Progression of clinical tuberculosis is associated with a Th2 immune response signature in combination with elevated levels of SOCS3

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Abstract   In this study, we explored the local cytokine/chemokine profiles in patients with active pulmonary or pleural tuberculosis (TB) using multiplex protein analysis of bronchoalveolar lavage and pleural fluid samples. Despite increased pro-inflammation compared to the uninfected controls; there was no up-regulation of IFN-γ or the T cell chemoattractant CCL5

Abbreviations: TB, tuberculosis; *M. tuberculosis, Mycobacterium tuberculosis*; HIV, Human Immunodeficiency Virus; Th, T helper; CTL, cytolytic T cell; NK, natural killer cell; Treg, regulatory T cell; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; Ig, immunoglobulin; SOCS, suppressors of cytokine signaling; JAK, Janus Kinases; STAT, Signal Transducers and Activators of Transcription; BAL, bronchoalveolar lavage; PBMC, peripheral blood mononuclear cell; TST, tuberculin skin test; LJ, Löwenstein–Jensen; BCG, Bacillus Calmette Guerin; FCS, fetal calf serum; OD, optical density; Ct, cycle threshold; M-CSF, macrophage colony-stimulating factor; GMCSF, granulocyte macrophage colony-stimulating factor; IL-1RA, IL-1 receptor antagonist; IL-2R, IL-2 receptor; MOI, multiplicity of infection; PTB, pulmonary tuberculosis.
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Immune response;
Cytokines

in the lung of patients with pulmonary TB. Instead, elevated levels of IL-4 and CCL4 were associated with high mycobacteria-specific IgG titres as well as SOCS3 (suppressors of cytokine signaling) mRNA and progression of moderate-to-severe disease. Contrary, IL-4, CCL4 and SOCS3 remained low in patients with extrapulmonary pleural TB, while IFN-γ, CCL5 and SOCS1 were up-regulated. Both SOCS molecules were induced in human macrophages infected with *Mycobacterium tuberculosis* in vitro. The Th2 immune response signature found in patients with progressive pulmonary TB could result from inappropriate cytokine/chemokine responses and excessive SOCS3 expression that may represent potential targets for clinical TB management.

1. Introduction

Tuberculosis (TB) is one of the most wide-spread human bacterial infections. The lethal synergy between TB and HIV infections is also a major challenge to public health and hence there is a need to understand the cellular and molecular mechanisms responsible for the progression of TB in both HIV-negative and HIV-positive individuals [1]. Successful control of *Mycobacterium tuberculosis* (*M. tuberculosis*) as well as HIV infection is dependent on cell mediated immunity that is orchestrated by multiple cytokines and chemokines involved in the activation and recruitment of cells to the site of infection [2,3]. Pro-inflammatory cytokines (IL-12, TNF-α) as well as Th1 (IL-2, IFN-γ) and Th17 (IL-17, IL-22) effector cytokines specifically promote classical activation of macrophages (M1) and activation of CD8+ cytolytic T cells (CTLs) or NK/NKT cells that trigger target cell and bacterial killing [3]. Here, HIV infection may impair cellular immunity at the site of *M. tuberculosis* infection by interfering with the recruitment and function of macrophages and CD4+ T cells [4].

We have previously reported an impairment of Th1/Th17 and CD8+ CTL responses in human granulomatous TB lesions in both the lymph nodes [5] and lung [6] that may be the consequence of an induction of Th2 and regulatory T cell (Treg) responses [5] Consistent with these findings, growing evidence suggests that a Th1/Th2 balance is crucial to control the progression of active TB disease [7,8]. IL-12, IFN-γ and TNF-α contribute to the induction of Th1-mediated protection in TB, while increased IL-4 levels promote the development of a Th2 response that efficiently antagonizes protective cytokines and results in loss of TB control [9]. Th2 cytokines have been shown to induce alternative macrophage activation (M2) that involves a less bactericidal state of the macrophage [10]. Th2 cytokines can also inhibit autophagy, which is a physiological process known to enhance intracellular degradation of mycobacteria [11]. On the contrary, Th2 responses promote antibody-mediated immunity that may fail to confer resistance in intracellular *M. tuberculosis* infection [12]. The Th1/Th2 balance is likely affected by different chemokines [13] produced by various cell types. Inflammatory chemokines such as CCL5, CXCL9 and CXCL10, selectively attract and recruit Th1 cells from blood to sites of infection while other chemokines such as CCL3 and CCL4 may promote Th2 immunity [13,14]. Although *M. tuberculosis* is a potent inducer of several inflammatory chemokines [15], little is known about the local chemokine profile in the chronic phase of human TB

infection. The Th1/Th2 balance could also be controlled by a family of regulatory proteins called suppressors of cytokine signaling (SOCS) [16]. SOCS are molecules induced by cytokines or other stimuli and function as negative feedback inhibitors by binding either to cytokine receptors or to associated Janus Kinases (JAK), to inhibit the activation of Signal Transducers and Activators of Transcription (STAT) [16]. SOCS1 and SOCS3 are the most studied members that inhibit STAT1 and STAT3 signaling, respectively [16]. Accumulating data support a central role of SOCS proteins in the regulation of immune polarization, which may be highly relevant for the outcome of different infectious diseases [16].

In this study, we aimed to discover adverse immune response signatures relevant to the progression and severity of active TB disease in HIV-negative and HIV-positive patients. We analyzed immune mediators locally in the lung or pleura in comparison with peripheral blood, in patients with pulmonary TB or pleural TB. While the lung is the most common site of *M. tuberculosis* infection, extrapulmonary TB including pleural TB without concomitant pulmonary infection, often represents a milder clinical form of disease [17]. Low levels of IFN-γ, IL-17 and CCL5 along with enhanced levels of IL-4, CCL4 and regulatory SOCS1 and SOCS3 proteins were observed in the lungs of patients with active pulmonary TB compared to the controls. The Th2-like immune signature in combination with enhanced SOCS3 expression was more prominent in severe forms of TB disease and was also associated with elevated antibody responses in patients with pulmonary TB. Future diagnostic and therapeutic approaches of clinical TB may therefore consider quantifying and targeting Th2 and SOCS3 regulated immune response pathways.

2. Material and methods

2.1. Study subjects and clinical diagnosis

The study subjects were recruited at the Chest Unit, Department of Internal Medicine, Black Lion University Hospital, Addis Ababa, Ethiopia, after providing signed informed consent. The study was approved by the National Ethical Committees in both Ethiopia and Sweden. Patients and controls were enrolled according to the flow chart illustrated in Fig. 1. Inclusion criteria: HIV-negative and HIV-positive sputum smear-negative individuals >18 years old with clinical symptoms of suspected TB. Exclusion criteria: patients with miliary TB, a history of previous TB, or >1 week of antimicrobial