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Tuberculosis distorts the inhibitory impact of interleukin-10 in HIV infection

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Abstract

Objectives—This study aimed to assess how *Mycobacterium tuberculosis* (MTB) coinfection alters the impact of interleukin-10 in chronic HIV infection.

Design—We assessed plasma cytokine levels (interleukin-10, interferon- γ , tumor necrosis factor- α , interleukin-2, interleukin-6 and interleukin-13) in 82 individuals presenting with HIV monoinfection, HIV-LTBI (latent MTB infection) coinfection or HIV-TB (active tuberculosis) coinfection. We also assessed the influence of MTB on the functional impact of interleukin-10 receptor alpha (interleukin-10R α) blockade on HIV and MTB-specific CD4⁺ T cells.

Methods—Plasma cytokine levels were measured by high sensitivity Luminex. We used an ex-vivo interleukin-10R α blockade assay to assess if functional enhancement of HIV and MTB-specific CD4⁺ T cells was possible following a 48-h stimulation with HIV gag or pooled ESAT-6 (6 kDa early secretory antigenic target) and CFP-10 (10-kDa culture filtrate protein) peptides. Cell supernatant was collected 48 h after stimulation and the cytokine profile was measured by Luminex.

Results—Plasma interleukin-10 levels were elevated in HIV-TB as compared with HIV monoinfection ($P<0.05$) and HIV-LTBI ($P<0.05$). Plasma interleukin-10 levels correlated to HIV viral load in HIV monoinfection ($P=0.016$) and HIV-LTBI ($P=0.042$), but not HIV-TB. Ex-vivo

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Conflicts of interest

The authors do not have any conflicts of interest.

blockade of interleukin-10R α significantly enhanced MTB and HIV-specific CD4⁺ T-cell function in HIV-LTBI individuals but not in HIV-TB individuals.

Conclusion—Tuberculosis disrupts the correlation between interleukin-10 and markers of HIV disease progression. In addition, HIV-TB is associated with a more inflammatory cytokine milieu compared with HIV monoinfection. Interestingly, interleukin-10R α blockade can enhance both HIV and MTB-specific T-cell function in HIV-LTBI, but not in HIV-TB coinfection.

Keywords

HIV coinfection; interleukin-10 receptor alpha blockade; immune regulation; interleukin-10; *Mycobacterium tuberculosis*

Introduction

Tuberculosis (TB) and HIV are devastating coepidemics in many countries and represent a tremendous challenge in patient care, public health and economic burden. TB is the main cause of HIV-related death worldwide, and coinfection with these two pathogens leads to an accelerated course for both diseases [1,2]. The responsible mechanisms are still largely unknown but may include synergistic detrimental effects on the immune response such as immune exhaustion [3].

Interleukin-10 is a cytokine produced by a variety of cells including monocytes and macrophages [4–7] and has been shown to inhibit antigen-specific T-cell responses [8,9] with a direct effect on T-cell proliferation and production of interleukin-2 and interferon (IFN)- γ [10,11]. In HIV infection, interleukin-10 plasma levels were found to increase upon HIV disease progression [6,12–14] and in-vitro blockade of interleukin-10 receptor alpha (interleukin-10R α) increased HIV-specific CD4⁺ T-cell cytokine secretion [6,14]. Elevated plasma interleukin-10 levels have also been reported in individuals with TB [15,16], but the impact of TB in regulating immune activation and interleukin-10 levels in HIV coinfection and the impact of this interplay on HIV-specific CD4⁺ T-cell responses is currently unknown.

Our study aimed to assess how infection with *Mycobacterium tuberculosis* (MTB) alters the impact of interleukin-10 in HIV infection by evaluating interleukin-10 plasma levels and its relationship with other cytokines [IFN- γ , tumor necrosis factor (TNF)- α , interleukin-2, interleukin-6 and interleukin-13] in HIV-infected individuals coinfecting with latent MTB infection (LTBI) or TB. We also assessed how TB affects the functional impact of interleukin-10R α blockade on HIV and MTB-specific CD4⁺ T cells.

Materials and methods

Individuals and sample processing

Eighty-two individuals were enrolled in Durban, South Africa: 22 HIV-positive individuals with TB (HIV-TB), 30 HIV-positive individuals with latent *M. tuberculosis* infection (LTBI) (HIV-LTBI patients) and 30 HIV mono-infected patients. All sample groups were matched for CD4⁺ cell count and viral load, and were comparable in sociodemographic

variables. All TB individuals were pulmonary TB cases identified by a positive sputum acid-fast bacilli smear or sputum culture. LTBI was defined as a positive ESAT-6 and/or CFP-10-specific IFN- γ enzyme-linked immunoSpot (ELISPOT) assay result in the absence of signs and symptoms of TB [17]. Ethical approval was obtained and written informed consent was obtained from all patients. All patients were antiretroviral therapy naive.

High sensitivity multiplex bead array assay (Luminex)

A high sensitivity bead array assay (Luminex) (Millipore, Billerica, Massachusetts, USA) was used to measure interleukin-10, interleukin-6, TNF- α , IFN- γ , interleukin-2 and interleukin-13 in plasma and cell supernatant according to the manufacturer's instructions. Plates were read using Bio-Plex 200 array system (Bio-Rad Laboratories, Hercules, California, USA).

Interleukin-10 receptor alpha blockade

To evaluate the impact of interleukin-10R α blockade on cytokine secretion by CD4⁺ T cells, we used CD8⁺ depleted peripheral blood mononuclear cells (PBMCs) (CD8⁺ T-cell positive selection, Dynalbeads; Invitrogen, Carlsbad, California, USA). Cells were incubated with either interleukin-10R α blocking antibody (clone 37607; R&D Systems, Minneapolis, Minnesota, USA) or IgG1 isotype (10 μ g/ml) for 15 min. 1×10^6 cells were incubated with ESAT-6 and CFP-10 (10 μ g/ml), Gag (10 μ g/ml) or no antigen at 37°C in 5% CO₂ for 48 h. Supernatants were collected and cytokine levels (IFN- γ , interleukin-2, interleukin-6, interleukin-13 and TNF- α) were detected as described above.

Statistical analysis

Cytokine levels were compared between patient groups with the nonparametric Kruskal–Wallis test, and Dunn's tests were performed for paired comparisons. CD4⁺ cell count and viral load were correlated to cytokine levels using Spearman correlations. The cytokine profiles (by percentage of total measured cytokine) were compared between patient categories and ratios of IFN- γ to interleukin-10 per patient were calculated by dividing IFN- γ level (pg/ml) by the corresponding interleukin-10 value. The contribution of each cytokine towards total cytokine measured was calculated by taking total cytokine level to be 100%. Fischer's exact test was used to assess differences between these cytokine profiles. For the interleukin-10R α blockade assay, differences between interleukin-10R α blocked and isotype control groups were determined using a Wilcoxon matched-paired test.

Results

HIV coinfection with tuberculosis, but not latent *Mycobacterium tuberculosis* infection, is associated with an altered interleukin-10 profile

Plasma interleukin-10 levels were measured in HIV-infected individuals with or without LTBI or TB. Figure 1a shows that coinfection with LTBI did not alter the levels of interleukin-10 compared with HIV mono-infection. However, TB coinfection resulted in higher levels of interleukin-10 compared with HIV mono-infection ($P < 0.05$) and HIV-LTBI ($P < 0.05$), suggesting a unique interplay between HIV and TB.

To further investigate the impact of TB on HIV disease, we evaluated the plasma levels of a selection of cytokines previously found to be distorted by interleukin-10 in both diseases (IFN- γ , interleukin-2, interleukin-6, interleukin-13 and TNF- α) [18]. We observed a higher IFN- γ /interleukin-10 ratio in HIV-TB coinfection compared with HIV mono-infection ($P<0.05$) (Fig. 1b).

We next modelled the cytokine profile (of those cytokines measured in this study) for all patient groups. In HIV mono-infection, interleukin-10 dominated the cytokine secretion profile (Fig. 1c). HIV-LTBI coinfection was associated with a lower contribution of interleukin-10 (38%) and a higher contribution of interleukin-13 (19%) and IFN- γ (12%) as compared with HIV mono-infection ($P<0.05$) – an indication of enhanced Th1 and Th2 responses. HIV-TB coinfection was characterized by a strikingly high contribution of IFN- γ (27%), almost equal to that of interleukin-10 (31%), which is significantly different from the HIV mono-infected group ($P<0.01$).

Plasma interleukin-10 levels in HIV mono-infection and HIV-LTBI coinfection correlated positively with viral load (data not shown and Fig. 1d). In contrast, plasma interleukin-10 levels in HIV-TB coinfection did not correlate with markers of HIV disease progression (Fig. 1e). Conversely, TNF- α levels did not correlate to markers of HIV disease progression in the HIV-LTBI group (Fig. 1. d) but did correlate to markers of HIV disease progression in HIV-TB coinfecting individuals (Fig. 1e). Overall these data suggest that MTB infection, whether LTBI or TB, alters the systemic cytokine milieu of HIV infection with TB having the larger impact, supported by the lack of interleukin-10 correlation with markers of HIV disease progression in HIV-TB (Fig. 1d and e).

Tuberculosis distorts the inhibitory effect of interleukin-10 on HIV and *Mycobacterium tuberculosis*-specific CD4⁺ T-cell function

To further investigate the above effect, we assessed the impact of interleukin-10R α blockade on cytokine secretion from HIV-specific and MTB-specific CD4⁺ T cells following antigen-specific stimulation. In-vitro blockade of the interleukin-10 pathway in HIV mono-infected individuals increased cytokine secretion by HIV-specific CD4⁺ T cells with the greatest impact on IFN- γ secretion, as we have previously reported [14] (data not shown). Blockade of interleukin-10R α in HIV-LTBI coinfection, enhanced HIV and MTB-specific CD4⁺ T-cell secretion of IFN- γ and TNF- α (Fig. 2a and c). In contrast, blockade of the interleukin-10 pathway in HIV-TB coinfection did not enhance HIV or MTB-specific CD4⁺ T-cell responses (Fig. 2b and d). These data strongly suggest that TB not only distorts the systemic cytokine milieu of HIV infection, but also abrogates the responsiveness to interleukin-10 blockade on HIV and MTB-specific CD4⁺ T-cell function.

Discussion

This study highlights the differences in systemic levels, proportions and functionality of interleukin-10 between states of HIV and MTB coinfection and its relationship to other key cytokines. First, we show increased levels of plasma interleukin-10 in HIV-TB coinfecting individuals. Furthermore, we observed that TB alters the cytokine milieu of HIV infection, leading to a more inflammatory cytokine profile than HIV mono-infection. Additionally, we

show that TB disrupts the correlation between markers of HIV disease progression and systemic interleukin-10 levels. Lastly, we show that interleukin-10R α blockade can enhance both HIV and MTB-specific T-cell function in HIV mono-infection and HIV-LTBI, but not in HIV-TB coinfection.

Elevated plasma interleukin-10 levels have been found to be associated with HIV disease progression [13,14]. We observed that absolute interleukin-10 levels were higher in HIV-TB coinfection as compared with HIV-LTBI and HIV mono-infection. This absolute elevation may be a homeostatic attempt to decrease the increased levels of pro-inflammatory cytokines observed in the HIV-TB group, which may in turn be due to increased pathogen load [19]. Furthermore, higher IFN- γ levels in HIV-TB, coupled with the significant rise in the ratio of IFN- γ /interleukin-10, suggest that TB may distort the inhibitory impact of interleukin-10 in HIV, shifting the cytokine profile towards a dominant systemic proinflammatory response, which could contribute to MTB-induced pathogenesis [18,19].

Interleukin-10 has been shown to correlate to HIV disease progression in HIV monoinfection [14]. In this study we show that interleukin-10 plasma levels correlated to HIV disease progression in HIV-LTBI but not HIV-TB, which suggests that TB may offset the typical characteristics of interleukin-10 in HIV infection. Unlike in HIV monoinfection, interleukin-10 did not dominate the measured cytokine profile in HIV-TB. Analysis of the percentage contribution of each cytokine indicated that the overall percentage of plasma interleukin-10 decreases progressively from HIV monoinfection to HIV-LTBI to HIV-TB, whereas at the same time IFN- γ and interleukin-6 increase progressively. This is a further indicator that TB may distort the inhibitory impact of interleukin-10 in HIV.

In contrast to HIV monoinfection, in which interleukin-10 is one of the dominant immunoregulatory pathways, in HIV-TB coinfection, the inhibitory impact of interleukin-10 appears to be distorted and other immunoregulatory pathways may dominate regulation of HIV-specific immune responses. This is supported by the lack of interleukin-10 correlation with markers of HIV disease progression in HIV-TB that is substituted with a TNF- α correlation. Although TNF- α does not correlate with HIV disease progression in the setting of monoinfection, the strong positive (though statistically nonsignificant) correlation observed in the setting of coinfection could be explained by studies showing that TNF- α increases HIV replication *in vitro* in monocytes in the setting of TB coinfection [20]. It is also possible that several factors or immunoregulatory pathways may enhance the effect of TNF- α either on CD4⁺ T-cell loss or enhancement of HIV replication.

In order to functionally assess if the interleukin-10 pathway was indeed distorted in HIV-TB, we performed ex-vivo blockade of the interleukin-10 receptor. Interleukin-10R α blockade has previously been shown to enhance HIV-specific CD4⁺ T-cell function in HIV monoinfection [6,14]. We show that this blockade enhances HIV (and MTB) specific CD4⁺ T-cell function in HIV-LTBI but not HIV-TB. This supports our observations that TB distorts the inhibitory impact of interleukin-10 in HIV infection. One possibility for this lack of functional enhancement is the downregulation of the interleukin-10 receptor on CD4⁺ T cells and monocytes. However, analysis of the mRNA levels in sorted cell subsets did not show any reduction of interleukin-10R α in CD4⁺ T cells and monocytes in the setting of

HIV-TB coinfection (data not shown). Therefore, another explanation could be that CD4⁺ T cells are more exhausted in TB coinfection, and multiple inhibitory pathways may be regulating CD4⁺ T-cell function. During exhaustion, hierarchical loss of T-cell functions mirrors the accumulation of inhibitory coreceptors, and it is proposed that restoration of immune function at late stages may reacquire manipulation of multiple inhibitory pathways [21,22]. To this extent, we recently showed that the function of HIV-specific CD4⁺ T cells from HIV-infected individuals, who do not respond to interleukin-10 blockade *in vitro*, was restored by blockade of the PD-1 (programmed cell death protein 1) pathway, suggesting that at this stage multiple pathways may be regulating CD4⁺ T-cell exhaustion [23]. More importantly, in the same individuals, combined blockade of PD-1 and interleukin-10 had a strong additive effect on CD4⁺ T-cell function compared with single blockade, suggesting that the interleukin-10 pathway was still active but masked by the effect of the PD-1 pathway. Concurrent blockade of both networks was more beneficial for CD4⁺ T-cell function. Data in this current study suggest that in HIV-TB coinfection, there is a more complex network of immunoregulatory pathways than are present in HIV monoinfection that may distort the inhibitory impact of interleukin-10 on CD4⁺ T cells. Alternatively, T cells in HIV-TB may not be functionally suited to interleukin-10 blockade enhancement. Further research is needed to characterize those immunoregulatory pathways in HIV-TB coinfection, including TNF- α , and evaluate their functional impact on T-cell function.

In summary, these data show for the first time that TB distorts the immunoregulatory networks that regulate the immune system during HIV infection and blocking the interleukin-10 pathway alone in HIV-TB will not enhance HIV-specific CD4⁺ T-cell function. Our data suggest that positively enhancing HIV and MTB-specific CD4⁺ T-cell function in HIV-LTBI-infected individuals, by interleukin-10 receptor α blockade, may be a potential immunotherapeutic strategy and requires further investigation.

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Fig. 1. Interleukin-10 levels are distorted in HIV coinfection with tuberculosis

IL-10 plasma levels significantly differed across all patient groups ($P=0.0130$) (a). Multiple comparison analysis revealed that IL-10 plasma levels were significantly higher in HIV-positive individuals co-infected with tuberculosis (TB) (HIV+/TB) individuals as compared with HIV mono-infected individuals ($P<0.05$) and HIV-positive individuals co-infected with latent *M. tuberculosis* infection (HIV-LTBI) ($P<0.05$). The ratio of IFN- γ /IL-10 was assessed (b). There was a significant difference in the IFN- γ /IL-10 ratios across patient groups ($P=0.0382$). Specifically, a greater IFN- γ /IL-10 ratio was observed in HIV-TB as compared with HIV mono-infection ($P<0.05$). The ratio of IL-10 was also correlated to markers of HIV disease progression. We created a cytokine secretion profile by calculating the percentage contribution of each measured cytokine to the overall pool. Both the cytokine profiles from HIV-LTBI ($P<0.05$) and HIV-TB ($P<0.01$) differed significantly from HIV mono-infection (c). In HIV mono-infection, IL-10 dominated the cytokine secretion profile. HIV-LTBI coinfection was associated with a lower contribution of IL-10 (38%) and a higher contribution of IL-13 (19%) and IFN- γ (12%). HIV-TB coinfection was characterized by a strikingly high contribution of IFN- γ (27%), almost equal to that of IL-10 (31%). A strong inverse correlation between CD4 $^{+}$ T-cell count and IL-10 plasma levels was observed in HIV-LTBI ($P=0.0002$, $r=-0.68$) with a correlation between log viral load and IL-10 ($P=0.01$, $r=0.46$) (d). These correlations were not present in HIV-TB (e). Conversely, TNF- α did not correlate to HIV viral load in HIV-LTBI ($P=0.27$) (d) but did correlate to HIV viral load in the HIV-TB group ($P<0.001$) (e). IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Fig. 2. Interleukin-10 receptor alpha blockade enhances HIV and *M. tuberculosis*-specific CD4⁺ T-cell function in HIV coinfection with latent *M. tuberculosis* but not tuberculosis

(a) Blockade of IL-10Rα in HIV coinfection with latent *M. tuberculosis* infection (LTBI), enhanced HIV-specific CD4⁺ T-cell secretion of (i) IFN-γ ($P=0.01$) and (ii) TNF-α ($P=0.02$) but not IL-2 (iii) ($P=0.93$). In contrast, blockade of the IL-10 pathway in HIV-TB coinfection did not restore HIV-specific CD4⁺ T-cell function (b). Enhancement of MTB-specific function was also assessed. (c) Blockade of IL-10Rα in HIV-LTBI coinfection enhanced MTB-specific CD4⁺ T-cell secretion of (i) IFN-γ ($P=0.007$), (ii) TNF-α ($P=0.007$) and (iii) IL-2 ($P=0.01$). In contrast, blockade of the IL-10 pathway in HIV-TB coinfection did not restore MTB-specific CD4⁺ T-cell responses (d). IFN, interferon; IL, interleukin; MTB, *Mycobacterium tuberculosis*; TNF, tumor necrosis factor.