Suppressor of cytokine signaling-3 is affected in T-cells from tuberculosisTB patients

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Abstract

T-cells and T-cell-derived cytokines are crucial mediators of protection against *Mycobacterium tuberculosis* infection, but these factors are insufficient as biomarkers for disease susceptibility. In order to define T-cell molecules involved in tuberculosis (TB), we compared gene expression profiles of T-cells from patients with active TB, healthy donors with latent *M. tuberculosis* infection (LTBIs) and non-infected healthy donors (NIDs) by microarray analysis. Pathway-focused analyses identified a prevalent subset of candidate genes involved in the Janus kinase (JAK)–signal transducer and activator of transcription signalling pathway, including those encoding suppressor of cytokine signalling (SOCS) molecules, in the subset of protection-associated genes. Differential expression was verified by quantitative PCR analysis for the cytokine-inducible SH2-containing protein (CISH), SOCS3, JAK3, interleukin-2 receptor α-chain (IL2RA), and the proto-oncogene serine/threonine protein kinase (PIM1). Classification analyses revealed that this set of molecules was able to discriminate efficiently between T-cells from TB patients and those from LTBIs, and, notably, to achieve optimal discrimination between LTBIs and NIDs. Further characterization by quantitative PCR revealed highly variable candidate gene expression in $CD4^+$ and $CD8^+$ T-cells from TB patients and only minor differences between $CD4^+$ and $CD8^+$ T-cell subpopulations. These results point to a role of cytokine receptor signalling regulation in T-cells in susceptibility to TB.

Keywords: Latent *Mycobacterium tuberculosis* infection, suppressor of cytokine signalling, T-cell subpopulations, T-cells, tuberculosis
Original Submission: 12 May 2010; **Accepted:** 8 July 2010
Editor: M. Drancourt
Article published online: 29 July 2010
Clin Microbiol Infect 2011; **17:** 1323–1331
10.1111/j.1469-0691.2010.03326.x

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Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality in humans worldwide, although only a minority of individuals infected with *Mycobacterium tuberculosis* develop active TB disease [1]. It is generally accepted that a functional T-cell response is crucial for protection against *M. tuberculosis*, but the precise underlying mechanisms remain elusive [2].

Interferon-γ and tumour necrosis factor-α are marker cytokines of T-helper type 1 cell responses, which form the basis of protective immunity against TB. Interferon-γ activates macrophages, and enables them to overcome *M. tuberculosis*-induced arrest of phagosome maturation and major histocompatibility complex class II-restricted antigen presentation [3].

The role of $CD8^+$ T-cells in the immune response against TB remains controversial. There is evidence that $CD8^+$ T-cells participate in the control of *M. tuberculosis* infection by expression of effector cytokines [4,5], as well as lysis of infected macrophages combined with killing of *M. tuberculosis* [6,7]. Yet, analyses of $CD8^+$ T-cell repertoires in children with TB have identified clonal expansion of $CD8^+$ terminally differentiated effector T-cells in severe forms of disease [8].

Hence, $CD8^+$ T-cells may serve targets of immune evasion by *M. tuberculosis*.

T-cell responses to pathogens are regulated by T-cell receptor affinity/avidity, co-receptor engagement (e.g. CD28 and cytotoxic T-lymphocyte antigen-4), and cytokine receptor signalling. Common cytokine γ-chain receptors, such as the interleukin (IL)-2 receptor, signal via the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathway [9], and the regulation of these processes is mediated by several molecules, including members of the suppressor of cytokine signalling (SOCS) family [10] and serine/threonine protein kinases (PIMs) [11,12]. The SOCS family comprises eight molecules, and at least four of these, namely CISH, SOCS1, SOCS2, and SOCS3, are expressed in T-cells [10]. SOCS molecules act as feedback inhibitors induced by different stimuli, including cytokines and Toll-like receptor ligands (reviewed in [13]). SOCS-mediated regulation of T-cell functions leads to immune polarization. In animal models, it has been shown, for example, that SOCS3 overexpression inhibits differentiation of T-helper type 17 (Th17) cells [14] and regulatory T-cells [15]. In $CD8^+$ T-cells, SOCS3 is involved in the control of IL-6-induced and IL-27-induced proliferation [16]. Like SOCS molecules, PIMs are induced by cytokine signalling via the JAK–STAT signalling pathway. PIM1 participates in cell survival, proliferation, and carcinogenesis [17]. It has antiapoptotic functions and interacts with several other proteins, including members of the SOCS family [18]. Therefore, SOCS and PIM molecules may be important targets of immune polarization induced by pathogens.

The present study determined the global gene expression profile of T-cells from TB patients, patients with latent *M. tuberculosis* infection (LTBIs), and non-infected healthy donors (NIDs), to determine novel candidates involved in protective immunity against TB.

Materials and Methods

TB patients, LTBIs, and NIDs

Forty-two TB patients, 42 LTBIs and ten NIDs were recruited at Tygerberg hospital, and six TB patients were recruited at the University Hospital Eppendorf, Hamburg. Diagnosis was based on chest radiography, tuberculin skin test, Quantiferon test, and laboratory confirmation of *M. tuberculosis* culture. All TB patients were human immunodeficiency virus-negative and had been included prior to chemotherapy. Features of patients and healthy donors are summarized in Table 1. All donors gave informed consent. This study was approved by local ethics committees in Stellenbosch (South Africa) (N05/11/187), Berlin (Germany) (EA 1/176/07), and Hamburg (WF-07/09) (Germany).

TABLE 1. Donor characteristics

Feature	TB patients	LTBIs	NIDs
Total no.	42	42	10
Gender			
Male	19	19	4
Female	23	23	6
Age (years), mean (SD)	29.0 (10.4)	28.2 (9.3)	25.0 (11.7)

TB, tuberculosis; LTBI, healthy donors with latent *Mycobacterium tuberculosis* infection; NID, non-infected healthy donors; SD, standard deviation.

Isolation of T-cells from peripheral blood

We isolated peripheral blood mononuclear cells from 40 mL of heparinized blood by Ficoll density centrifugation (Biochrom, Berlin, Germany), following the manufacturer's instructions. We separated T-cell populations by magnetic cell sorting with magnetically labeled αCD3, αCD4 or αCD8 antibodies (Miltenyi Biotech, Bergisch Gladbach, Germany) and enrichment columns (MS columns; Miltenyi Biotech), following the manufacturer's instructions. To avoid any influence on T-cell RNA expression, all steps were performed on ice. The purity of $CD3^+$ T-cells was 98.9% ± 0.9% for TB patients, 98.0% ± 2.4% for LTBIs, and 99.2% ± 0.6% for NIDs. Isolation of $CD4^+$ and $CD8^+$ T-cell subpopulations was performed by fluorescence-activated cell sorting (FACS), with the following antibodies: CD4-APC-Cy7, CD8-PerCP-Cy5.5, CD45RO-Pe-Cy7, and CD62L-APC (all BD Biosciences, Franklin Lakes, NJ, USA). Staining procedures have been described previously [19]. A FACS-Aria was used, and the purity was found to be >95%.

Preparation of RNA from T-cells and T-cell subpopulations

After isolation, T-cells were immediately mixed with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and frozen at −80°C until RNA was extracted according to the manufacturer's instructions. RNA content, purity and integrity were determined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Forster City, CA, USA).

Microarray procedures: experimental design

Total RNA was labelled with a Fluorescent Linear Amplification Kit (Agilent Technologies), following the manufacturer's instructions. After photometric quantification of cRNA and determination of labelling efficiency, 2-μg portions of samples were fragmented and hybridized for 20 h on whole genome oligonucleotide microarrays (Agilent Technologies). Each T-cell RNA sample from TB patients was co-hybridized with T-cell RNA from a gender-matched and age-matched LTBI to avoid age-dependent or gender-dependent biases. Because of the lower number of NIDs recruited, each NID T-cell