Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

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

Review article

Origin and production of inflammatory perivascular macrophages in pulmonary hypertension

Jonathan Florentin[a], Partha Dutta[a,b,*]

[a] Center for Pulmonary Vascular Biology and Medicine, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, Division of Cardiology, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

ARTICLE INFO

Keywords:
Alveolar macrophages
Interstitial macrophages
Inflammation
Pulmonary hypertension
HSC progenitors

ABSTRACT

Myeloid cells, including monocytes and macrophages participate in steady state immune homeostasis and help mount the adaptive immune response during infection. The function and production of these cells in sterile inflammation, such as pulmonary hypertension (PH), is understudied. Emerging data indicate that pulmonary inflammation mediated by lung perivascular macrophages is a key pathogenic driver of pulmonary remodeling leading to increased right ventricular systolic pressure (RVSP). However, the origin of these macrophages in pulmonary inflammation is unknown. Inflammatory monocytes, the precursors of pathogenic macrophages, are derived from hematopoietic stem and progenitor cells (HSPC) in the bone marrow and spleen during acute and chronic inflammation. Understanding the role of these organs in monocytopoiesis, and the mechanisms of HSPC proliferation and differentiation in PH are important to discover therapeutic targets curbing inflammation. This review will summarize the current limited knowledge of the origin of lung macrophage subsets and over-production of inflammatory monocytes in PH.

1. Introduction

PH is a progressive cardiopulmonary disease leading to right ventricular failure, multi-organ dysfunction and often death. The vascular manifestations of pH are associated with cellular and soluble inflammatory mediators [1–3]. There is growing appreciation that pulmonary vascular inflammation, involving multiple immune cells, plays a key role in the development and progression of vascular remodeling in PH [4–6]. This review will focus on the relevance of myeloid cells, especially monocytes and macrophages in PH and will shed light on the recent related discoveries.

Monocytes constitute an important part of the innate immune system. They are generated by hematopoietic progenitor cells, which are located in hematopoietic organs such as bone marrow and spleen. In mice, monocytes fall into two main subsets: proinflammatory Ly-6C^{high} Cx$_3$cr1^{low} [7] and patrolling Ly-6C^{low} Cx$_3$cr1^{high} [8]. Humans have three distinct monocyte subsets: CD14^{high} CD16^{low} (inflammatory, classical), CD14^{high} CD16^{int} (intermediate), and CD14^{int} CD16^{high} (patrolling, non-classical) [9]. Monocytes can trigger inflammation in various diseases. Human classical and intermediate monocytes are thought to be inflammatory [10–12]. However, in some inflammatory diseases, human non-classical monocytes have been shown to have pro-inflammatory features [13–15]. Chemokines, such as CX$_3$CL1 and CCL2, help monocytes egress from the bone marrow and migrate to tissues where they help orchestrate adaptive immune responses through their role of antigen presentation. Once in injured tissues, they may differentiate into macrophages and dendritic cells. However, this notion has been challenged by a recent study [16] reporting that there is a minimum differentiation of monocytes as they survey tissues and carry antigens to lymph nodes. Macrophages are phagocytic cells that participate in the clearance of tissue debris and foreign materials, and tissue homeostasis. Recent evidences showed the importance of macrophages in aggravating inflammation in PH.

2. Origin of monocytes and macrophages

Monocytes in adult mammals are produced from hematopoietic stem cells (HSC) in hematopoietic organs such as bone marrow (BM) and spleen. However, most tissue resident macrophages are not derived from HSC in the steady state. Ontogenically, macrophages can be divided in two classes: embryo- and monocyte-derived macrophages. Tissue resident macrophages, such as microglia, Kupffer cells, and peritoneal, splenic and lung macrophages, arise from two successive steps of hematopoiesis: primitive and definitive hematopoiesis during fetal period [17–19]. During primitive hematopoiesis, which occurs in

* Corresponding author.
E-mail address: duttapa@pitt.edu (P. Dutta).

http://dx.doi.org/10.1016/j.cyto.2017.08.015
Received 15 August 2017; Accepted 19 August 2017

early embryonic stage, erythroid myeloid progenitors from the yolk sac seed different organs and can differentiate into macrophages without intermediate monocyte stage [16,20]. These cells self-maintain by proliferation and perform homeostatic functions in tissues at steady state [21]. During later embryonic stage, macrophages can be produced by fetal liver monocytes. Seeding of fetal liver monocytes in different tissues depends on plasmalemmal vesicle-associated protein [22]. Macrophages can also be derived from bone marrow-derived monocytes at steady state. Monocytes are continuously recruited into organs, such as the intestine [23], that have high macrophage turnover. Once in tissues, monocytes differentiate into macrophages [24]. It is likely that the origin of macrophages depends on the availability of niches [23]. For example, yolk sac-derived erythroid myeloid progenitors seed the brain during embryonic day 8 when the blood brain barrier is not established and differentiate into microglia. Moreover, during inflammatory processes that compromise the blood brain barrier, the local demand of myeloid cells especially macrophages is high and tissue resident macrophages disappear due to their increased apoptosis, resulting in empty macrophage niches. To fill the empty niches, monocytes rapidly egress from the bone marrow and are recruited to inflamed tissues where they can differentiate into macrophages. Various reports showed that human classical monocytes or their murine counterpart Ly-6C^{high} monocytes are the source of macrophages in pathological context such as cancer [25,26], atherosclerosis [27] and metabolic diseases [28]. Embryonic macrophages can be slowly replaced by circulating monocytes at steady state [29]. Recent studies indicate that, irrespective of the origin of macrophages, their phenotype and function depend on the local environment where they reside [30,31].

3. Function and origin of lung macrophages

The lungs consist of mainly two kinds of macrophages: interstitial macrophages and alveolar macrophages. Alveolar macrophages are found in alveoli lumen. They participate in local immune homeostasis and have certain surfactant properties [32,33]. Additionally, alveolar macrophages are important cellular assets to protect the lungs from viral infections such as Influenza [34]. Interstitial macrophages reside in lung interstitium where they interact with interstitial T lymphocytes and trigger an adaptive immune response [35–37] and are considered as antigen presenting cells in the lungs [38].

Initial histological and immunohistochemical studies showed that embryonic lung macrophages derived from the yolk sac sequentially differentiate into interstitial macrophages and then alveolar macrophages after birth [39–43]. Alveolar macrophages are renewed by patrolling monocytes with interstitial macrophages as intermediates. However, this theory of alveolar macrophage development has been challenged by recent studies using cell fate mapping and parabiosis experiments. These studies provide evidence that alveolar macrophages can divide and self-renew without being replaced by circulating monocytes in the steady state [44–47]. Further adoptive transfer and lineage tracing experiments show that alveolar macrophages are derived from fetal liver monocytes, not yolk sac [48,2]. PPAR-γ expression, on fetal liver monocytes, driven by GM-CSF in embryonic lungs is important for the development of alveolar macrophages [49].

It has been shown that interstitial macrophages are derived from blood monocytes at steady state [50]. However, the origin of the lung macrophage subsets in inflammatory conditions is not well understood. Recently, Misharin et al. observed a novel population of alveolar macrophages, which are derived from blood monocytes in the context of pulmonary fibrosis [51]. This population of monocyte-derived alveolar macrophages coexists with embryonic alveolar macrophages and express high levels of pro-fibrotic genes. Monocyte-derived alveolar macrophages persist in the lungs long after the resolution of fibrosis and gradually become similar to lung-resident macrophage subset. Besides fibrosis, interstitial and alveolar macrophages play a major role in lung inflammation [35] and function [35,52–55]. Moreover, their interplay is relevant in the context of pH. In line with this, Kurt Stenmark's group recently showed that, in a hypoxic mouse model of pH, activation and expansion of lung alveolar and interstitial macrophages was time and space dependent [56].

4. Importance of bone marrow-derived cells in PH pathogenesis

Plexiform lesions of the vasculature of pH patients contain markers of inflammation [57,58]. Monocytes and macrophages are the main effector cells contributing to local lung inflammation in the context of pH [59–61]. Recruitment of immune cells to the lungs contributes to vascular destruction and remodeling in the pathogenesis of chronic lung diseases, such as PH [62,63]. In response to stimuli, endothelial cells overproduce chemokines such as fractalkine (CX_3CL1) [64–69]. This triggers the attachment of leukocytes expressing CX_3CR1, the CX_3CL1 receptor, to lung endothelial cells [69–73]. The importance of monocyte infiltration into the lungs in PH is relatively understudied. Recently, Amsellem et al. showed that Cx_3cr1 plays a major role in hypoxia-induced PH by modulating monocyte recruitment, macrophage polarization and smooth muscle cell proliferation in the lungs [74]. Congruently, *cx$_3$cr1*-deficient mice had an attenuated PH pathogenesis. Consistent with the importance of bone marrow-derived leukocytes in PH pathogenesis, a recent study has shown that TGF-β activation in the lungs by hematopoietic cell-derived thrombospondin-1 triggers *Schistosoma mansoni*-induced PH [75]. Prior work has suggested that expansion of lung macrophages in PH is dependent on circulating progenitors originated in the bone marrow [2,76,77]. However, the contribution of hematopoietic stem cells (HSCs) in the origin of inflammatory perivascular lung macrophages in pulmonary hypertension is unknown.

5. Generation of inflammatory myeloid cells in the bone marrow

HSCs are the source of myeloid cells. At steady state, most HSCs are quiescent as only approximately 5% of them are in the cell cycle [78]. However, stress can induce HSC proliferation and differentiation into hematopoietic progenitor cells [79], which can further differentiate into mature hematopoietic cells. Multiple studies reported that circulating alarmins, such as interferons, secreted during infection or injury drive quiescent HSCs into cell cycle [80–82]. However, how HSCs sense danger still remains unclear. This is an interesting question in the context of cardiovascular diseases since we previously showed that myocardial infarction leads to HSC activation and differentiation [83,84]. HSCs express high levels of pattern recognition receptors (PRR), such as Toll-like receptors, which help them sense danger [85]. When HSCs are stimulated with ligands for Toll-like receptors, they enter the cell cycle and differentiate into committed progenitors such as granulocyte and monocyte progenitors (GMP) [86], which are precursors of myeloid cells. Any alteration in bone marrow microenvironment can also affect HSC quiescence. In the context of myocardial infarction, decreased levels of HSC retention factors, such as Cxcl12, Scf, Ang-1 and Vcam-1, result in increased HSC proliferation and release from their niches. These retention factors are secreted by bone marrow niche cells, such as macrophages [87,88], regulatory T cells [89], endothelial cells [90,91] and CXCL12-abundant reticular cells [92], which are located in hematopoietic tissue in close proximity to HSCs and hematopoietic progenitors. Retention factors secreted by these niche cells maintain the hematopoietic niche, and regulate HSC proliferation and differentiation (Fig. 1). Subtle modifications in HSC niche in disease conditions alter hematopoiesis. For example, in the context of atherosclerosis, high levels of G-CSF suppress CXCL12 production by osteoblasts, leading to HSC proliferation and release [93,94]. As discussed above, multiple studies reported blood and lung monocytosis in the context of pH [61,74,75]. The blood monocyte expansion depends on their generation in the bone marrow from hematopoietic stem and progenitor cells. Indeed, immunodeficient mice