intestinal inflammation that contributes to ileal pathology [149]. Since Ly6C^{hi} monocytes assume a parallel role in dampening neutrophil activation [93], these studies collectively emphasize the dual pro-inflammatory and regulatory functions of these cells during acute intestinal toxoplasmosis.

In mice infected with the protozoan parasite *Trypanosoma brucei*, the causative agent of sleeping sickness in humans, IL-10 production inhibits the differentiation of (bone marrow-derived) Ly6C^{hi} monocytes into TNF⁺ and iNOS⁺ effector cells in the spleen, liver, and LNs [150, 151]. While cell-intrinsic MyD88 and IFN- γ signals promote Ly6C^{hi} effector functions, IL-10 limits this process and associated tissue damage. Similarly, in the *P. berghei*

mouse model of cerebral malaria, selective depletion of Ly6C^{hi} monocytes prevents associated immunopathology [152].

5.2.3. Monocytes and antigen-presenting cell functions during parasitic infections—While *ex vivo* T cell stimulation studies suggested that activated Ly6C^{hi} monocyte may have the capacity to present peptide-MHC complexes to prime protective Th1 CD4⁺ T cells during *L. major* infection in C57BL/6 mice, in depth analysis of *L. major*-derived peptide-MHC complexes localized such Ag T cell specific complexes on infected CD11c⁺ DCs and not on CD11c[−] monocytes, macrophages, or myeloid cell populations in susceptible Balb/c mice [144, 153]. Differences in mouse strains, parasite loads may account for some of the experimental variability observed. Furthermore, Ly6C^{hi} monocytes can up-regulate CD11c upon entry into inflamed tissues [32] and the use of CD11c is, per se, may not be adequate to distinguish reliably monocyte-derived and conventional DC subsets in inflamed tissues.

Ly6C^{hi} monocytes can secrete physiologically relevant amounts of bioactive IL-12 after *L. major* and *T. gondii* infections [144, 154], though the exact sensing pathway has not been elucidated. In mice inoculated with *T. gondii* cysts, NK cell-derived IFN- γ promoted Ly6C^{hi} monocyte maturation into F4/80⁺ macrophages and IL-12-producing Mo-DCs [154]. Recent work using a model of attenuated *T. gondii* tachyzoite vaccination reported that Ly6C^{hi} monocytes in the extrafollicular splenic compartment secrete a late wave of IL-12 that promotes the formation of terminally differentiated (KLRG1⁺) *T. gondii*-specific effector CD8⁺ T cells [155].

6. Summary

Ly6C^{hi} monocytes are essential for innate and adaptive host defense against a wide range of microbial pathogens. During the past decade, researchers have delineated the mechanisms by which infectious stimuli re-direct the homeostatic trafficking, maturation, and differentiation of monocytes and promote their deployment to portals of pathogen entry. A wide range of bacterial, fungal, and parasitic pathogens induce tissue- and pathogen-specific monocyte effector functions, including the production of reactive oxygen and nitrogen species, as well as direct uptake and killing, as visualized by fluorescence microscopy and flow cytometry techniques. Monocytes play critical roles in transporting microbial antigens to tissue-draining lymph nodes, an essential step for priming T cell responses, and in conditioning the cytokine milieu to direct T cell differentiation. The role of Ly6C^{lo} monocytes in host defense remains enigmatic, with the exception of vascular and endothelial surveillance functions. Much remains to be learned about the precise control of tissue inflammation, microbial killing, and tissue repair by the major monocyte subsets and the development of improved experimental strategies that target monocytes and their derivative cells with high specificity will undoubtedly lead to new advances in the field.

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Abbreviations

Ab	antibody
Ag	antigen
BM	bone marrow
CCR2	-CC- chemokine receptor 2
CDP	common DC progenitor
CLR	C-type lectin receptor
cMoP	common monocyte progenitor
DC	dendritic cell
DT	diphtheria toxin
LSL	lox-stop-lox
MDP	macrophage-DC progenitor
M-CSF	monocyte colony-stimulating factor
Mo	monocyte
NO	nitric oxide
NOS	nitric oxide synthase
ROS	reactive oxygen species
Tg	transgene
TLR	Toll-like receptor

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Highlights

- Monocytes are essential for host defense against bacteria, fungi, and parasites
- Monocytes can sense, engulf and kill these pathogens
- Monocytes regulate innate and adaptive immune responses
- Monocyte responses are specific to pathogens, bacterial commensals, and tissue context

Figure 1. Monocyte functions during bacterial, fungal, and parasitic infections

Ly6C^{hi} bone marrow monocytes arise from progressively committed hematopoietic progenitors that include MDPs and cMoPs. Ly6C^{hi} monocytes exit the bone marrow and enter the circulation in response to CCR2 ligands and traffic to peripheral tissues (A–D). Ly6C^{lo} monocytes exhibit vascular and endothelial surveillance functions. (A) During systemic listeriosis, Ly6C^{hi} monocytes differentiate into TNF⁺ and iNOS⁺ effector cells in the spleen, termed Tip-DCs, under the influence of NK cell-derived IFN- γ . Tip-DCs mediate cell-intrinsic and cell-extrinsic listerial killing through the production of

reactive nitrogen and oxygen species and inflammatory cytokines and direct T cell differentiation. Memory T cells in turn potentiate Tip-DC and neutrophil-mediated listerial killing during recall infections. (B) Fungal conidia are engulfed by Ly6C^{hi} monocytes or their Mo-DC derivatives and are killed directly in the lung or transported to lung-draining LNs for CD4 T cell priming by LN-resident DCs. Mo-DCs direct CD4 T cell differentiation by regulating T-bet expression and have the capacity to enhance neutrophil-mediated conidial killing through undefined mechanism(s). (C) During intestinal helminth infections, Ly6C^{hi} monocyte-derived macrophages (Mo-Mac) promote inducible Treg development through the production of retinoic acid (RA) and dampen Th2 via PDL2-dependent mechanisms. (D) During acute toxoplasmosis, tissue-infiltrating Ly6C^{hi} monocytes dampen neutrophil-dependent ileitis and tissue damage upon exposure to commensal bacteria via induction of PGE₂ and IL-10. Ly6C^{hi} monocytes are the major cell type infected by *T. gondii* and mediate proinflammatory responses and parasite killing as well.

Table I

Bacterial, fungal, and parasitic diseases and important monocyte functions.

	Organism	Common Clinical Syndromes	Features of monocyte-mediated defense
Bacteria	<i>Listeria monocytogenes</i>	Gastroenteritis; bacteremia and meningitis in pregnant women, neonates, and immune compromised hosts	<ul style="list-style-type: none"> • Identification and functional characterization of “Tip-DCs” as monocyte-derived effector cells • Memory CD8 T cell and NK cell activation and tissue recruitment • CD4 T cell polarization
	<i>Mycobacterium tuberculosis (Mtb)</i>	Pneumonia; extrapulmonary disease following dissemination	<ul style="list-style-type: none"> • <i>Mtb</i> transport to lung-draining LNs • <i>Mtb</i> Ag transfer to LN DCs
	<i>Klebsiella pneumoniae</i>	Pneumonia; bacteremia in immune compromised patients	<ul style="list-style-type: none"> • Essential for pulmonary clearance of specific human clinical isolates
Fungi	<i>Aspergillus fumigatus</i>	Pneumonia and systemic disease in immune compromised hosts; Allergic disease in atopic hosts	<ul style="list-style-type: none"> • Transport of conidia to lung-draining LNs • Direct conidial killing • Innate immune crosstalk with neutrophils to regulate killing • Regulation of Th1/Th17 CD4 T cell differentiation
	<i>Blastomyces dermatitidis</i>	Pneumonia and mucocutaneous disease	<ul style="list-style-type: none"> • Monocyte recruitment subverted by fungus-induced chemokine proteolysis
	<i>Candida albicans</i>	Mucositis, dermatitis and vulvovaginitis; systemic disease primarily in immune compromised hosts	<ul style="list-style-type: none"> • Monocytes contribute to renal and CNS fungal clearance • Fungal Ag trafficking from mucosal sites
	<i>Cryptococcus neoformans</i>	Pneumonia and meningitis, usually in immune compromised hosts	<ul style="list-style-type: none"> • Monocyte-derived exudative macrophages protect hosts against serotype A strains
	<i>Histoplasma capsulatum</i>	Pneumonia and systemic disease in immune compromised hosts	<ul style="list-style-type: none"> • CCR2 signaling prevents detrimental T helper 2 cytokine environment in lung
	<i>Schistosoma spp.</i>	Acute and chronic schistosomiasis; Urinary tract or portal fibrosis is associated with tissue injury and organ failure	<ul style="list-style-type: none"> • Differentiate into macrophages with M2 phenotypic characteristics • Induction of Tregs and regulation of Th2 responses
Parasites	<i>Toxoplasma gondii</i>	Cervical lymphadenopathy, chorioretinitis, congenital toxoplasmosis; disseminated disease and encephalitis in immune compromised patients	<ul style="list-style-type: none"> • Parasite killing in the intestine • Regulation of neutrophil activation
	<i>Plasmodium sp.</i>	Malaria	<ul style="list-style-type: none"> • Blood stage parasite elimination

Table II

Common mouse strains and reagents to examine monocyte function during microbial challenge.

Strain or Reagent	Uses	Limitations	References
CCR2^(-/-)	<ul style="list-style-type: none"> Analysis of CCR2-dependent monocyte trafficking BM monocyte retention leads to loss of Mo-derived effector cells in target tissues during infections 	<ul style="list-style-type: none"> CCR2-independent monocyte functions cannot be investigated Partial phenotype (20–50% of circulating Ly6C^{hi} monocytes remain) 	[29, 30]
Anti-CCR2 Ab (clone MC-21)	<ul style="list-style-type: none"> Interferes with CCR2-dependent monocyte migration 	<ul style="list-style-type: none"> Does not target CCR2-independent Mo functions 	[31]
CCR2 Reporters	<ul style="list-style-type: none"> Fluorescent transgene expression controlled by CCR2 promoter Isolation of BM or circulating monocytes by negative selection for adoptive transfer studies Label Ly6C^{hi} monocytes more brightly than Ly6C^{lo} monocytes 	<ul style="list-style-type: none"> CCR2 expression in non-monocytic cells (subsets of NK and T cells) Transgene expression by myeloid progenitor cells (MDPs) that give rise to cDPs and to cMoPs 	[32–35]
CX3CR1 Reporter	<ul style="list-style-type: none"> GFP expression under control of the CX3CR1 promoter Label Ly6C^{lo} monocytes more brightly than Ly6C^{hi} monocytes 	<ul style="list-style-type: none"> CX3CR1 expression in NK cells, activated T cells, microglia, tissue-resident DCs, MDPs, and CDPs 	[36]
CCR2-DTR CX3CR1-DTR	<ul style="list-style-type: none"> Ablation of circulating monocytes upon DT administration Complementation experiments (using sorted DT-resistant Mo) can address specificity of infectious phenotypes in CCR2 or CX3CR1 deleter mice 	<ul style="list-style-type: none"> DT-induced ablation includes partial NK cell, splenic conventional DC, T cell subsets, and MDPs and derivative cells; notable upon repeated DT administration 	[32, 37]
LysM-Cre x CD115^{LSL-DTR} (MM^{DTR}) CX₃CR1-Cre x CD115^{LSL-DTR}	<ul style="list-style-type: none"> Ablation of circulating Mo and Mo-derived macrophages Improved specificity by intersectional gene targeting approach DT treatment does not appear to ablate splenic conventional DCs Cre activity can be monitored by mCherry expression 	<ul style="list-style-type: none"> Intersectional gene targeting approach increases murine husbandry Approach has not been adapted for Mo conditional gene targeting 	[22, 23]
CCR2-Cre-ERT2	<ul style="list-style-type: none"> Enables conditional gene targeting in Ly6C^{hi} monocytes ~90% recombinase activity in Ly6C^{hi} monocytes achieved with homozygous Cre-ER^{T2} Tg 	<ul style="list-style-type: none"> CCR2 expression by progenitor cells (e.g. MDPs) and non-monocytic cell populations 	[25]

Strain or Reagent	Uses	Limitations	References
CX3CR1-Cre CX3CR1-Cre-ERT2	<ul style="list-style-type: none"> Conditional and inducible monocyte gene deletion 	<ul style="list-style-type: none"> Constitutive Cre activity in Ly6C^{hi} Mo (~40%) and Ly6C^{lo} Mo (>90%) Tamoxifen induction leads to reduced Cre activity, particularly in Ly6C^{lo} Mo 	[4, 24, 38]
CD115-Cre CD115-mER-Cre mER	<ul style="list-style-type: none"> Conditional and inducible monocyte gene deletion 	<ul style="list-style-type: none"> Constitutive CD115-Cre targets Ly6G⁺ neutrophils and CD3⁺ lymphoid cells <i>In vivo</i> efficacy of inducible CD115-Cre targeting strategy needs to be defined for specific uses 	[39, 40]
LysM-Cre	<ul style="list-style-type: none"> Widely used for conditional gene deletion in monocytes and neutrophils, though specificity is problematic 	<ul style="list-style-type: none"> Cre transgene more active in neutrophils than Mo ~40–50% efficiency in circulating Ly6C^{hi} and Ly6C^{lo} monocytes 	[24, 41]
Clodronate liposomes	<ul style="list-style-type: none"> Intravenous injection transiently depletes circulating monocytes 	<ul style="list-style-type: none"> IV route targets splenic macrophages and other mononuclear phagocytes in contact with the vasculature 	[42, 43]
Anti-Ly6C/G Ab (clone RB6-8C5)	<ul style="list-style-type: none"> Widely used for <i>in vivo</i> neutrophil depletion studies Depletes circulating Ly6C^{hi} monocytes at high doses 	<ul style="list-style-type: none"> Low doses deplete only neutrophils but not Ly6C^{hi} monocytes At high doses, phenotypes attributed to neutrophil depletion may be due to Ly6C^{hi} monocyte depletion 	[44–47]
Nr4a19^{gfp-Cre} (Nur77)	<ul style="list-style-type: none"> Homozygotes for the Tg display a reduction in the frequency of circulating Ly6C^{lo} monocytes 	<ul style="list-style-type: none"> Nur77 is widely expressed in stromal, T cell, and myeloid cell populations 	[48]