Journal of Immunological Methods 403 (2014) 7–16

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Temporal and spatial characterization of mononuclear phagocytes in circulating, lung alveolar and interstitial compartments in a mouse model of bleomycin-induced pulmonary injury

Wen-Jie Ji [a,b,*,1], Yong-Qiang Ma [a,b,1], Xin Zhou [b,c], Yi-Dan Zhang [a,b], Rui-Yi Lu [b,c], Hai-Ying Sun [b,c], Zhao-Zeng Guo [b,c], Zhuoli Zhang [d], Yu-Ming Li [b,c], Lu-Qing Wei [a,**]

[a] Department of Respiratory and Critical Care Medicine, Pingjin Hospital, Logistics University of the Chinese People's Armed Police Forces, 220, Cheng-Lin Road, Tianjin 300162, China
[b] Tianjin Key Laboratory of Cardiovascular Remodeling and Target Organ Injury, 220, Cheng-Lin Road, Tianjin 300162, China
[c] Institute of Cardiovascular Disease and Heart Center, Pingjin Hospital, Logistics University of the Chinese People's Armed Police Forces, 220, Cheng-Lin Road, Tianjin 300162, China
[d] Department of Radiology, Northwestern University, 737 N. Michigan Ave, Chicago, IL 60611, USA

ARTICLE INFO

Article history:
Received 31 August 2013
Received in revised form 11 October 2013
Accepted 18 November 2013
Available online 23 November 2013

Keywords:
Pulmonary fibrosis
Monocyte subsets
Macrophage polarization
Lung macrophage
Bleomycin

ABSTRACT

The mononuclear phagocyte system, including circulating monocytes and tissue resident macrophages, plays an important role in acute lung injury and fibrosis. The detailed dynamic changes of mononuclear phagocytes in the circulating, lung alveolar and interstitial compartments in bleomycin-induced pulmonary injury model have not been fully characterized. The present study was designed to address this issue and analyzed their relationships with pulmonary pathological evolution after bleomycin challenge. A total of 100 male C57BL/6 mice were randomly divided to receive bleomycin (2.5 mg/kg, $n = 50$) or normal saline ($n = 50$) via oropharyngeal approach, and were sacrificed on days 1, 3, 7, 14 and 21. Circulating monocyte subsets, polarization state of bronchoalveolar lavage fluid (BALF)-derived alveolar macrophages (AMφ) and lung interstitial macrophages (IMφ, derived from enzymatically digested lung tissue) were analyzed by flow cytometry. There was a rapid expansion of circulating Ly6C^{hi} monocytes which peaked on day 3, and its magnitude was positively associated with pulmonary inflammatory response. Moreover, an expansion of M2-like AMφ (F4/80+CD11c+CD206+) peaked on day 14, and was positively correlated with the magnitude of lung fibrosis. The polarization state of IMφ remained relatively stable in the early- and mid-stage after bleomycin challenge, expect for an increase of M2-like (F4/80+CD11c−CD206+) IMφ on day 21. These results support the notion that there is a Ly6C^{hi}-monocyte-directed pulmonary AMφ alternative activation. Our result provides a dynamic view of mononuclear phagocyte change in three compartments after bleomycin challenge, which is relevant for designing new treatment strategies targeting mononuclear phagocytes in this model.

Abbreviations: BLM, bleomycin; NS, normal saline; IS, inflammation score; CVF, collagen volume fraction; Hyp, hydroxyproline; FSC, forward-scattered light; SSC, side-scattered light; AMφ, alveolar macrophage; IMφ, interstitial macrophage.

* Correspondence to: W. J. Ji, Department of Respiratory and Critical Care Medicine, Tianjin Key Laboratory of Cardiovascular Remodeling and Target Organ Injury, Pingjin Hospital, Logistics University of the Chinese People's Armed Police Forces, 220, Cheng-Lin Road, Tianjin 300162, China. Tel.: +86 022 60578746; fax: +86 022 24716466.
** Correspondence author. Tel.: +86 022 60577559; fax: +86 022 24716466.
E-mail addresses: wenjieji@gmail.com (W.-J. Ji), luqing-wei@163.com (L.-Q. Wei).
[1] Authors have contributed equally to this work.

0022-1759/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jim.2013.11.012

1. Introduction

The mononuclear phagocytes are originated from bone-marrow-derived myeloid cells, which then circulate in the blood as monocytes and populate tissues as macrophages (Mφ) in the steady state and during inflammation (Geissmann et al., 2010). It is generally acknowledged that mouse monocytes can be divided into two subpopulations: the inflammatory monocytes ($Ly6C^{hi}$) exhibiting a strong capacity of phagocytosis and chemotaxis, and the anti-inflammatory monocytes ($Ly6C^{lo}$) participating in extracellular matrix remodeling and tissue repair (Gordon and Taylor, 2005; Wynn et al., 2013). Mounting evidence revealed that during inflammation, $Ly6C^{hi}$ monocytes are recruited and differentiate into Mφ with functional diversity at the site of the inflammatory lesion.

Tissue Mφ plays an essential role in the innate immune response in the lung (Misharin et al., 2011; Schneberger et al., 2011). Based on the anatomical locations in the lung, there are two broad subpopulations of Mφ, i.e., alveolar Mφ (AMφ) and interstitial Mφ (IMφ). The function of AMφ is to remove particulates and microorganisms from the alveolar space, and interstitial Mφ (IMφ) might have a role in limiting inflammation, fibrosis and antigen presentation (Tschernig and Pabst, 2009; Schneberger et al., 2011). Furthermore, the activation state of Mφ can be generally characterized as classically activated Mφ (M1 polarization) that are associated with a Th1 immune response, or alternatively activated Mφ (M2 polarization) that are associated with Th2 immune response. In lung tissue, M1-like Mφ are the first to respond to lung injury and are later replaced by M2-like Mφ that contribute to tissue repair and fibrosis (Misharin et al., 2011).

Mice challenged with bleomycin are the most widely used pulmonary injury and fibrosis model and can provide useful insights into the biology of lung injury and fibrosis (Moore and Hogaboam, 2008). Up to now, the detailed kinetics of mononuclear phagocytes in the circulating, lung alveolar and interstitial compartments has not been fully characterized in this model. Therefore, in the present study, we sought to address this issue by serial flow cytometry analyses and explored their relationships with pulmonary pathological evolution.

2. Materials and methods

2.1. Reagents

Bleomycin A5 (BLM) was obtained from Taihe Pharmaceutical Co. Ltd (Tianjin, China). Fluorescein isothiocyanate (FITC)-conjugated anti-mouse Ly6C (clone HK1.4), phycoerythrin (PE)-conjugated anti-mouse CD11b (clone M1/70) and CD206 (clone C068C2), PE/Cy5-conjugated anti-mouse F4/80 (clone BM8), PE/Cy7-conjugated anti-mouse CD11c (clone N418), and their respective isotype controls (Rat IgG2c-FITC, Rat IgG2b-PE, Rat IgG2a-PE, Rat IgG2a-PE/Cy5, Armenian Hamster IgG-PE/Cy7) were purchased from Biolegend (San Diego, CA, USA).

2.2. Animals

C57BL/6 male mice (8–10 weeks, 18–20 g) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). Animals received humane care in compliance with the Regulations for Management of Experimental Animals (Tianjin Municipal Science and Technology Commission, revised June 2004) which was in accordance with Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Pub. no. 85-23, revised 1996). All experimental procedures were performed with the authorization of the Animal Use and Care Committee of the Logistics University of the Chinese People's Armed Police Forces.

2.3. Model of bleomycin-induced lung injury

A total of 100 mice were used in the present work. Mice were lightly anesthetized with ether (inhalation for 30 s to 1 min) and followed by oropharyngeal installation of BLM 40 μL (2.5 mg/kg, BLM group, $n = 50$) or sterile saline 40 μL (normal saline, NS group, $n = 50$) as described previously (Lakatos et al., 2006). On days 1 (1 day after operation), 3, 7, 14 and 21 after bleomycin/saline treatment, animals were euthanized by exsanguinations while under an overdose of pentobarbital sodium (100 mg/kg, $n = 10$ for each group at each time point). Their blood, bronchoalveolar lavage fluid (BALF) and lung tissue were collected for the following assays.

2.4. Pathological examinations of lung

The left lobe from non-lavaged lung was fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.2–7.4) for 24 h. Fixed lungs were embedded in paraffin, and sequential 5 μm sections were stained with hematoxylin eosin (HE) and Masson's trichrome. The severity of alveolitis was blindly determined using the HE-stained sections according to previously published criteria (Szapiel et al., 1979). Briefly, each lung section was systematically scanned under a magnification of ×100; five successive fields were graded according to the degree of inflammatory infiltration and the area of involved lesions: grade 0, normal tissue; grades 1–3, the presence of pulmonary inflammation with the extent of pathology graded as 1 (<20% of the slide), 2 (20% to 50% of the slide) or 3 (>50% of the slide). After examination of the entire section, the mean score from all examined fields was calculated as the inflammation score (IS). A semi-quantitative assessment for pulmonary fibrosis was performed by calculating collagen volume fraction (CVF) using Masson-stained sections by the Image Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA). Five fields were randomly selected from each section under a magnification of ×200.

2.5. BALF analysis

The BALF was collected through an intratracheal cannula with three sequential 1 mL of 0.9% sterile saline and centrifuged at 300 *g* for 10 min at 4 °C. The cell-free supernatant was stored at −80 °C for analysis of cytokines. The cell pellet was resuspended in 0.9% sterile saline for total cell counts, differential cell counts and flow cytometry analysis.