# Synergistic Upregulation of Systemic IL-10 and Functional Enhancement of CD4+ T Cells by IL-10 Receptor Blockade in HIV+/MTB Co-Infection

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## Introduction

**HIV and MTB co-infection**

Co-infection with *M. tuberculosis* (MTB) and HIV is a devastating co-epidemic in many countries and represents a tremendous challenge in patient care, public health and economic burden. TB is the main cause of HIV-related death worldwide (Karim et al, 2009). Co–infection with these two pathogens leads to an accelerated course for both diseases by decreasing CD4 count and increasing pathogen load (Bartlett, 2007). The molecular mechanisms leading to this rapid clinical deterioration are still largely unknown. They may include both synergistic detrimental effects on innate and adaptive immune responses and a facilitating impact of each pathogen on the replication of the other through modulation of immune activation and the cytokine microenvironment (Kaufmann, 2005).

**The role of IL-10 in HIV and MTB pathogenesis**

Interleukin-10(IL-10), produced mainly by macrophages, is a regulatory cytokine required to modulate pro-inflammatory responses and prevent the detrimental effects of excessive inflammation (Moore *et al,* 2001). However IL-10 may dampen the immune response and prevent effective clearance of pathogen (Moore et al, 2001). A recent human study by Brockmann, *et al* (2009) reports that HIV replication enhances IL-10 production and demonstrates that *in vitro* blockade of IL-10 receptor (IL-10Rα) leads to increased antigen specific CD4+ T cell function. In Tuberculosis research, studies have demonstrated that higher IL-10 levels in latent TB infected (LTBI) mice increased susceptibility to re-activation disease (Turner *et al,* 2002). Beamer *et al* (2008) went on to show that systemic IL-10 promotes disease progression in MTB infected mice. In humans, IL-10 has been shown to be indicative of immune suppressed or anergic states in LTBI and active TB disease (Boussiotis *et al,* 2000) . However, no comprehensive assessment has been done on the role of IL-10 in HIV+/TB co-infection. *This study therefore aimed to characterize the role of IL-10 in HIV+/MTB co-infection as compared to HIV+/MTB to mono-infection.*

## Methods

**Patient Information**

This was an IRB approved study and all patients recruited provided written consent. From a 400 patient chronic untreated HIV+ and HIV- cohort at McCord's Hospital (Durban, South Africa), the following subsets of patients were indentified; 22 HIV+/TB active patients, 30 HIV+/LTBI patients, 30 HIV+ mono-infected patients, 10 Active TB mono-infected patients, 7 LTBI mono-infected patients and 22 Healthy controls. In the absence of a gold standard diagnostic for latent TB we used an in-house Interferon gamma release assay (IGRA) in the form of the RD-1 ELISPOT in conjunction with the absence of symptoms of active disease. The RD-1 antigens used in these assays have previously been shown to detect latent TB (Liu et al, 2004).

**Analysis of plasma cytokine levels by Luminex**

We performed bead array assays (Luminex) on frozen plasma from the aforementioned individuals. We used the high sensitivity multiplex kit (Millipore) and measured IL-10 and a selection of other cytokines (IL-6, IL-2, IFNγ, TNFα and IL-13). Kruskal-Wallis and Dunn's post test was used to compare cytokine levels between groups. A cytokine secretion assay was performed to ascertain if IL-10Rα blockade enhanced the cytokine secretion capacity of CD4+ T cells in response to stimulation with HIV and MTB antigens. CD8+ T cells were firstly depleted from frozen whole PBMC using anti-CD8+ T cell magnetic beads. CD4+ T cells were incubated with the MTB specific RD-1 combined antigens (ESAT-6 and CFP-10), Gag or no antigen in the presence of either IL-10Rα blocking antibody isotype control at for 48 hours. Supernatant was collected and IFNγ, IL-2, IL-6, IL-13 and TNFα detected using the described Luminex assay.

## Results

**Synergistic upregualtion of IL-10 and pro-inflammatory cytokines in HIV+/TB active subjects.**

IL-10 plasma levels differed significantly across the patient groups (1A) (Kruskal-Wallis, p= 0.0328). Specifically, elevated IL-10 was observed in HIV+/TB active subjects as compared to active TB ($p<0.05$, Dunn's multiple comparison). In addition to IL-10, pro-inflammatory cytokines (IFNγ, TNFα, IL-6 and IL-2) and another anti-inflammatory cytokine (IL-13) were simultaneously measured. There were significant differences across the subject groups for IFNγ (Figure 1B) (Kruskal-Wallis, $p<0.0001$), TNFα (1C) ($p=0.0001$, Kruskal-Wallis), IL-2 (1D) ($p=0.0001$, Kruskal-Wallis) IL-6 (Figure 1E) ($p=0.0003$, Kruskal-Wallis), and IL-13(1E) ($p<0.0001$, Kruskal-Wallis). As compared to healthy controls, HIV mono-infection significantly decreased plasma levels of IFNγ ($p<0.001$, Dunn's multiple comparison), TNFα ($p<0.05$, Dunn's multiple comparison), IL-2 ($p<0.001$, Dunn's multiple comparison), IL-6($p<0.05$, Dunn's multiple comparison) and IL-13($p< 0.001$, Dunn's multiple comparison) but not IL-10(ns $p>0.05$, Dunn's multiple comparison). However, a significant decrease in the leukocytotrophic cytokine IL-2 and an increase in TNFα and IL-6 was noted in HIV+/TB active co-infection as compared to other disease states. Summarily, IL-10 was uniquely not suppressed in HIV mono-infection as compared to healthy donors and an imbalance towards pro-inflammatory cytokines appears to be present in HIV+/Active TB.

## Results-Continued

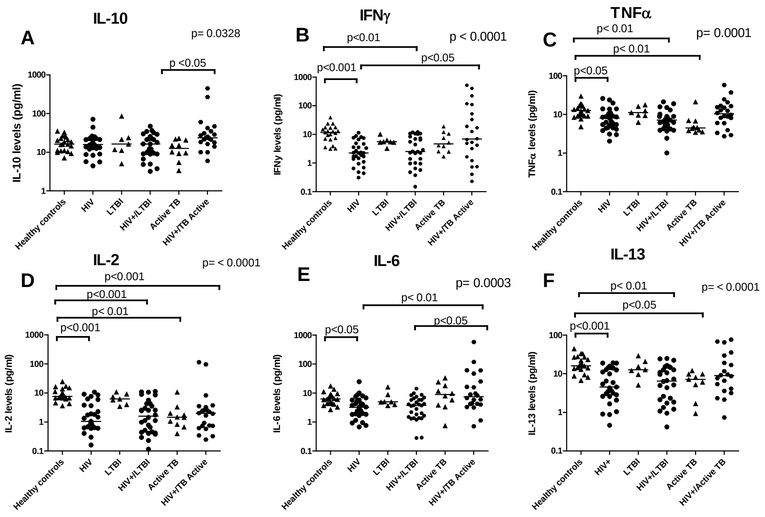


**Figure 1: Comparison of plasma cytokine levels between a constellation of HIV+ and MTB co- and mono-infected subjects.**

**Comparison of individual cytokine contributions to overall cytokine pool between groups.**

Assessment of the proportions and relationship between cytokines was postulated to allow for further characterization of systemic differences between subject groups. The contribution of each cytokine towards total cytokine measured was calculated by taking total cytokine level to be 100%. When compared to healthy controls, HIV mono-infection more than doubled the proportion of IL-10 from 26 % (2A) to 54% (2B) whilst decreasing IFNγ (16% to 6%). In LTBI, co-infection with HIV was observed to increase the percentage of IL-10 from 23% to 38% (2C & 2D) which lead to a dominance of anti-inflammatory cytokines in HIV+/LTBI. In active TB, a massive shift towards pro-inflammatory responses was noted (64% pro-inflammatory as compared to 36% anti-inflammatory (2E) whilst HIV+/Active TB co-infection decreased the pro-inflammatory percentage whilst increasing the IL-10% (19% in Active TB as compared to 31% in HIV+/Active TB, (2E &F).

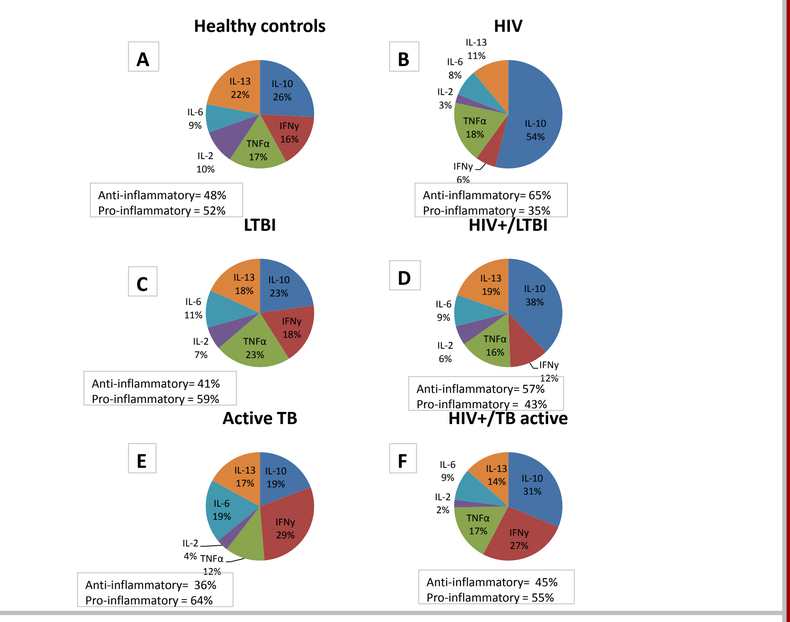


**Figure 2: Comparison of cytokine contributions to overall systemic pool between groups.**

**Inverse relationship between IL-10 and TNFα in relation to HIV disease progression depends on MTB infection state.**

When plasma levels of IL-10, a modulator of pro-inflammatory cytokines such as TNFα, was correlated to HIV disease progression in HIV+/TB active and HIV+/LTBI individuals, the inverse of what was seen with TNFα was observed. IL-10 plasma levels correlated to HIV disease progression in HIV+/LTBI but not HIV+/TB active individuals(Figures 3A-D) and TNFα levels correlated to HIV disease progression in HIV+/TB active but not HIV+/LTBI subjects(Figures 3E-H). This data indicates differential changes of IL-10 between HIV+/TB disease states but provides no indicator of possible differences in IL-10 function.

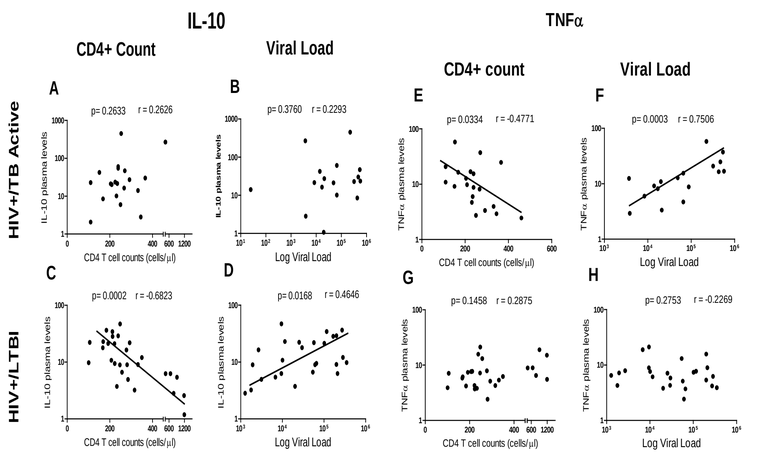


**Figure 3: Correlation of CD4 count and viral load to IL-10 and TNFα plasma levels in HIV+/TB active and HIV+/LTBI subject groups.**

## Results-Continued

**IL-10Rα blockade enhances MTB specific CD4+ T cell function in HIV+/LTBI but not HIV+/TB active patients**

To functionally characterise the MTB specific function of IL-10 between different states of HIV+/TB co-infection, an IL-10 blockade assay was performed and the functional cytokines measured by Luminex. IL-10Rα blockade was found to enhance MTB specific pro-inflammatory responses in HIV+/LTBI CD4 T cells as compared to isotype control: IFNγ ($p=0.0078$, median fold increase=5.63, Paired samples t test) (4A) , TNFα ($p=0.0078$, median fold increase=2.23, Paired samples t test, (4C), IL-6 ($p=0.00313$, median fold increase=1.56, Paired samples t test, (4E) and IL-2($p=0.0156$, median fold increase= 1.71, Paired samples t test, (4G). However, IL-10Rα blockade did not have an effect MTB specific pro-inflammatory responses in HIV+/LTBI CD4 T cells as compared to isotype control: IFNγ (4B), TNFα (4D), IL-6(4F) and IL-2 (4H).

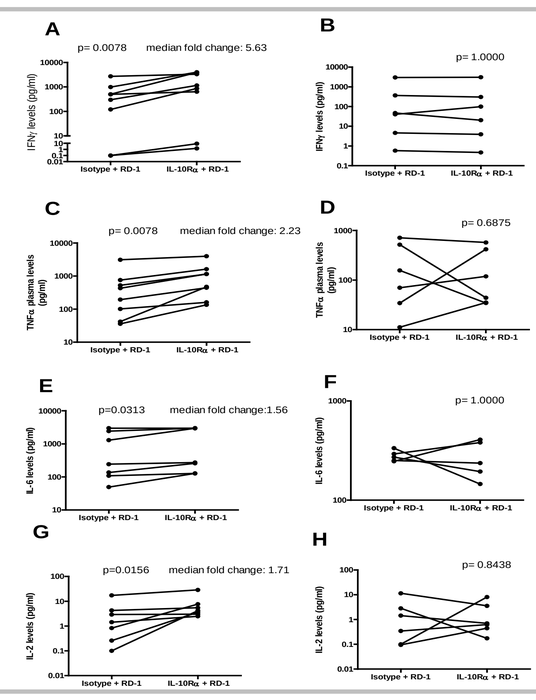


**Figure 4: IL-10Rα blockade enhances MTB specific CD4+ T cell function in HIV+/LTBI but not HIV+/TB active patients**

**IL-10Rα blockade enhances HIV specific pro-inflammatory cytokine production by CD4 T cells in HIV+/LTBI patients**

To functionally characterise the HIV specific function of IL-10 between different states of HIV+/TB co-infection, an IL-10 blockade assay was performed and the functional cytokines measured by Luminex. IL-10Rα blockade enhanced HIV specific pro-inflammatory responses in HIV+/LTBI CD4 T cells as compared to isotype control. IFNγ (5A) ($p=0.0156$, median fold increase=1.55, Paired samples t test) TNFα ($p=0.0234$, median fold increase=1.66, Paired samples t test) (5B) and IL-6($p=0.00313$, median fold increase= 3.30, Paired samples t test, (5C) were all significantly upregulated when blocked with IL-10. However, we found no significant change in IL-2 levels upon blockade (5D) ($p=0.9375$, Paired samples t test). Additionally, IL-10Rα blockade did not restore HIV specific responses in HIV+/TB active subjects: IFNγ (5B), TNFα (5D), IL-6 (5F) and IL-2 (5H).

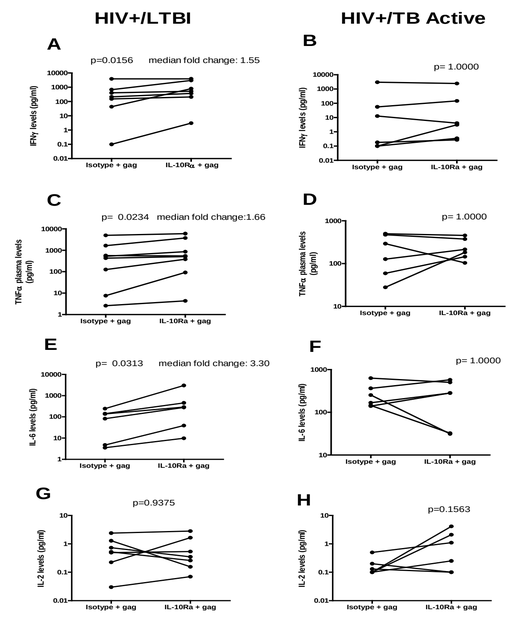


**Figure 5: IL-10Rα blockade enhances HIV specific CD4+ T cell function in HIV+/LTBI but not HIV+/TB active patients**

## Conclusion

This study concluded that in HIV+/LTBI subjects, who have IL-10 dominant cytokine profiles, *ex vivo* antigen specific CD4 T cell enhancement is possible via IL-10 blockade. However, in HIV+/TB active individuals, where elevated levels of both inflammatory and anti-inflammatory cytokines are present, IL-10 receptor blockade does not enhance CD4 T cell function. Dysregulation of the IL-10 pathway in HIV+/TB active subjects may lead to detrimental levels of pro-inflammatory cytokine and dominant IL-10 in HIV+/LTBI may lead to suppresses levels of CD4 T cell function.