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Increased density of macrophage migration inhibitory factor (MIF) in tuberculosis granuloma

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ABSTRACT

Granulomas, the pathologic hallmarks of tuberculosis, are composed of tightly numerous immune cells that respond to a variety of persistent stimuli during pathogen–host interaction. The granuloma is essential for host containment of mycobacterial infection, however, the mechanism of host and pathogen determinants to recruit immune cells at the site of inflammation and the formation of granulomas remains elusive until now. Macrophage migration inhibitory factor (MIF), a cytokine produced by many cell types, modulates cellular and humoral immune responses and promote lymphocytes migration to the site of infection. In this study, we evaluate the expression of MIF in tuberculous granulomas by three different models of diseases: mouse, human tissues and zebrafish. The overall results demonstrated that the expression of MIF positive signals markedly increased in the tissues which have been infected with mycobacterium, whereas a few presence of MIF in the PBS-treated animals (means the control group). In the mycobacterial-infected animals, the MIF positives distributed extensively within the granuloma especially in the multinucleated giant cells. Thus, three independent lines of evidence support the hypothesis that MIF may be an important player in aggregate immune cells to the granuloma microenvironments in these animal models of tuberculosis.

Introduction

Tuberculosis (TB), one of the major health threats over the world until now, accounts for the annual death of 2 million individuals. Granulomas, the hallmark of tuberculosis, were organized by numerous immune cell aggregates (Adams, 1976). Traditionally, the tuberculosis granuloma have been thought to be essential for host to contain mycobacterial dissemination, however, the granuloma remains enigmatic, particularly the relative contributions of host and pathogen determinants to the migration and aggregation of immune cells and the formation of granulomas is not well understood.

Macrophage migration inhibitory factor (MIF), originally described in 1966 as a factor produced by activated T lymphocytes that exhibited macrophages-regulation properties (Bloom and Bennett, 1966; David, 1966). For a long time, MIF has been considered as a mysterious cytokine due to its biological activities and origins. Recently, MIF has been characterized as a multifunctional biological cytokine, an enzyme or a hormone (Swope and Lolis, 1999). Indeed, some obtained results demonstrated that MIF plays a major role in the regulation of glucocorticoid-mediated cytokine production (Calandra et al., 1995). In recent years, it has been proofed that MIF is an integral component of the host immune system that promotes the pro-inflammatory functions of immune cells (Calandra and Roger, 2003). More recently, some experiments revealed that MIF is essential for normal embryonic development and that it functions as a growth factor for the proliferation and differentiation of embryonic tissues in vertebrates, such as rodents, chicken lens and zebrafish (Kobayashi et al., 1999; Suzuki et al., 1996; Wistow et al., 1993). Furthermore, an increasing numerous studies of MIF from animal models and human diseases demonstrated that MIF is an important mediator of pathogenesis, such as delayed-type hypersensitivity (Bernhagen et al., 1996), adjuvant and antigen-induced arthritis (Leech et al., 1998; Mikulowska et al., 1997), acute lung injury (Makita et al., 1998), adult respiratory distress syndrome, asthma, tuberculosis (Lai et al., 2003; Rossi et al., 1998; Yamada et al., 2002a; Yamaguchi et al., 2000).

Tuberculosis granuloma, the numerous macrophage aggregates originated from pathogen–host interplaying during mycobacterium infection, however, its detailed formation process and role still greatly appeal to the biomedical community. MIF, the first reported cytokine inhibits the random migration of macrophages and promotes the delayed-type hypersensitivity reaction. Importantly, Yamada et al.

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demonstrated an elevated level of serum MIF in pulmonary tuberculosis patients (Yamada et al., 2002a). However, there is no report whether MIF is involved in the tuberculosis granuloma formation until now. Thus, the goal of the current study was to evaluate the kinetics of MIF during granuloma formation using mice and zebrafish infection models and tissues from tuberculosis patients.

Materials and methods

Collection of patient tissues

16 adult tissues samples were collected undergoing surgery at Shanghai Public Health Clinical Center affiliated to Fudan University. All the patients were diagnosed on the basis of a combination of radiologic, endoscopic, histologic, and clinical guidelines, including response to treatment. Formalin-fixed, paraffin-embedded diagnostic lymph node biopsies were collected from the files of the Department of Pathology, Shanghai Public Health Clinical Center. The study was approved by the Fudan University Ethical Review Committee.

Mouse aerosol infection with H37Rv

Twenty-eight SPF female C57BL/6 mice aged 6 to 8 weeks were purchased from Sino-British Sippr/BK lab animal Ltd., Co (SCXK(Hu) 2008-0016). The animals were housed in a sterile cage under biosafety-level-3 (BSL-3) conditions at the Shanghai Public Health Clinical Center (No. CNASBL0016), Shanghai, China. Experimental animals will be provided special feed and sterile water ad libitum. The mice were randomly divided into two groups: 20 mice in the H37Rv-infected group and 8 mice as the PBS control. Mice were infected by atomizing with an aerosol generator (Glas-Col Inc. Terre Haute, IN, USA). The H37Rv strains were cultured to mid-log phase (optical density at 600 nm of 0.5 to 1.0). The cultures were washed one time and resuspended in phosphate-buffered saline (PBS) with 0.05% Tween 80 (PBST) to a concentration of 1×10^7 CFU/ml. The bacterial suspension was placed into the nebulizer jar of a whole-body exposure aerosol chamber and mice were exposed for 30 min. We expect that these conditions would lead to about 100 CFU of bacteria in each animal. Two animals in each group will be sacrificed 1 day after infection to confirm that 100 CFU of bacteria was inhaled. Detailed documentation were maintained to record the mental status of the mice in each group over the eight weeks course of the experiment. Five mice from the H37Rv-infected group and two mice from the control group, were randomly sampled on weeks 4, 6 and 8. Tissue samples of lung, liver and spleen were collected and fixed in 4% paraformaldehyde solution immediately after mice were weighed and killed. This study will be approved by the Institutional Animal Care and Use Committee of Shanghai Public Health Clinical Center.

Zebrafish infection model with Mycobacterium marinum (M. marinum)

M. marinum M strain (ATCC BAA-535) was kindly provided by Dr. L. Ramakrishnan (University of Washington, Seattle). Adult wild-type AB zebrafish (*Danio rerio*) were originally obtained from Institute of Brain Science of Fudan University. The attenuated strain 05A3 and its complementation, and the zebrafish infection model were established as our previously described (Yu et al., 2011). All animal work was approved by the ethics committee of Fudan University.

Histology and MIF immunohistochemistry of patient, murine and zebrafish samples

For all tissue samples, 5 μm serial paraffin sections were prepared and immersed in three consecutive washings in xylol for 5 min to remove paraffin, and then hydrated with five consecutive washings with alcohol 100, 95, 80, 70% and PBS, respectively. All the sections were subsequentially processed by hematoxylin–eosin and Ziehl–Neelsen for detection of histopathology and mycobacterium distribution. For detection of MIF expression, paraffin sections (5 μm) were dewaxed in xylene, rehydrated in a series of ethanol washes, and the following steps were carried out in a moist chamber. Sections were coated with blocking buffer (Zymed Laboratories Inc., San Diego, USA) containing 20% normal donor bovine serum (Gibco) and 80% PBS (0.01 M, pH 7.4) at room temperature for 30 min. Then we discarded the bovine serum and incubated the sections with MIF rabbit-anti-rat polyclonal antibody (Invitrogen, Co., USA) at 4 °C overnight. The MIF pAB was used at a dilution of 1:2000 in PBS (0.01 M PBS, pH 7.4), which gave optimal staining results. The following steps were performed as our established protocols. In parallel, the tissue specimens in which the primary antibody was replaced by PBS served as negative controls. Specificity was established by demonstrating the loss of immunoreactivity in matched tissue sections.

The number of MIF positives in mice and zebrafish was counted in 10 fields of view under the microscope (40× magnification) and the means calculated. Sampling of the sections was unbiased, with the samples coded and examinations performed by one investigator. The area density of MIF-positive cells in the mice and zebrafish was measured using the Motic Images 2000 (Motic China Group Co., China). The results were expressed as area density (area of the MIF positives/area of the whole field).

Statistical analysis

Experimental data were analyzed by one-way analysis using GraphPad PRISM statistical program (Graph Pad Software, Inc.; USA), version 4.0. The results were expressed as means and standard errors. Differences were considered significant at $p<0.05$ or $p<0.01$.

Results

High homology of MIF in NCBI database

The homology of protein and nucleotide sequence of MIF between human and mouse are 88% and 85%, respectively. Even in the zebrafish, the identity of amino acid is 69%. All the data from NCBI database demonstrated that MIF is highly conserved protein among different species.

MIF expression in patient tissues

Firstly, we analyzed the expression of MIF in biopsy samples of tuberculosis patients. For this purpose, paraffin-embedded patient lymph node biopsies were collected according to the following strict inclusion criteria: (a) a clinically suspected infectious process, (b) the histopathological diagnosis of an infection (no malignancy, no autoimmunological disease) and (c) a microbiologically positive laboratory result confirming the presence of specific pathogens in the lesions. In total, 16 tuberculosis patient's samples were investigated by MIF-specific immunohistochemistry (Fig. 1).

Up-expression of MIF in the granuloma of H37Rv-infected mice

Severe inflammatory infiltrate was noted in H37Rv-infected mice at autopsy performed at weeks 8 after infection. At this time points, histopathological parameters such as peribronchiolitis and alveolitis were observed in the lung of most animals, while granuloma formation was always evident. All histopathological parameters increased in severity in the course of infection especially the lung pathology (Fig. 2-A), and Ziehl–Neelsen stain demonstrated that red-rod bacteria distributed extensively in the granuloma (Fig. 2-C). Accordingly, the markedly elevated expression of MIF was detected in the lung of H37Rv-infected mice (Fig. 2-B), whereas the mild inflammatory lesions and absent acid-fast bacteria were observed and weak expression of MIF was detected in the lung of PSB-treated mice (Fig. 2-D). These differences between the control and H37Rv-infected groups were significant ($p<0.01$, Fig. 3).