Mycobacterium tuberculosis and human immunodeficiency virus type 1 interaction: Pathogenesis and disease modulation in dual infection
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Addendum
Discussion
HIV and MTB infections are a major cause of illness globally with the highest rates in developing countries; each infection potentiating the morbidity and mortality associated with the other. The main mechanism by which HIV worsens MTB infection is by down modulating the cell-mediated immunity (CMI). However, the mechanism by which MTB disease worsens HIV-1 disease remains unclear. This thesis focuses on the immunological interaction of HIV and tuberculosis, potential mechanisms of worsening HIV-1 disease in active tuberculosis, as well as the clinical effects of dual HIV-1/MTB co-infection and disease. The work outlines some pathogenetic mechanisms of tuberculosis and HIV dual infection, and discusses some in-vitro and in-vivo mechanisms of ameliorating the impact of MTB disease on HIV infection.

Chapters 2 and 3.
To define factors that may affect viral activity among HIV-1-infected adults with active TB, we assessed plasma viral load in HIV-1 infected patients with PTB, and asymptomatic HIV-1 infected controls at different CD4 T cell counts. We also evaluated the status of HIV-1 gene expression in peripheral blood mononuclear cells (PBMC) and serum from HIV-1/PTB patients and CD4-matched healthy HIV-1 infected controls. Finally, we looked at the effect of treatment of pulmonary TB on HIV-1 activity in HIV-1/PTB subjects with CD4 cell counts above 100 cells/microL in vitro.

When we evaluated the impact of TB on HIV-1 disease, we showed that HIV-1 transcriptional activity is enhanced in HIV-1/PTB patients, with a significantly higher viral load in HIV-1/PTB compared to HIV-1 patients alone at any CD4 T cell level. Also, in our study 25% of HIV-1/PTB patients had CD4 counts >500/microl, indicating that a significant number of HIV-1-infected subjects develop TB when the immune system is generally “intact”. In this subgroup of HIV-1 patients with high CD4 T cell levels, active PTB was associated with an approximately 20-fold higher viral load compared to those with HIV-1 alone. By contrast, viral load was comparable in HIV-1/PTB and non-TB symptomatic HIV-1 patients whose CD4 was < 500/ml. Patients with HIV-1 infection who progressed to active PTB had significant increase in viral load (average 2.5-fold). This increase in viral load in HIV-1/PTB compared to HIV-1 infected subjects was seen in both mononuclear cells and serum, most notably at higher CD4 T cell
counts. At low CD4 T cell counts, the HIV-1 load was high among both the HIV-1/PTB and HIV-1/non-TB subjects indicating that with advanced HIV-1 disease, PTB may not have further significant effect on the viral load.

The HIV-1 gene expression (determined by polymerase chain reaction, PCR) was higher in cells and serum from HIV-1/PTB patients compared to CD4-matched HIV-1 infected controls, again with the difference more significant at higher CD4 T cell counts.

Overall, whereas all HIV-1 infected patients who developed TB had fairly high viral loads at the time of diagnosis of PTB (5-6 log on average); those with low initial viral levels (< 50,000 copies/ml) had a more dramatic increase in their viral loads and tended to have a poorer clinical outcome. As TB is often an early HIV-1 opportunistic infection, it may particularly favour early viral replication and dissemination, and therefore contribute to progression of HIV-1 disease.

We postulate that even MTB infection without disease may have a significant contribution to the HIV-1 load in dually infected subjects, making the benefit of latent TB treatment even more relevant in high endemic countries.

The mechanism for the high prevalence of active TB at high CD4 T cell levels is unclear. Although MTB virulence is a possibility, it is highly likely that other than numbers of CD4 T cells, their functional disarray in HIV-1 infection may be a major contributing factor causing disturbances in the normal host immune-surveillance against MTB infection. The pathogenesis of TB during HIV-1 infection includes both reactivation of prior MTB infection, and progressive primary MTB infection [1]. At high CD4 T cell counts, TB in early HIV-1 infection is characterized by zonal infiltrates typical of reactivation PTB; at intermediate CD4 T cell counts, pleural effusion is common while at CD4 T-cells below 200/microL, lower or mid-zone infiltrates, adenopathy, interstitial pattern or even normal radiograph are the characteristic features [2]. The high prevalence of typical PTB pattern in HIV infection at high CD4 T cell counts may be related to early functional defects in the CD4 Th1 responses, even before the effect of the gradual decline of CD4 T cell counts comes into play [3]. This has been demonstrated in Simian Immune Deficiency [4], where loss of memory CD4 T cells is demonstrated in primates and may be conducive to development of active TB [5, 6, 7]. In CD4 T cells cultured with ESAT-6, an MTB antigen, CD4 T cell-specific IFN-gamma secretion was lower in culture-positive active PTB patients than in latently infected healthy contacts or in
subjects with minimal disease and low bacterial burdens [8]. Others have shown that high mycobacterial load is associated with progressive impairment of MTB-specific T cell responses [9], a further indication of dual interaction of HIV-1 and MTB. The functional defect in CD4 T cells in early HIV infection may be related to rapid HIV-induced MTB-specific Th1 cell depletion contributing to progression of latent MTB infection [10].

In early HIV-1 infection, active tuberculosis through activation of macrophages harboring the HIV provirus may be a major factor in the high HIV-1 load seen in dual infection. Our data indicates that the HIV-1 transcriptional activity is enhanced by active PTB mainly in early HIV-1 disease at higher CD4 T cell counts.

Monocytes/macrophages are markedly activated in MTB/HIV-1 co infection, compared to the lymphocytes [11, 12]. Thus it is possible that macrophages with HIV-1 provirus, on activation by TB disease, may be a major source of the high viremia seen in dual infection. In patients with advanced HIV-1 infection, which is characterized by mutation from macrophage tropic (M tropic) to T lymphocyte tropic (T tropic) strains of HIV and declining CD4 T cell levels, there is exponential increase in the viral load, and the added effect of active TB seems to have minimal contribution to the viral load levels. Thus TB as an early HIV-1 opportunistic infection may particularly precipitate early viral replication and dissemination, and thus contribute to progression of HIV-1 disease.

We showed that successful treatment of pulmonary TB was associated with a decrease of HIV-1 load in some HIV-1/PTB patients. However among these “responders” the decrease in viral load was not sustained, returning to baseline 12 months after start of anti-TB treatment. This shows that anti-TB treatment alone is inadequate in reversing the increased HIV-1 transcription seen in macrophages of dually infected persons, and that the associated immune activation is not readily reversed by successful anti-TB treatment. Others have shown that macrophages contribute significantly to viral load during HIV-1/TB co-infection, particularly at sites of MTB infection [13].

In our studies, about half the patients with HIV-1/PTB had no significant decrease of HIV-1 load with successful anti-TB treatment (figure 9). It seems patients with lower mycobacterial load and faster resolution of PTB are the ones more likely to decrease HIV load with anti TB treatment.
subjects with minimal disease and low bacterial burdens [8]. Others have shown that high mycobacterial load is associated with progressive impairment of MTB-specific T cell responses [9], a further indication of dual interaction of HIV-1 and MTB. The functional defect in CD4 T cells in early HIV infection may be related to rapid HIV-induced MTB-specific Th1 cell depletion contributing to progression of latent MTB infection [10]. In early HIV-1 infection, active tuberculosis through activation of macrophages harboring the HIV provirus may be a major factor in the high HIV-1 load seen in dual infection. Our data indicates that the HIV-1 transcriptional activity is enhanced by active PTB mainly in early HIV-1 disease at higher CD4 T cell counts. Monocytes/macrophages are markedly activated in MTB/HIV-1 co-infection, compared to the lymphocytes [11, 12]. Thus it is possible that macrophages with HIV-1 provirus, on activation by TB disease, may be a major source of the high viremia seen in dual infection. In patients with advanced HIV-1 infection, which is characterized by mutation from macrophage tropic (M-tropic) to T lymphocyte tropic (T-tropic) strain(s) of HIV and declining CD4 T cell levels, there is exponential increase in the viral load, and the added effect of active TB seems to have minimal contribution to the viral load levels. Thus TB as an early HIV-1 opportunistic infection may particularly precipitate early viral replication and dissemination, and thus contribute to progression of HIV-1 disease. We showed that successful treatment of pulmonary TB was associated with a decrease of HIV-1 load in some HIV-1/PTB patients. However among these “responders” the decrease in viral load was not sustained, returning to baseline 12 months after start of anti-TB treatment. This shows that anti-TB treatment alone is inadequate in reversing the increased HIV-1 transcription seen in macrophages of dually infected persons, and that the associated immune activation is not readily reversed by successful anti-TB treatment. Others have shown that macrophages contribute significantly to viral load during HIV-1/TB co-infection, particularly at sites of MTB infection [13]. In our studies, about half the patients with HIV-1/PTB had no significant decrease of HIV-1 load with successful anti-TB treatment (figure 9). It seems patients with lower mycobacterial load and faster resolution of PTB are the ones more likely to decrease HIV load with anti-TB treatment [14]. In Uganda, most patients present late, with advanced or far advanced chest X-ray features of PTB, a factor that may contribute to the poor impact of anti-TB treatment on HIV-1 load. We found that older patients were more likely to lower viral load with anti-TB treatment. This was in contrast to studies among HIV-1 infected, non-PTB patients for whom age has been identified as a negative prognostic factor of progression of HIV-1 disease [15]. Cavitating PTB is a disease of young adults with a peak at 25-35 years. The reason for this is unclear. PTB without HIV-1 infection is predominant in males, whereas HIV-1/PTB is seen equally in males and females [16] [4]. In our study, there was no gender difference among those who lowered viral load with anti-TB treatment and those who did not. This emphasizes the findings that HIV-1 has equalized the prevalence and presentation of PTB in males and females [17] [18] [19].

Figure 9: Anti tuberculosis treatment alone has a positive effect on older patients with HIV-1/PTB who show an effect on the viral load as well as activation markers.
The role of macrophage activation in HIV-1/PTB disease in supporting viral production is particularly notable in responders who in addition to having lower viral load at 6 months of anti-TB treatment, also had significantly lower soluble CD14 (sCD14), a serum marker of macrophage activation, by the end of anti-TB treatment. In the non-responders, sCD14 also decreased by the end of treatment, but not significantly, suggesting higher persistent macrophage activation. Macrophage infection with MTB up-regulates various surface markers, cytokine and chemokine molecules aimed at controlling MTB progression. However, with a compromised immune response in HIV-1 infection, these molecules activate the macrophage and enhance HIV-1 entry and replication, leading to increased viral load [20]. Thus, regardless of the initial response in lowering the viral load, sustained generalized immune activation may continue to promote viral replication even after successful treatment of active TB [21]. This persistent cellular activation in HIV-1/PTB co-infected patients may underlie the increased HIV-1 replication in PTB, and may be responsible for the excessive mortality associated with dual infection [22].

Our findings strengthen the need for prevention of active TB in early HIV infection. In fact it is possible that even latent MTB may lead to sufficient immune stimulation to contribute to low-grade increase in HIV-1 load and viral mutation over the years. This mechanism may be one possible reason for the high morbidity and mortality seen in countries where both HIV-1 and MTB infection are common. Considering the effect of TB on viral load in early HIV infection, it seems logical that efforts directed at treatment of latent TB should be particularly targeted to HIV-1 infected patients who have higher CD4 counts where prevention of active PTB may delay progression to AIDS. This would be more imperative in countries where both MTB and HIV-1 infection are common, especially when HAART for all HIV-infected patients is not feasible. There is currently little evidence for the potential role of latent TB in the pathogenesis of HIV-1 disease, and this theory needs further investigation.

Our studies also underscore the need for early HAART among patients dually infected with HIV-1 and pulmonary TB. Early HAART in HIV/PTB would abrogate the deleterious impact of TB on HIV-1 disease, especially in developing countries where both HIV-1 and MTB infection rates remain high. The use of HAART can induce sustained recovery of CD4 T-cell reactivity against opportunistic pathogens in severely immune-suppressed patients, which depends on amplitude...
and duration of viral-load reduction and is associated with increase in the memory CD4 T cells [23]. This suggests that early HAART in patients with active TB may positively impact on HIV-1 progression. WHO has recommended initiation of HAART for all HIV-1 infected patients with active PTB (http://www.who.int/tb/strategy/en/), but this has not yet been globally implemented.

Chapter 4 and 5.

In the previous studies, we showed that active MTB disease is associated with an increase in HIV-1 load, more significant at high CD4 T cell counts. Also successful anti TB treatment has variable effect on HIV-1 load, and this is related to the degree of immune activation. To further elucidate the immune mechanisms of the effect of MTB on HIV-1, we determined the expression and regulation of MIP-1-alpha and RANTES beta-chemokines and their CCR5 receptor in patients with active PTB with and without HIV-1 infection. We also compared the production of the CD4 Th1 cytokines TNF-alpha and IFN-gamma by peripheral blood mononuclear cells from HIV-1 infected and uninfected patients with newly diagnosed PTB, and the relationship of the cytokine profiles with the clinical presentation of tuberculosis. We showed that MTB induced high levels of beta-chemokines MIP-1-alpha and RANTES and increased the expression of CCR5 receptor on CD4 T cells. Viral load was significantly higher in HIV-1/PTB compared to HIV-1 alone, but they did not seem to be a direct correlation between the viral load and the chemokines and CCR5 induced by active PTB.

MIP-1-alpha induction was highest in PTB subjects, followed by the HIV-1/PTB patients, and lowest in HIV subjects; suggesting that in PTB, there is an increased ability of PBMC to produce MIP-1-alpha compared to PBMC from HIV-1 infected subjects. There conversely appeared to be a limitation in MTB-induced production of beta-chemokines in patients with HIV infection, regardless of the presence of PTB, even at high CD4 T cell levels (figure 10). The cell sources of MIP-1-alpha are multiple; CD4 T cells, possibly CD8 T cells, macrophages and even platelets may contribute to the production of MIP-1alpha [24 - 29]. The restriction in chemokine production in HIV/PTB subjects compared to PTB-only subjects may constitute an HIV related defect in lymphocyte cellular responses, both CD4 and CD8, and has been shown in other chronic diseases [30]. Low levels of CD4 T cells and impaired function of both CD4 and CD8 T cells, and macrophages may contribute to the decreased production of beta-chemokines in HIV-1.
disease. It is also possible that HIV-1 infection per se may down-regulate MIP-1-alpha production.

Figure 10: Dual HIV-1/PTB is associated with increased expression of CCR5, but seems to limit the MTB alone induced beta chemokine expression. The macrophage activating cytokine IFN gamma show variable expression at different CD4 levels, and this is associated with CXR presentation of PTB. TNF alpha is high irrespective of infection with HIV-1 in PTB infection, or the CD4 counts

We showed a significant correlation between the ability of PTB to up regulate MIP-1-alpha production in PBMC among subjects with high CD4 T cell counts (over 500 cells/μL), compared to patients with PTB at low CD4 T cell counts in the absence of HIV-1 infection, supporting the role of the CD4 cells in the production of MIP-1-alpha upon stimulation within tissues.
Additionally, CD4 cells may up-regulate production of MIP-1-alpha in CD8 positive T cells or monocytes/macrophages. This increase in subjects with PTB infection is in keeping with the role of MIP-1-alpha as an inflammatory chemokine. The increase of beta-chemokines in active TB does not seem to be related to virulence of MTB, where levels were shown to be comparable in cells stimulated with the virulent or avirulent strains of MTB [31]. The restricted release of beta-chemokines we demonstrated among patients with HIV-1/PTB at high CD4 T cell counts may contribute to the observed MTB dissemination in early HIV-1 infection, since the chemokines, as mediators of mononuclear cell chemotaxis have a role in the control of MTB spread and disease. High beta-chemokine (MIP-1-alpha, -beta and RANTES) levels may decrease HIV-1 entry into cells by their effect on interaction with, and blocking of, the HIV-1 coreceptor CCR5 [32] [33], an effect that seems to occur through interaction of all three beta-chemokines collectively [34]. M-tropic virus infectivity may be reduced with elevated levels of the CCR5 ligand cytokines RANTES and MIP-1β [33]. These beta-chemokines however do not block cell entry of the T tropic HIV which uses CXCR4 receptors. Thus the limited production of beta-chemokines in HIV-1/PTB may contribute to the higher viral load in dual disease.

The up-regulation of chemokines has been shown to be higher in alveolar macrophages compared to PBMC when these cells were cultured with MTB in vitro, indicating a greater effect in the lungs, and a potential for involvement of the beta-chemokines in granuloma formation [28], and is associated with high expression of CXCR4 mRNA (which supports T tropic HIV-1 entry into cells) and increased viral entry into alveolar macrophages in vitro [35]. Thus MTB, through up-regulation of beta-chemokines may promote change from M tropic to T tropic HIV-1, which may be another mechanism by which active TB promotes progression of HIV-1 disease in dual infection, by supporting change in viral tropism to the more pathogenic syncytial inducing HIV-1 T tropic strains.

It is also possible that high expression of MIP-1-alpha per se in HIV/PTB may contribute to an increase in viral load through its inflammatory role, by activating latently HIV-1 infected cells, and paradoxically turn on viral replication in recruited macrophages. Schmidtmayerova et al showed that RANTES, MIP-1-alpha, MIP-1-beta enhance HIV replication in macrophages as well as virus induced inflammation [36]. Thus the role of beta-chemokines seems unclear, and
the effect on HIV may be related to the viral load levels as well the stage of HIV infection and the degree of immune activation.

The pattern of expression of RANTES, another beta-chemokine was compared to that of MIP-1-alpha in PBMC cultured with or without MTB stimulation. Unlike MIP-1-alpha, RANTES was expressed in cultured PBMC from PTB subjects, even without MTB antigen stimulation, with significant up-regulation of RANTES when cultured with MTB. Addition of H37Ra did not appreciably increase RANTES in PBMC of subjects with HIV or healthy controls. These results suggest that RANTES levels ex vivo (in plasma) may be a potential correlate of MTB activity in the body. It would be interesting to determine the levels, and thus utility of RANTES among patients with mycobacteriosis.

The beta-chemokine receptor CCR5 showed significant up-regulation on the surface of CD4 T cells from HIV-1/PTB subjects. This effect of PTB up-regulation of chemokine receptors on CD4 T cells has also been shown with other HIV inter-current infections [33], so does not seem to be unique to mycobacterial infections. This increased CCR5 receptor expression may contribute to the mechanism of MTB accelerating the course of AIDS as reported in other studies [33] [35], since CCR5 is an essential co-receptor for HIV-1 entry into cells [34] [37]. Thus it is possible that MTB disease, through its effect on chemokine receptors could further potentiate the viral infectivity of cells and accentuate progression of HIV-1 disease. Some chemokine receptors, such as CXCR3, regulate migrati

TNF-alpha levels were comparable in PTB and HIV-1/PTB patients, regardless of CD4 T cell count. TNF-alpha is an inflammatory cytokine, produced by CD4 Th1 cells and monocytes/macrophages, and is increased in active PTB [39]. Since CD4 T cells are low in advanced HIV disease, it would have been expected that there would be less ability to produce TNF-1-alpha in HIV-1/PTB at low CD4 T cell levels, but this was not the case. TNF-alpha and beta-chemokines have been shown to interact in the control of MTB disease. MTB-induced chemokine secretion is partly dependent on TNF-alpha, where neutralizing antibody to TNF-alpha partially reduces beta-chemokine secretion in MTB stimulated alveolar macrophages [40].
Chemokines together with TNF-alpha may influence granuloma formation and thus contribute towards limiting spread of MTB at high CD4 T cell count [42] with a possible direct link between chemokine expression and level of MTB disease [43]. Thus TNF-alpha seems to play a potentiating role in the pro-inflammatory functions of beta-chemokines which would be beneficial in HIV-1/PTB. However both TNF-alpha and beta-chemokines may also contribute to increased viral load in HIV-1 infection. TNF-alpha, through its permissive role in the translocation of Nuclear Factor kappa B (NFkB) into the nucleus, promotes transcription of HIV provirus integrated in the human chromosome. Hence molecules which are important in the immune response to MTB are also used by HIV-1 for its replication and propagation. This underscores the way HIV-1 makes use of the human immune system for its survival, where in addition to leading to lower CD4 T cell function and numbers it also uses other immune systems for its survival.

When we looked at IFN-gamma regulation in HIV-1/PTB, we found marked variability in MTB-stimulated IFN-gamma production by PBMC from HIV-1–infected PTB patients, which related to the degree of immunodeficiency. HIV-1/PTB patients with high (>500) CD4 T cells had low IFN-gamma production similar to that of PTB (non HIV) patients, whereas more than two-thirds of patients with more advanced HIV-1 disease (200–500 CD4 cells/microL) had high IFN-gamma production. This finding was unexpected, as IFN-gamma is produced mainly by activated CD4 T cells. It is postulated that in at high CD4 T cells, early immune response may lead to “cellular exhaustion” with resultant decline in IFN production over time. This may explain why some patients despite a good immune response are unable to control their PTB infection. In advanced HIV-1, a possible decrease of IFN-gamma use may contribute to more severe MTB disease in the presence of high levels of this cytokine. Conversely it is possible that CD4 T cells are not the major source of IFN-gamma, and in advanced HIV-1 disease a combination of functional defects due to the lack of a permissive role of the “helper CD4 T cells” may contribute to the dissemination of TB disease. Schoenborn showed that IFN-gamma is produced predominantly by natural killer cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific
immunity develops. [44]. It is possible that with advanced HIV infection, and a deranged adaptive immune response, there is increased activity of the innate system, with high IFN-gamma levels. This hypothesis needs further study.

Both the clinical picture and the MTB stimulated IFN-gamma profile in PBMC of HIV-1/PTB patients with “normal” CD4 levels (>500 cells/mL) resembled those of PTB patients. Patients with low IFN-gamma production were more likely to have reactivation cavitating TB, compared to those with higher IFN-gamma production, which was associated with atypical non cavitating PTB in HIV-1 infection. A correlation between presence of cavities and low IFN-gamma production has been described in other TB patients [45]. When IFN-gamma knockout mice are infected with MTB, they can form granulomas, but are unable to produce reactive nitrogen radicles or to restrict growth of the bacilli [46].

TNF-alpha plays a role in tissue necrosis and cavitation. In advanced HIV-1 disease, with decreased CD4 T cell and function, less TNF-alpha function and or levels, may explain the lack of cavitation in advanced AIDS. Thus it appears that in AIDS with low CD4 T cell levels, TNF-alpha is decreased while IFN-gamma is not. These data suggest the potential pathogenic influence of these macrophage activating cytokines in TB pathology in HIV-1 infected patients.

High IFN-alpha in advanced HIV-1 disease as shown in our results, may up regulate CCR5 and accelerate HIV-1 disease progression as was shown in other studies [47]. IFN-gamma induced secretion of beta-chemokines (RANTES, MIP-1-alpha, and MIP-1-beta) by mononuclear phagocytes may also suppress HIV entry into macrophages. [48]. Lee et al showed attenuation of production of MIP-3-alpha by TNF-alpha or IFN-gamma [49], suggesting that excess production of these Th1 cytokines in patients with PTB may have a negative impact in active disease. Thus an interplay between chemokines and cytokines seems to determine the expression of dual HIV-1 and PTB disease.

In early HIV infection, rapid depletion of MTB-specific Th1 CD4 cells may be a key to the risk of TB at this stage [10]. It is unclear if high MTB induced IFN-gamma production in the lower CD4 T cell group (200–500 CD4 cells/mL) of HIV-1/PTB patients is due to “overactivity” of the immune response during this stage of HIV-1 disease or to over production of IFN-gamma by
non-CD4 cells in response to MTB. A non-CD4 basis for excessive IFN-gamma production in MTB-infected mice that were rendered CD4 deficient has been suggested [50].

Another hypothesis is that since the majority of HIV-1/PTB patients with 200–500 CD4 cells/mL also had radiographic evidence of atypical TB, which may be consistent with primary TB, high IFN-gamma levels in this group may reflect higher induction of this cytokine during primary TB, as seen in children [51]. Understanding the biology of IFN-gamma production and responsiveness among HIV-1/PTB patients is important, because this cytokine may have a predominant protective role in MTB infection, and is emerging as a possible immunotherapeutic agent for patients with hard-to-treat TB [46]. The heterogeneity of HIV-1/PTB patients in the capacity to produce IFN-gamma in response to MTB needs to be considered in potential immunotherapies.

The mononuclear cell derived cytokines may modulate chemokines, and their receptor expression and function. IFN-gamma upregulates CCR5 leading to increased HIV tropism and infection of macrophages [47], [48]. IFN-gamma also seems to increase the beta-chemokine induced chemotaxis [48], while in vitro blockage of TNF-alpha and IFN-gamma inhibit upregulation of CCR5 and CXCR4 receptors by antigenic stimulation [33].

Chapter 6.

To assess the impact of dysregulations in chemokines and Th1 cytokines in HIV-1 activation during MTB infection, we examined the role of TNF-alpha cytokine and MCP-1 chemokine interaction in vitro on MTB-induced HIV-1 transcriptional activation in mononuclear cells of HIV-1/TB subjects with pleural TB.

The basis of HIV-1 expansion at sites of active TB in dually infected HIV-1/TB subjects includes both transcriptional activation of infected cells and spread of virus to newly recruited uninfected mononuclear cells. Monocytes from tuberculosis patients express high MCP-1 mRNA and protein compared to cells from tuberculin positive healthy persons [52]. This MCP-1 is chemotactic for monocytes and T lymphocytes, and plays a role in the immune response to MTB. Patients who produce large amounts of MCP-1 are more likely to progress to active MTB disease [53]. Also, HIV-1 viral load has been shown to be positively associated with MCP-1 levels [54]. In active TB, both MCP-1 and TNF-alpha are elevated, and both are implicated in
transcriptional activation of HIV-1. We therefore examined the role of MCP-1 and TNF-alpha in activation of HIV-1 during TB and by MTB in mononuclear cells from HIV-1/TB subjects with pleural TB. We showed very high levels of MCP-1 (as compared with TNF-alpha) protein and mRNA in pleural fluid and pleural fluid mononuclear cells (PFMC), and higher HIV-1 transcription in pleural fluid, compared to PBMCs, of patients with HIV-1/TB pleurisy. Both MTB and its products, as well as HIV, are known to induce MCP-1 from multiple cell types [28][55]. Thus the high levels of MCP-1 in pleural fluid of HIV-1/TB subjects may be due to both active MTB and HIV-1 infection, and may contribute to the increased HIV-1 transcription at sites of MTB disease. MTB infected patients whose cells are genetically programmed to produce large amounts of MCP-1 are more likely to develop active disease [53].

In order to dissect out the roles of MCP-1 and TNF-alpha in promoting HIV-1 transcription we conducted in vitro studies where we neutralized either or both molecules in cell cultures from HIV-1 pleural-TB patients. We found that neutralization of MCP-1 (but not TNF-alpha) resulted in lower MTB-induced HIV-1 mRNA transcription, suggesting a predominant role of MCP-1 over TNF-alpha in HIV-1 transcription in dually infected subjects. It thus seems that MCP-1 activity may be critical to activation of HIV-1 at sites of TB (figure 11).

![Diagram](image)

**Figure 11**: Inhibition of MCP, but not TNF alpha is associated with a decreased HIV-1 transcription in mononuclear cells.
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Figure 11: Inhibition of MCP, but not TNF alpha is associated with a decreased HIV-1 transcription in mononuclear cells.

MCP-1 has been shown to be associated with high HIV-1 load in other conditions. Levels of MCP-1, as well as MIP-1 alpha and beta and RANTES, were found to be higher in HIV-1 associated dementia, where MCP-1 levels correlated with CSF viral load and severity of dementia [56][57].

It is possible that viral activity in HIV-1/PTB dual infection may be inhibited by molecules that block the bioactivity of MCP-1, and this may be helpful in decreasing viral activity in dually infected HIV-1 /TB subjects, taking into consideration the interplay of MCP-1 and TNF-alpha.

Chapter 7, 8.
Next we looked at effects of immune modulation mechanisms on HIV-1 load in HIV-1/PTB patients. We assessed the effect of blockage of CCR5 receptor using RANTES analogues on HIV-1 entry into cells in vitro, and in vivo prednisolone immunoadjuvant therapy on HIV load and cytokine expression in HIV-1/PTB patients.

The beta-chemokines MIP-1-alpha, MIP-1-beta and RANTES may inhibit HIV-1 infection of CD4 T cells and macrophages by inhibiting interactions between the virus and CCR5 [32][37]. RANTES has pro-inflammatory properties through chemotaxis of mononuclear cells and granulocytes to inflammatory sites. Also, through its effect on CCR5 receptor, RANTES may suppress HIV-1 infection of cells. When the amino terminus of RANTES is chemically modified, it loses many of its biological activities, and produces RANTES analogues which are able to sequester CCR5 into the cells, thus down-modulating its ability to enhance HIV-1 entry into cells [58].

In our studies, we showed higher expression of CCR5 on pleural monocytes than on the CD4+ lymphocytes, as well as higher HIV-1 infection in macrophages at sites of MTB infection. When we co-cultured mononuclear cells with MTB and chemically modified RANTES analogues (AOP and NYY RANTES), we showed significant decrease in HIV-1 in the cells (figure 12).
Other researchers have shown inhibition of HIV-1 entry via blockage of the CCR5 co-receptor by RANTES analogues, or by the more recently described small molecule CCR5 inhibitors [59]. These studies suggest potential chemotherapeutic activity of these analogues, which may be useful in the management of HIV-1/TB patients. Blockage of CCR5 receptors is unlikely to be associated with deleterious effects in humans, as persons with a mutant form of the CCR5 gene (CCR5-delta32) or congenital absence of this receptor on cell surface expression have no harmful phenotypes in humans.

In other studies, topical RANTES analogues have been shown to protect rhesus monkeys against SIV infection, and have potential in the prevention of HIV infection [60] [61]. These chemically modified RANTES analogues are more potent than RANTES at inhibiting the entry of primary HIV-1 isolates into host mononuclear cells through inducing CCR5 sequestration [62]. Also
studies have shown that HIV-1 isolates are highly susceptible to inhibition by these topical RANTES analogues, the effect varying up to 2 log decrease [63]. Although our studies showed blockage of HIV-1 replication in cells by AOP and NYY RANTES analogues this effect was variable, with higher inhibition in macrophages compared to lymphocytes of MTB-induced cultures from HIV-1 pleural TB subject. This suggests that some Tropic HIV-1 isolates from HIV-1/PTB co-infected patients may be less susceptible to blockage by these RANTES analogues. However, variable effect of beta-chemokines on the inhibition of HIV-1 infection in mononuclear cells has been shown by others. In one study, (non TB cells) TB RANTES significantly increased the antiviral effects in lymphocytes, but not in macrophages in cells from HIV-1 infected patients without tuberculosis [64]. Other beta-chemokines and their analogues can often enhance HIV-1 replication independent of the entry step [65]. Thus it appears that MTB with its activation of macrophages affects the response to the chemokines. In vitro cultures of cells from HIV-1 patients showed an initial restriction of HIV-1 entry in macrophages in vivo, but this is lost during long-term in vitro cultures. This potential variable effect of beta-chemokines in HIV-1 infection of cells was further amplified by studies by Kelly et al in which HIV-1 replication was increased in cells that had been exposed to beta-chemokines before HIV infection, but the viral replication was inhibited in cells that had been exposed to beta-chemokines either simultaneously with or after HIV infection [66]. It is thus possible that in patients with latent TB (which is as high as 70% in sub-Saharan Africa) who then acquire HIV-1 infection, low-level chronic stimulation of beta-chemokines may control MTB spread, but may potentiate HIV-1 replication. Latent TB infection chemoprophylaxis is not given to healthy adults in sub-Saharan Africa, but in the face of HIV-1 infection, this issue may call for further review.

Our study suggested that the CCR5 beta-chemokine inhibitors may be helpful during HIV-1/TB in preventing the expansion of HIV-1 reservoirs, with the potential to inhibit rapid progression of HIV-1 disease. However whether the HIV-1 inhibitory effects of RANTES analogues observed in this study can be extended in vivo in chemotherapeutic trials of HIV-1/TB patients remains unclear, and needs further elucidation in view of these and other studies where the effect varies with different conditions.
We next looked at in vivo modulation of immune responses in HIV-1/PTB patients treated with standard anti-TB treatment without HAART randomized to adjunct prednisone, as well as its effect on viral load. As shown in our prior studies, PTB in HIV-1 infected persons is associated with increased viral load compared to patients with HIV-1 alone at comparable CD4 T cell levels. Also successful treatment of PTB seems to have little impact on the PTB associated increase in viral load, and a poorer prognosis after active TB compared to HIV-1 infection without active TB. Patients with HIV-1 and active TB respond well to anti-TB therapy, but without HAART the prognosis remains poor with subsequent progressive HIV disease [67 - 70].

Active TB, through up-regulation of cytokines such as TNF-alpha and beta-chemokines activates mononuclear cells with transcription of HIV-1 provirus in these cells and subsequent increase in HIV-1 load is associated with cellular immune activation [71], followed by stimulation of HIV replication in cells latently harboring both MTB and HIV-1 provirus and a significant increase in viral load with progression of HIV disease. Studies using TNF inhibitors such as etanercept did not appreciably ameliorate this interaction [72].

To counteract TB induced immune activation and its deleterious effect, we hypothesized that global immune suppression in HIV-1 /PTB may ameliorate the impact of PTB in HIV-1 infection. We thus conducted a randomized trial to determine the effect of global immune modulation with immunoadjuvant glucocorticoids in HIV-1/PTB. In a phase 2 clinical trial, patients with HIV-1/PTB were randomized to addition of prednisone (or placebo) for 2 months together with standard anti-TB treatment in the absence of HAART. This two month adjunct prednisolone therapy reduced levels of immune activation as evidenced by a significant reduction in TNF-alpha levels, tended to produce higher CD4+ T cell counts, and was associated with faster clearance of MTB in sputum. However we also noted an increase in HIV load which receded with end of corticosteroid therapy, but with no increase in HIV-associated conditions or mortality. Patients on prednisone also had more side effects, mainly leg edema, transient hyperglycemia, and in one case worsening of existing hypertension (figure 13).
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Figure 13: In a double blind randomized clinical trial, high dose prednisone showed beneficial effects on MTB induced immune activation, increase in CD4 T cell counts, but was associated with higher HIV-1 load and had significant adverse effects.

Prednisone has been used anecdotally by different HIV-1 care providers both in absence of TB and in various extra-pulmonary tuberculosis situations in the hope of improving clinical outcome, limit sequelea and sometimes in an attempt to ameliorate the “wasting syndrome” seen in AIDS. In HIV-1 associated pleural TB, adjunct prednisone was associated with rapid clearance of the pleural effusion but with high risk of HIV-1 progression and Kaposi sarcoma. [73]. Although the potential for serious side effects and progression of HIV-1 may limit use of corticosteroids in HIV-1/AIDS, they still have a role in specific HIV-1 related infections.
In TB-associated immune reconstitution inflammatory syndrome (TB-IRIS), prednisone has been shown to be beneficial in patients with severe symptomatic immune activation. In a South African study, prednisone reduced the need for hospitalization and therapeutic procedures, and hastened improvements in symptoms, CXR and quality of life among patients with paradoxical TB-IRIS [74]. However low dose prophylactic adjunct prednisolone in patients on HAART and anti-TB treatment showed no appreciable benefit [75].

Prednisone in HIV-1 has also been shown to have a role in HIV-associated nephropathy (HIVAN), especially with heavy proteinuria where it limits the risk of progression to end stage renal disease [76 - 78]. In addition, among patients with HIVAN on HAART, prednisone use has been associated with reduced rate of decline in creatinine clearance [79].

Other conditions where adjunct prednisone is used among AIDS patients include severe pediatric lymphocytic interstitial pneumonitis (LIP) and dilated cardiomyopathy with evidence of inflammation [3]. Among patients with herpes zoster ophthalmicus on acyclovir, corticosteroids may diminish ocular sequelae although their use among HIV-infected patients are still uncertain, but may be beneficial in uveitis [80, 81].

In a study among patients with tuberculous meningitis aged 14 years and above, adjunctive dexamethasone improved survival irrespective of HIV-1 infection, but did not seem to prevent severe disability. In the same study, serious adverse events were fewer in the corticosteroid group compared to the placebo group [82, 83].

Our data does not show clinical benefit of oral prednisone in HIV-1/PTB patients without HAART. This study however does have clinical implications. In resource-limited settings, health care workers still use prednisone in HIV-1 and AIDS patients as a supplement to improve appetite and weight, especially where HAART is not available in various HIV associated conditions. This may probably not be advisable especially in a high TB endemic area, with limited facilities for diagnosing TB and with rising drug resistant TB. However, results of our study would guide clinicians on activity and side effects of prednisone use among carefully selected patients where oral corticosteroids are recommended in various HIV-1/AIDS infections with and without the use of HAART. Also because of the potential for increase in viral load, it is advisable to use corticosteroids together with HAART whenever possible.
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