International Immunopharmacology 11 (2011) 783–788

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Review

Myeloid cell population dynamics in healthy and tumor-bearing mice

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ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 2 March 2011
Accepted 3 March 2011
Available online 12 March 2011

Keywords:
Tumor
Myeloid cell
Macrophage
Chemokine
Migration
Myelopoiesis

ABSTRACT

Tumor growth is often associated with the aberrant systemic accumulation of myeloid-derived suppressor cells (MDSCs), which are a heterogenous population of cells composed of polymorphonuclear neutrophils, monocytes, macrophages, dendritic cells and early myeloid precursors. These MDSCs are thought to suppress anti-tumor T cell responses in both tumor tissues and secondary lymphoid tissues. Accumulation of MDSCs in these target tissues is a dynamic process associated with medullary and extramedullary myelopoiesis and subsequent cellular migration. Here, we review the current understanding of the cellular, molecular, hematological and anatomical principles of MDSC development and migration in tumor-bearing mice. We also discuss the therapeutic potential of chemokines that influence the balance between MDSC subpopulations.

Contents

1. Introduction

Myeloid cell subpopulations — polymorphonuclear neutrophils (PMNs), monocytes (MOs), macrophages (Mϕs; extravasated blood MOs are defined as Mϕs) and dendritic cells (DCs) — are key mediators of inflammatory and immune responses [1]. They share several common properties, namely phagocytic activity, immunophenotype, origin and relatively rapid turnover. However, they also have distinct roles in inflammatory responses: PMNs and Mϕs play an important role in the elimination of pathogens and components of damaged tissue via phagocytosis; PMNs amplify inflammation by releasing cytotoxic granules, whereas Mϕs terminate inflammation

Abbreviations: BrdU, 5-bromodeoxyuridine; CDP, common DC precursor; CFU-GM, colony-forming unit granulocyte-macrophage; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; DC, dendritic cell; GMP, granulocyte-macrophage progenitor; HEV, high endothelial venule; HSC, hematopoietic stem cell; LN, lymph node; Mϕ, macrophage; MDP, macrophage-DC progenitor; mDC, migratory DC; MDSC, myeloid-derived suppressor cell; MO, monocyte; MCP, monocyte chemoattractant protein; PCV, post capillary venule; pDC, plasmacytoid DC; PMN, polymorphonuclear neutrophil; rDC, resident DC; TipDC, TNFα/iNOS-producing DC.

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doi:10.1016/j.intimp.2011.03.003

and restore tissue integrity after removal of the inflammatory stimuli [2,3]. Myeloid cells also play an important role in adaptive immune responses; DCs activate antigen specific T cells during inflammatory responses, whereas PMNs, Mϕs and some immature myeloid cells suppress T cell responses, which has led to the concept of myeloid-derived suppressor cells (MDSCs) [4–7]. Balance between myeloid cell subpopulations is critical to achieve optimal immune responses and host defense.

Tumors are environments of unresolved inflammation initiated by malignant cells. During tumor development, MDSCs accumulate massively in both tumor and secondary lymphoid tissues, where they regulate inflammatory and immune responses [5–7]. Since the systemic accumulation of myeloid cells is often associated with poor prognosis and immunosuppression, MDSCs have attracted considerable interest as potential targets in the development of cancer therapies that may abrogate MDSC-mediated immunosuppression [8].

Tumor-induced MDSC accumulation is due to increased MDSC proliferation and differentiation in bone marrow, and subsequent migration from bone marrow to blood and from blood to target tissues [9]. This increased turnover of MDSCs is regulated by inflammatory mediators produced within the tumor, including cytokines, growth factors and chemokines. Since the molecular and cellular biology of MDSCs have been reviewed elsewhere [5–7], this review will focus on the turnover and trafficking of MDSCs *in vivo* from hematological and anatomical viewpoints.

2. Immunophenotype of MDSC subpopulations in mice

Immunophenotyping has been used to characterize MDSCs in tumor-bearing mice and in patients with cancer. Mouse MDSCs have been characterized as $CD11b^{+}Gr\text{-}1^{+}$ cells composed of PMNs, MOs/Mϕs, DCs and early myeloid precursors. Since the heterogeneity of the MDSC subpopulation often makes it difficult to interpret MDSC dynamics, we begin by characterizing mouse MDSC subpopulations into hematological classification according to their immunophenotype and cell morphology. The functional properties of the various myeloid cell subpopulations have been reviewed in detail elsewhere [5–7].

PMNs, a major subpopulation of MDSCs in bone marrow, blood and spleen, constitute about 25% of circulating leukocytes in specific pathogen free healthy male mice. Multi-color flow-cytometry analyses have enabled identification of mature PMNs with segmented or band nuclei as $SSC^{lo}lineage^{-}CD11b^{+}Gr\text{-}1^{hi}Ly\text{-}C^{lo}$ cells. “Lineage” positive or negative defines expression of CD3, CD19, CD49b, NK1.1 and Ter119. Exclusion of NK cells by a $lineage^{-}$ flow-cytometry gating is required to precisely identify myeloid cell populations, since NK cell subsets express CD11b and Ly-6 C [10,11]. The expression levels of Gr-1 in PMN lineage cells correlate with their maturation stage. Tumor development often results in accumulation of immature $Gr\text{-}1^{int}$ PMN lineage cells in the bone marrow and spleen. Recently, spleen $CD11b^{+}Gr\text{-}1^{int}$ cells in tumor-bearing mice, mainly comprising monocytes and myeloid precursors, have been reported to be capable of suppressing $CD8^{+}$ T cell activation [12]. However, cellular dominance within the $CD11b^{+}Gr\text{-}1^{int}$ cell population can shift from monocytes to immature PMN lineage cells as the tumor develops, and these subsets become difficult to distinguish by CD11b/Gr-1 expression. Additional markers such as Ly-6 G, Ly-6 C, CD115 or CX_3CR1 help to segregate these two independent cell lineages and to delineate the function of $CD11b^{+}Gr\text{-}1^{int}$ cells in mice at different stages of tumor development.

MOs/Mϕs, which predominate in the tumor-infiltrating MDSC subpopulation, constitute about 3% of circulating leukocytes in specific pathogen free healthy male mice. MOs are immunophenotypically identified as $SSC^{lo}lineage^{-}CD11c^{-}F4/80^{-}CD11b^{+}Gr\text{-}1^{lo}$ cells and can be further subdivided into $Ly\text{-}6C^{hi}$ classical “inflammatory” MOs and $Ly\text{-}6C^{int\ -\ low}$ non-classical “resident” MOs [13,14]. It has been reported that expression levels of chemokine receptor CCR2 and CX_3CR1 also distinguish $CCR2^{+}CX_3CR1^{int}$ inflammatory MOs and $CCR2^{-}CX_3CR1^{hi}$ resident MOs [15]. However, the narrow difference in the expression levels of CX_3CR1 between these two MO populations makes this a less useful marker of phenotype. PMNs and MOs are rarely detected in peripheral lymph nodes (LNs) under steady-state conditions, but increase slightly under inflammatory-conditions. Although a recent study demonstrated that *Trypanosoma brucei* infection-associated $CD11b^{+}Ly\text{-}6C^{+}CD11c^{+}$ inflammatory DCs are the main population of TipDCs and are derived from $CD11b^{+}Ly\text{-}6C^{+}$ MOs [16,17], the lineage relationship among inflammatory MOs, resident MOs, TipDCs [18] and inflammatory DCs [19] remain to be determined.

DCs are released from bone marrow as precursor cells, which are hardly detectable in the circulation. These precursor cells terminally differentiate into mature DCs after migration into peripheral tissues [14,20]. Recent findings on the function, progenitors, life-cycle and molecular dependency of DC subsets in mice have revealed that mouse LNs contain type-I interferon-producing plasmacytoid DCs (pDCs) and two types of potent antigen presenting DC subsets; tissue-derived migratory DCs (mDCs) and blood-derived resident DCs (rDCs) [20]. Immunophenotypically, pDCs are $lineage^{-}CD11c^{low}Siglec\text{-}H^{+}$. CD317/PDCA1 is a useful marker of pDCs under steady-state conditions but is upregulated on various cell types under inflammatory-conditions. mDCs and rDCs can be distinguished by CD11c and MHC class II expression levels; mDCs are $lineage^{-}Siglec\text{-}H^{-}CD11c^{low}MHC\ II^{hi}$, and rDCs are $lineage^{-}Siglec\text{-}H^{-}CD11c^{hi}MHC\ II^{int}$. rDCs can be further subdivided into $CD8\alpha^{+}$ and $CD8\alpha^{-}$ subsets, although the functional significance of these subsets is still controversial. Since mDCs and a proportion of $CD8\alpha^{-}$ rDCs express CD11b, a $CD11c^{-}$ gate is required for identification of Mϕs in peripheral lymphoid tissues.

Importantly, classification of cells by their immunophenotype should reflect distinct cell lineages with distinct life-cycles under the control of specific transcription factors, cytokines, growth factors, and chemokines.

3. Origin and development of MDSC subpopulations

With the exception of tissue-resident cells such as brain microglia and epidermal Langerhans cells, which are locally maintained under steady-state conditions, PMNs, MOs/Mϕs and DCs in peripheral tissues develop from myeloid progenitors by a process called myelopoiesis. Development of PMNs or MOs specifically is referred to as granulopoiesis or monopoiesis, respectively. In this section, we discuss the developmental pathways of myeloid cells in bone marrow under steady-state conditions, and tumor-induced medullary and extramedullary myelopoiesis.

3.1. Myeloid progenitors and medullary myelopoiesis

Recent advances in immunophenotyping have revealed a developmental pathway of myeloid cells from hematopoietic stem cells (HSCs) [21–23]. In mice, primitive HSCs give rise to $CD34^{+}Fc\gamma RII/III^{lo}$ $Sca\text{-}1^{-}c\text{-}kit^{+}lineage^{-}$ common myeloid progenitors (CMPs) and $lineage^{-}IL\text{-}7R^{+}Flt3^{+}$ common lymphoid progenitors (CLPs). $CD34^{+}Fc\gamma RII/III^{hi}$ $Sca\text{-}1^{-}c\text{-}kit^{+}lineage^{-}$ granulocyte-macrophage progenitors (GMP) are descended from CMPs. Importantly, these immunophenotypic classifications of myeloid progenitors are not always applicable to inflammatory conditions, since inflammatory mediators such as IFNs directly induce expression of the Sca-1 antigen on various cell types [24,25].

Recently, $lineage^{-}c\text{-}kit^{+}CX_3CR1^{+}$ or $lineage^{-}CD115^{+}$ macrophage-DC progenitors (MDPs) were identified as common progenitors for MOs/Mϕs and DCs, although the immunophenotype of MDPs overlaps those of CMPs and GMPs. MDPs give rise to MOs and common DC precursors (CDP) [14,20]. The developmental pathway and lineage