Abstract

The dual epidemic of human immunodeficiency virus type-1 (HIV-1) infection and Mycobacterium tuberculosis (M.tuberculosis) in the past few decades have shown to have a syndemic interaction that exacerbates the morbidity and mortality associated with each pathogen alone. HIV-1 infection is the most powerful known risk factor predisposing individuals to M.tuberculosis infection and progression to active disease, which increases the risk of active disease by 20-37 times, depending upon the prevalence of HIV in the population. The mechanism behind the breakdown of immune defense and synergistic effect is still not well understood. Any input into these mechanisms will lead to better understanding of the disease and will be of clinical importance as far as designing of newer and effective controlling strategies are concerned. In this study, we aim to highlight few of the immunological events that may influence and accelerate the development of the disease in the presence of co-infecting organism.

A cross-sectional study was performed on 67 individuals including antitubercular therapy (ATT) naïve active pulmonary tuberculosis (PTB) individuals, antiretroviral therapy (ART) naïve HIV-1 infected individuals at different stages of disease, ATT and ART naïve HIV-PTB co-infected individuals and healthy controls. The profile (immunophenotypic and functional) of T-regulatory (Treg) cells in HIV-1 at different stages of the disease and HIV-PTB co-infected individuals in peripheral blood was evaluated. The expression of CCR5 and CxCR4 on CD4 T-cell subpopulations were evaluated in the peripheral blood to assess their possible role or association with change in Treg cell frequency during the HIV-1 disease progression and PTB co-infection. Further we also investigated the association of FoxP3 splice variants, heme-oxygenase 1, NF-κB and Rac1 with disease state and their possible role in inducing HIV-1 replication or shift in the viral tropism.

There was a significant decrease in the frequency of Treg cells in early phase of HIV-1 infection that correlated with significant decrease in levels of Foxp3 expression. This lower frequency of Treg cells correlated well with significantly higher CCR5 expression on CD4+CD25^{high} Treg cells in HIV-1 subjects in early stage
of the disease. However, with the disease progression these correlations were reversed. We noticed a significant inverse relationship of CD4 count with respect to the frequency of Treg cells and FoxP3 expression making these subjects more immuno-compromised. These findings are in agreement with the Treg function and FoxP3 variant 1 mRNA expression and not with variant 2 or the whole FoxP3 gene expression. The immunosuppressive environment in the advanced stage of the disease is further exacerbated and made favorable for TB co-infection with enhanced expression of HO-1. Higher CxCR4 expression on CD4^+CD25^{low/negative} T-cells (non-Treg cells) and increase in the Rac1 expression in later stage of the disease could explain the tropism shift to X4 type. This is supported by a decrease in the percent of CxCR4^+ cells in CD4^+CD25^{low/negative} population within HIV-1 groups as the disease progresses. With the onset of PTB co-infection in HIV-1 individuals, we observed further increase in Foxp3 expression in CD4 T-cells in comparison to individuals infected only with HIV-1 with similar CD4 count (<250 cells/µl). Besides, there was a concomitant increase in the frequency of FoxP3 positive cells among CD4 T-cells and increased mRNA expression of FoxP3 variant 1 in PBMC when compared to healthy controls. PTB co-infection was also associated with significant increase in CCR5 expression on Treg cell population and increased CxCR4 expression on CD4^+CD25^{low/negative} subset. There was a decrease in the HO-1 expression which correlates with significant higher expression of redox sensitive NF-κB in these co-infected individuals when compared to only HIV-1 infected individuals with similar CD4 count.

Apparently, the study provides novel inter-relationships of co-receptor expression and their regulatory genes in therapy naïve individuals and how the virus manipulates the host machinery to its advantage. Treg cells expressing high CCR5 co-receptor during early phase of HIV-1 infection made these cells preferential initial targets. This study lends support and indicates significant positive correlation of inhibitory factor like HO-1 with FoxP3 in HIV infected patients, while making the environment favorable for mycobacterial infection. Decrease in HO-1 expression associated with *M.tuberculosis* infection in HIV subjects result in the increase in redox stress which correlates with increase in NF-κB expression in this group that in
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Reactivation of HIV-1 provirus resulting in high plasma viral load. Further, PTB co-infection also associates with significant down-regulation of Rac1 expression making CxCR4 non-usable by HIV, which explains the predominance of R5 viral variants in HIV infected individuals with active TB. Our study is a modest attempt to delineate the inter-relationships between genes that may play important role in establishing a synergism between these two killer bugs in a common human host.