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# Tuberculosis specific responses following therapy for TB: Impact of HIV co-infection

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**Abstract** Characterizing perturbations in the immune response to tuberculosis in HIV can develop insights into the pathogenesis of coinfection. HIV+ TB+ and TB monoinfected (TB+) subjects recruited from clinics in Bamako prior to initiation of TB treatment were evaluated at time-points following initiation of therapy. Flow cytometry assessed CD4+/CD8+ T cell subsets and activation markers CD38/HLA-DR. Antigen specific responses to TB proteins were assessed by intracellular cytokine detection and proliferation. HIV+ TB+ subjects had significantly higher markers of immune activation in the CD4+ and CD8+ T cells compared to TB+ subjects. HIV+ TB+ had lower numbers of TB-specific CD4+ T cells at baseline. Plasma IFNγ levels were similar between HIV+ TB+ and TB+ subjects. No differences were observed in in-vitro proliferative capacity to TB antigens between HIV+ TB+ and TB+ subjects. Subjects with HIV+ TB+ coinfection demonstrate in vivo expansion of TB-specific CD4+ T cells. Immunodeficiency associated with CD4+ T cell depletion may be less significant compared to immunosuppression associated with HIV viremia or untreated TB infection.
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## 1. Introduction

The immune response to tuberculosis infection is a complex interaction of host and pathogen engaging multiple elements of the innate and adaptive immune systems. Exposure to mycobacterium tuberculosis (Mtb) results in infection within alveolar macrophages and myeloid DCs [1]. Activation of CD4+ T cells and migration of effector T cells to the disease site are important for disease control [2,3]. Additionally Th1 responses to mycobacterial antigens are essential for an effective immune response. In mouse models, disruption of the gene for IFNγ results in necrotic granulomas and failure to control mycobacterial replication. Survival is prolonged

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with the exogenous administration of IFNγ [4,5]. Data from murine models shows that survival may be directly linked to IFNγ from CD4+ T cells [3]. Lack of TNFα in mice during the chronic phase of infection causes breakdown of granulomas and enhancement of pro-inflammatory responses [6]. IL2 is essential for differentiation and survival of activated cells and maintenance of effector function. Patients with tuberculosis may have lower levels of IL2 as compared to healthy volunteers [7,8]. IL10 has been shown to decrease Th1 responses and enhance disease [9].

In 50% of individuals with close contact to individuals with tuberculosis no infection is demonstrable based on current diagnostics indicating that a significant proportion of individuals clear the infection effectively [10]. Among otherwise healthy individuals with detectable immune responses after exposure to tuberculosis, 95% develop latency with a 10% lifetime risk of reactivation. This paradigm of disease control is disrupted by HIV infection leading to higher rates of tuberculosis infection and a lifetime reactivation risk as high as 15% per year [11].

The pathogenesis of this lack of immunologic control of tuberculosis in HIV infection is not well understood. Viral load drives activation of CD4+ T cells causing apoptosis and depletion of the CD4+ T cell pool. Decrease in antigen specific IFNγ+ CD4+ T cells has been reported in the peripheral blood of patients with HIV and latent tuberculosis. A decrease in polyfunctional (IFNγ+ IL2+ and TNFα+) antigen specific cells has also been noted in the airways of patients with HIV [12–14]. Such findings strengthen the hypothesis that HIV reduces the number and function of Mtb specific CD4+ T cells [4,5,15–19]. Other studies have demonstrated higher numbers of Mtb antigen specific CD4+ T cells in coinfected patients despite lower total CD4+ T cell counts [20–22].

The dynamics of the immune system, particularly the function of CD4+ T cells after commencement of treatment for either HIV or TB are quite complex. Evidence supports improved CD4+ T cell function after commencement of HAART in coinfected patients. This improvement may lead to an exacerbation of symptoms described as the immune reconstitution inflammatory syndrome (IRIS) [23–25]. Commencing treatment for HIV in the first few months of TB treatment, when the antigen burden is high appears to trigger extensive T cell and Th1 responses perhaps due to the reconstitution of CD4+ T cells and robust cytokine responses [26,27]. Similar increases in TB-specific immunity have also been reported following the initiation of treatment for TB monoinfected patients [28].

It is almost universally agreed, that starting treatment for both diseases sooner rather than later leads to better outcomes despite some suggestion that patients with HIV may continue to have higher susceptibility to tuberculosis [29,30]. Improved prognosis could be a consequence of decreased antigen burden, restoration of CD4+ T cell count, reduction of immune activation, qualitative and functional improvement in immune responses or a combination of these [31–34].

Comparison of immune responses in subjects with active tuberculosis or HIV alone to subjects who are coinfected with HIV and tuberculosis can help in understanding the differences in immune function as a result of TB or HIV infection. Comparisons over time, afford the ability to describe the sequence of quantitative and qualitative immune events during coinfection and its treatment. The present study conducted in Bamako Mali examined TB specific responses in the context of HIV coinfection, to compare immune responses between individuals with mono or coinfection with TB and or HIV. We compared immunophenotypic and plasma cytokine profiles, cytokine responses and proliferation to in-vitro stimulation with tuberculosis antigens PPD, ESAT-6 and CFP-10 among groups.

## 2. Materials and methods

### 2.1. Recruitment of study subjects

The NIAID institutional review board (IRB) and Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry (FMPOS), University of Bamako approved the protocol. Subjects were enrolled in 4 groups: (a) Healthy volunteers, HIV negative and tuberculin skin test negative (HIV− TST−), (b) HIV positive and TST negative (HIV+), (c) active pulmonary tuberculosis but not HIV+ (TB+) and (d) co-infected with HIV and pulmonary tuberculosis (HIV+TB+) (Table 1). Subjects were excluded if they were under 18 years, or had a 1) hemoglobin ≤7.5 g/dl, 2) TST+, with no symptoms and negative chest X-ray, 3) previous history of TB, 4) known underlying bleeding disorder, 5) psychiatric illness, 6) used immunomodulators or cytotoxic agents, 7) positive test for HIV2 or 8) difficult to access veins. Subjects had been previously BCG vaccinated. Subjects with disseminated tuberculosis were not included in the analysis.

Subjects were recruited between August 2004 and December 2009, from the Hospital of Point G and six referral health centers in the District of Bamako. The first study visit occurred within 1 week of screening. Thereafter subjects were followed at 4, 13 and 52 week intervals. TB+ and HIV+ TB+ subjects had an additional visit at 72 h to collect sputum samples for confirmation of diagnosis. Treatment of TB commenced after enrollment. HIV+ subjects received treatment from their clinicians. Treatments were according to Malian National guidelines for TB and HIV.

### 2.2. Laboratory procedures

#### 2.2.1. HIV and TB diagnostics

After informed consent participants underwent baseline screening, including medical history, physical examination, complete blood count (Coulter A$^c$ T diff, Beckman Coulter, Miami, FL), and HIV testing using a rapid test (Determine® HIV-1/2, Abbott Laboratories, Matsudo-Shi, Chiba, Japan). Confirmation of HIV seropositivity was by ELISA (Genscreen, HIV-1/2 version 2 Assay, Bio-Rad Laboratories, Marnes—La Coquette, France) and Western blot (New LAV Blot I and II, Bio-Rad Laboratories, Marnes—La Coquette, France). Subjects without active TB had tuberculin skin testing. Chest X-rays were performed as clinically indicated. Sputum samples were examined by auramine—rhodamine smear and confirmed by culture on liquid (Mycobacterium Growth Indicator Tube, BBL™ MGIT™, BD, Spark, MD, USA) and solid (Middlebrook 7H11 agar and selective Agar plate) media. Drug susceptibility testing was performed using the AST/SIRE system (BD, Spark, MD, USA).

#### 2.2.2. Immunophenotype staining

Briefly, 100 μl of peripheral whole blood collected in EDTA tubes (BD, Franklin Lakes, NJ, USA) was stained with the