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Immune regulation by monocytes

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ABSTRACT

Monocytes emerging from the bone marrow are the progenitors of monocyte-derived macrophages. An essential function of monocytes is to seed tissues with sufficient macrophages to replace loss from infection and tissue damage. Recent work from diverse inflammatory and homeostatic settings has shown monocytes also possess direct protective and pathogenic activities. Thus, monocytes are not simply needed to generate macrophages, but instead contribute to the overall orchestration of immunity. Some recently described properties of monocytes are both surprising and mechanistically specific; for example, inflammatory monocytes are required for the efficacy of transferred activated cytotoxic T cells, but can have potent tissue damaging effects while patrolling monocytes are required for anti-tumor immunity in some cases, but in another example provokes resistance to chemotherapy and thereby aid tumor growth. This summary will therefore focus on new findings about the regulatory activities of monocytes themselves.

1. Introduction

Over the last few years, the understanding of monocyte development in the bone marrow has advanced to the point where the specialized monocyte progenitors have been characterized and the cellular steps from the heterogeneous 'granulocyte-monocyte progenitor' or GMP pool are now amenable to definition with single cell resolution [1,2]. Furthermore, changes in the bone marrow monocyte progenitor pool following trauma, stress and infection have become clearer [3]. While two populations of circulating monocytes have been defined as 'inflammatory' or Ly6C^{hi} and 'patrolling' or Ly6C^{lo} (Fig. 1) [4], the relationship between these two populations has only recently been resolved in favor of a model where most, if not all patrolling monocytes, develop from Ly6C^{hi} cells rather than being a separate lineage that arises in the bone marrow (discussed below). For simplicity, the two main monocyte populations will be referred to throughout with restricted terms: 'inflammatory' monocytes or Ly6C^{hi} cells and 'patrolling' monocytes or Ly6C^{lo} cells. Fig. 1 provides further terms used in the literature to refer to the distinct populations in mice and humans. Furthermore, for simplicity this summary will refer exclusively to mouse monocytes as Zielgler-Heitbrock and colleagues have comprehensively summarized the properties of monocytes across species [5–7].

In addition to the advances in monocyte developmental biology, powerful tools are available to track and manipulate monocytes in living animals (Table 1). Collectively, the subject of bone marrow monocyte development has been covered extensively in the literature and will only be mentioned herein with the goal of articulating how monocyte development is associated with inflammation. In addition, the recent identification of splenic pools of monocytes poised to traffic to tissue damage and infection suggests an additional layer of complexity about monocytes exists [8]; however, tools to readily discriminate between circulating versus resident monocytes are not widely used, but will clearly be important for future dissection of monocyte biology.

Numerous immunoregulatory activities have been ascribed to inflammatory and patrolling monocytes and this review will thus focus on examples where monocytes provoke or inhibit immune and inflammatory responses. The activities of the progeny of monocytes, mature macrophages, have also been exhaustively summarized in the recent literature.

1.1. Experimental dissection of two controversial aspects of monocyte development

Ly6C^{lo} 'patrolling' monocytes are dependent on the orphan nuclear hormone receptor transcription factor NR4A1 (encoded by *Nr4a1* and also called Nur77) [9]. Ly6C^{lo} monocytes also require the transcription factor C/EBPβ [10]. However, the activity of C/EBPβ is required in many contexts in hematopoietic cells. In the absence of NR4A1, Ly6C^{lo} monocytes are almost absent from the blood. This observation led to the proposal that patrolling monocytes could develop from a bone marrow monocyte progenitor distinct from the source of Ly6C^{hi} monocytes [9,11]. Patrolling monocytes have a suite of functions distinct from Ly6C^{hi} monocytes as discussed later, and therefore the notion of distinct

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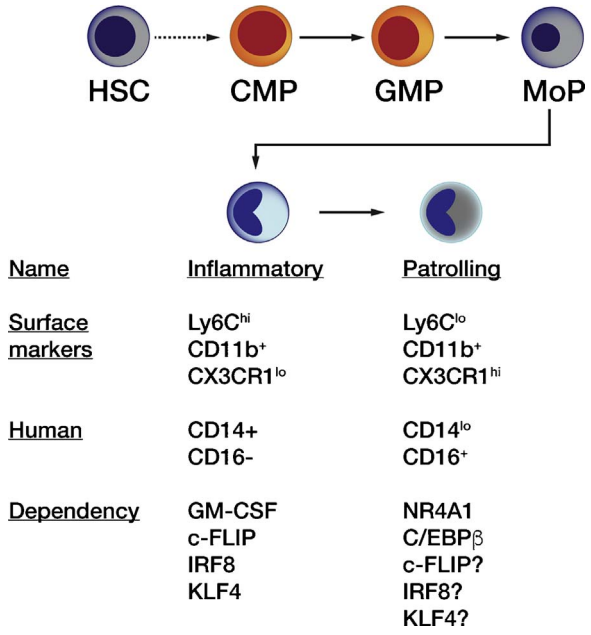

Fig. 1. Monocyte subsets: Monocytes develop from bone marrow progenitors consisting sequentially of the CMP (Common myeloid progenitor) then the GMP (granulocyte-monocyte progenitor) and finally the common monocyte progenitor (MoP) defined by Hettinger et al. [12]. The two main monocytes in mouse are shown as having a sequential relationship as defined by lineage tracing [13]. However, as noted in the text, the role of IRF8 and KLF8 makes this precise step-wise development unclear. Also shown are the known dependencies of inflammatory and patrolling monocytes defined by genetics.

monocyte developmental lineage was reasonable. We now know that the weight of evidence argues for a model where Ly6C^{lo} monocytes derive from Ly6C^{hi} monocytes rather than having a distinct ontogeny. Several lines of evidence have clarified the interconnections between the two types of monocytes. First, gene expression studies in monocytes derived from single cell sorted bone marrow monocyte progenitors demonstrate that *Nr4a1* expression occurs late in monocyte development, and after the expression of cardinal monocyte markers [12]. These data argue that *Nr4a1* expression is not unique to a distinct bone marrow monocyte progenitor. However, the most convincing data came from lineage tracing studies using an inducible fate tracker (Table 1) that showed how the Ly6C^{lo} monocyte pool first had to transition through the Ly6C^{hi} population [13]. Therefore, the simplest interpretation of these data is that the bone marrow produces Ly6C^{hi} monocytes (in steady state and inflammation) and that a sub-population of these cells downregulate Ly6C and upregulate *Nr4a1* (and C/EBPβ) coincident with the acquisition of the unique properties of patrolling monocytes. Ly6C^{lo} cells also upregulate the expression of Cx3CR1, a chemokine receptor, which can be useful to track populations enriched in patrolling monocytes, but is not specific for these cells (Table 1). A caveat to this model has recently been articulated by Hanna and colleagues [14] who pointed out that mice lacking the transcription factors IRF8 or KLF4 have severe defects in Ly6C^{hi} monocyte numbers but not Ly6C^{lo} cells [15,16], perhaps suggesting that another pathway exists to make one monocyte versus the other. KLF4 is upregulated during monocyte maturation while IRF8 is downregulated consistent with the idea that IRF8 is required at an earlier step in monocyte development while KLF4, NR4RA1 and C/EBPb function later in the steps that leads to patrolling monocyte development [12]. A counter-argument is that normal bone marrow myeloid development in the KLF4 and IRF8 is aberrant and cells with the properties of patrolling monocytes are made by a process unrelated to normal patrolling monocyte development. Perhaps in the future, more definitive experiments using mixed bone marrow chimeras and single cell methods will address the question of how IFR8 and KLF4 regulate the two different monocyte pools.

Left unanswered are three additional questions about normal patrolling monocyte development. First, is the transition process stochastic or instructive? In other words, is the development of patrolling monocytes programmed during development, or are distinct cues needed to enforce the developmental switch? One curious aspect of patrolling monocytes is that their circulating numbers are stable, suggesting mechanisms exist to set the number of cells with precision. A second question concerns the role of NR4A1. As mentioned above, the ligand of NR4A1, if it exists, is unknown because this nuclear hormone receptor has yet to be deorphanized. An intriguing possibility is that a ligand for NR4A1 might help instruct the patrolling monocyte developmental switch. Or, do patrolling monocytes represent a developmental endpoint and thus requires continuous new supply from the Ly6C^{hi} pool? Further use of fate mapping tools are a sound way to resolve this issue.

A second controversial aspect of monocyte development concerns

Table 1
[a]Genetic tools for monocyte investigation.

Gene	allele	Type	References	Notes
Ccr2	Complete knockout	Complete loss-of-function		Several groups have constructed loss-of-function alleles (Mouse Genome Informatics entry: MGI: 106185)
Ccr2	*Ccr2*^{RFP}	Knock-in Reporter allele	[67]	Reporter allele in heterozygosity
Ccr2	*Ccr2*^{GFP}	BAC transgene	[68,69]	Reporter allele in heterozygosity
Ccr2	*Ccr2*$^{Cre-ERT2}$	Not described	[70]	Inducible deletion of conditional alleles in CCR2-expressing cells; fate mapping tool
Ccr2	*Ccr2*^{DTR}	BAC transgene	[68,69]	Inducible deletion of CCR2-expressing cells by diphtheria toxin
Nr4a1	Complete knockout	Complete loss-of-function	[9]	Mice have near complete absence of patrolling monocytes
Nr4a1	*Nr4a1*^{GFP}	Knock-in Reporter allele into a BAC transgene	[71]	Originally developed to measure TCR signal strength
Cx3cr1	Complete knockout	Complete loss-of-function		Several groups have constructed loss-of-function alleles (Mouse Genome Informatics entry: MGI:1333815)
Cx3cr1	*Cx3cr1*^{GFP}	Knock-in Reporter allele	[72]	Reporter allele in heterozygosity
Cx3cr1	*Cx3cr1*$^{Cre-ERT2}$	Knock-in allele (Littman) or BAC allele (Jung)	[13,73]	Fate mapping tool or inducible deleter in Cx3CR1+ cells or of Cx3CR1+ cells when crossed to a *Rosa26* LoxP-STOP-LoxP-DTR mouse
Cx3cr1	*Cx3cr1*$^{STOP-DTR}$	Knock-in	[74]	Mice have DTR knocked into the *Cx3cr1* locus controlled by a STOP codon that must be removed by Cre-mediated excision
Cflar	*Cflar*$^{Flox/Flox}$	Conditional allele	[32,75,76]	When crossed to *Lyz2*^{CRE} (LysM^{CRE}), mice are devoid of monocytes and produce excess neutrophils
Klf4	Complete knockout and *Klf4*$^{Flox/Flox}$	Complete loss-of-function | Conditional allele	[15,77–79]	Several groups have constructed loss-of-function alleles (Mouse Genome Informatics entry: MGI:1342287)

[a] Information in the table does not claim to be comprehensive for all alleles made for the genes listed, not the potential usefulness in monocyte analysis.