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Evidence for M2 macrophages in granulomas from pulmonary sarcoidosis: A new aspect of macrophage heterogeneity

Masoud Shamaei[a], Esmaeil Mortaz[a,b], Mihan Pourabdollah[c], Johan Garssen[b,d], Payam Tabarsi[a], Aliakbar Velayati[e], Ian M. Adcock[f,g,*]

[a] Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, Netherlands
[c] Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Nutricia Research Centre for Specialized Nutrition, Utrecht, Netherlands
[e] Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[f] Airways Disease Section, National Heart & Lung Institute, Imperial College London, London, UK
[g] Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, The University of Newcastle, Newcastle, NSW, Australia

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ABSTRACT

Background: Sarcoidosis is a granulomatous disease of unknown etiology. Macrophages play a key role in granuloma formation with the T cells, having a significant impact on macrophage polarization (M1 and M2) and the cellular composition of the granuloma. This study evaluates macrophage polarization in granulomas in pulmonary sarcoidosis.

Materials and Methods: Tissue specimens from the Department of Pathology biobank at the Masih Daneshvari Hospital were obtained. Paraffin sections from 10 sarcoidosis patients were compared with those from 12 cases of tuberculosis using immunohistochemical staining. These sections consisted of mediastinal lymph nodes and transbronchial lung biopsy (TBLB) for sarcoidosis patients versus pleural tissue, neck, axillary lymph nodes and TBLB for tuberculosis patients. The sections were stained for T-cells (CD4+, CD8+) and mature B lymphocytes (CD22+). CD14+ and CD68+ staining was used as a marker of M1 macrophages and CD163+ as a marker for M2 macrophages.

Results: Immunohistochemical staining revealed a 4/1 ratio of CD4+/CD8+ T-cells in sarcoidosis granuloma sections and a 3/1 ratio in tuberculosis sections. There was no significance difference in single CD4+, CD8+, CD22+, CD14+ and CD68+ staining between sarcoidosis and tuberculosis sections. CD163 expression was significantly increased in sarcoidosis sections compared with those from tuberculosis subjects.

Conclusion: Enhanced CD163+ staining indicates a shift towards M2 macrophage subsets in granulomas from sarcoidosis patients. Further research is required to determine the functional role of M2 macrophages in the immunopathogenesis of sarcoidosis.

1. Introduction

Sarcoidosis is a multiorgan granulomatous disorder that most often affects the lungs. Although sarcoidosis has unknown etiology, the association of sarcoidosis with specific occupations, genetic susceptibility, and various infectious pathogens has been described [1]. Classical sarcoidosis is characterized by well-formed, tightly packed, non-necrotizing granulomas surrounded by lamellar hyaline collagen [2]. Granulomas are a closed, centrally organized collection of macrophages and epithelial cell surrounded by lymphocytes [3]. Chronic cytokine stimulation of macrophages leads to their differentiation into multinucleated giant cells over a period of time [4].

Macrophages have been classically defined as M1 and M2 dependent upon their functionality [5]. M1 macrophages activated by lipopolysaccharide (LPS) and interferon γ (IFN-γ) release interleukin 12 (IL-12) and tumor necrosis factor α (TNF-α) [6]. M2 macrophages are produced in the presence of Th2 cytokines (IL-4 and IL-13) and have a suppressive and immunoregulatory function being able to produce both

* Corresponding author.
E-mail address: Ian.adcock@imperial.ac.uk (I.M. Adcock).

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IL-10 and IL-1ra [7]. In addition to activation by Th2 cytokines, M2 macrophages are also activated by immune complexes [6,8]. Although there are no gold standard means of detecting macrophage subtypes, M1 and M2 macrophages have been detected historically by immunohistochemical (IHC) staining using anti-CD68 for M1, and anti-CD163 antibodies for M2 cells [9–14]. Granulomas are recognized as Th1-mediated and Th2-mediated granulomas according to predominant type of participating T-cell helper cell present [15].

Human CD163 is a restricted monocytes-macrophage lineage with high expression in macrophages like as red pulp, bone marrow, liver (kupffer) and lungs [16]. The most well-known stimulants of CD163 expression are glucocorticoids, IL-6, IL-10 and heme/Hb, whereas IL-4, LPS, TNF-α, INF-γ, CXC chemokine ligand 4 (Cxcl4) and granulocyte-macrophage colony-stimulating factor (GM-CSF) downregulate CD163 expression [17–21]. CD163 is a member of the scavenger receptor cysteine-rich family (SRCR) and human CD163 expression is restricted to the monocyte–macrophage lineage [16]. Some studies suggest that CD163 is a pattern recognition receptor and plays a critical role as a part of innate immunity [22,23]. IFN-γ and IL-12 are their predominant cytokines found in Th1-mediated granulomas and a low level of CD163 expression is expected [24].

Tuberculosis (TB) is often considered a Th1 cell-mediated immune response both in active and latent tuberculosis infection [25] although a wide spectrum of alveolar macrophage activation and immune activation in lymph nodes exists [26,27]. Polarization to a Th2 cell-mediated immune response is seen in severe tuberculosis (miliary TB) [28] and active pulmonary tuberculosis (mixed Th1/Th2) [29,30]. In sarcoidosis, after the initial Th1-mediated granuloma formation there is a relative increase in the expression of IL-4 and IL-10 which can induce CCL18 within the granuloma [31]. This may reflect a shift from a Th1-mediated to a Th2-mediated immune response resulting in fibrosis due to the persistence of this inflammatory milieu [32,33].

Macrophage polarization plays a crucial role in chronic inflammatory disease. Overall, the persistence, resolution or progression of granulomas and the conversion to fibrosis is a balance between various inflammatory, regulatory, apoptotic, Th1/Th2 cytokine responses and M1/M2 polarization [34]. It is important to understand this micro-environment in sarcoidosis, to improve our knowledge in the diagnostic and therapeutic aspects of patient management.

To the best of our knowledge, despite the clinical and histopathological similarities between sarcoidosis and tuberculosis [35,36] there are no previous studies evaluating the relativeness of CD163 expression between sarcoidosis and tuberculosis in lung and mediastinal lymph nodes. The aim of the present study is to identify macrophage polarization in pulmonary sarcoidosis in comparison with tuberculosis as a disease with similar clinical, radiological and pathologic patterns.

2. Material and methods

2.1. Patient's selection

Formalin-fixed paraffin-embedded human tissues were obtained from the archives of the department of pathology, Masih Daneshvari Hospital, a pulmonary tertiary referral hospital in Tehran-Iran. Tissue samples consisted of pleura, lymph nodes and transbronchial lung biopsy (TBLB). The medical records of sarcoidosis and TB patients were reviewed based on their clinical, radiological and pathological information by at least two pulmonologists and two pathologists. After review, tissue blocks from 33 sarcoidosis and 27 TB patients with confirmed diagnosis were selected and sections prepared from those with sufficient tissue for immunohistochemical staining. All samples were analysed by PCR for MTB DNA. Samples from 10 sarcoidosis and 12 TB patients were eventually used as providing good quality analysis for all antibodies with the presence of at least three granulomas. TB tissue samples comprised of 4 pleural tissues (33.3%), 6 lymph nodes (50%) and two transbronchial lung biopsy (TBLB) specimens (16.6%) while sarcoidosis tissue specimens contained 4 mediastinal lymph nodes (40%) and 6 cases of TBLB (60%) which are addressed in Table 1. Immunohistochemical analysis was performed in two batches of lymph node and non-lymph node tissue for both TB and sarcoidosis patients.

Table 1
Demographic characteristics and PCR assay of study subjects.

Sarcoidosis (n = 10)	Tuberculosis (n = 12)	Variable
8/2	8/4	Sex (F/M)
42.8 ± 14.3	40.1 ± 16	Age (Mean ± SD)
0	10 (83.3%)	Positive MTB PCR[†]
4 (40%)	6 (50%)	Tissue sample type
6 (60%)	2 (16.6%)	Lymph node
–	4 (33.3%)	TBLB[‡]
		Pleural tissue

[†] Real-time polymerase chain reaction (PCR) assay targeting insertion sequence *IS6110*.
[‡] Transbronchial lung biopsy (TBLB).

Inclusion criteria for pulmonary sarcoidosis patients were clinical data matching lung involvement with radiology findings (i.e. hilar adenopathy, reticulonodular infiltration or pulmonary fibrosis). Histopathologic findings compatible with the ATS definition of sarcoidosis were considered as the presence of tight and well-formed perilymphatic and interstitial granulomas with a rim of lymphocytes and fibroblasts in the outer margin of the granuloma [37]. All microbial factors were excluded for sarcoidosis cases and confirmed by H & E staining in addition to routine tests for bacteria, fungi, and culture for acid-fast bacilli (AFB) and auramine O staining. TB patients entered in the study had a positive sputum smear or culture for AFB, positive Ziehl–Neelsen staining or a positive molecular test for MTB DNA (IS 6110) along with a positive response to standard anti-TB therapy. This study was conducted with the approval of Masih Daneshvari Hospital ethic committee and utilizing protocols approved by the respective institutional review boards (SBMU1.REC.1393.60).

2.2. Real time PCR

DNA was extracted using the Qiagen QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *Mycobacterium Tuberculosis* detection was performed using a *Mycobacterium tuberculosis* PCR Kit (GeneProof, Brno, Czech Republic) based on amplification of the specific multi-copy insertion sequence (IS6110) according to the manufacturer's instructions. All PCRs were performed using StepOne™ Real-Time PCR Systems (Roche Diagnostics Deutschland GmbH, Sandhofer Straβe, Mannheim, Germany). The kit specifically detects *M. tuberculosis, M. bovis, M. africanum* and *M. microti* strains in addition to vaccination strains (e.g. BCG).

2.3. IHC analysis

Ten sarcoidosis patients with appropriate tissue blocks with 12 TB patients were entered into the study. Five-μm tissue sections were deparaffinized and stained with monoclonal antibodies for CD4, CD8, CD22, CD14, CD68, and CD163 (Leica Microsystems Newcastle Ltd, STANSFIELD, Suffolk, UK). The antibody clone, dilutions, and incubation times are shown in Table 2. After deparaffinization and fixation with 70–99% alcohol, specimens were rinsed with distilled water and PBS and treated with methanol and 3% H_2O_2 for 10 min to remove endogenous peroxidase. After antigen retrieval and cooling, the tissues were rinsed with distilled water and PBS and specimens blocked with control autologous antiserum. Antibodies were prepared in Tween 20 + Tris. The primary antibodies were incubated with issue sections in a water saturated environment at room temperature for 30 min and, after rinsing the specimens with PBS, were incubated with secondary antibody provided in kits (DAKO EnVision™ Detection Kit, Peroxidase/