Latent and Active TB Infection Increase Harmful Immune Activation in Individuals Co-Infected with HIV

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BACKGROUND

In HIV infection, surface markers of T-cell activation (CD38, HLA-DR) and several soluble markers of inflammation and fibrosis (sCD14, IL-6, IL-8, CRP and hyaluronic acid) have been found to independently predict progression to AIDS and mortality. Co-infections such as CMV and active TB have been shown to contribute to immune activation; but the impact of latent TB infection, which is widely endemic in the areas hardest hit by the global AIDS epidemic, has not been evaluated. We hypothesize that active and latent states of *M.tb.* co-infection are associated with elevated immune activation as measurable by these markers.

METHODS

84 HIV-positive, antiretroviral naïve individuals from the iThimba cohort in KwaZulu-Natal, South Africa were initially classified as having:

(1) No evidence of TB infection (**No TB**)
(2) Latent TB infection (**LTBI**)
(3) Active culture-positive pulmonary TB (**Active TB**).

The **Active TB** group in this cohort had lower CD4 counts than the **No TB** or **LTBI** group; thus additional patients were selected from two other Durban cohorts to create groups for CD4-matched comparison:

(4) A group with no evidence of active TB and lower CD4 counts (**No active TB**) and
(5) A group with active TB and higher CD4 (**Active TB, higher CD4**).

Plasma concentrations of sCD14, CRP, IL-6, IL-8 and IP-10 were measured by multiplexed bead-based immunoassays and plasma concentration of hyaluronic acid was measured by ELISA. Expression of CD38 and HLA-DR on cryopreserved CD4+ and CD8+ T lymphocytes were measured by flow cytometry. Comparisons were made using the Mann Whitney U test (non-parametric), student's t-test (parametric) and ANOVA (three groups). All p-values were adjusted for multiple hypothesis testing using the Bonferroni correction.

In HIV patients matched by CD4 cell count, soluble markers of Inflammation are elevated in active, but not latent, TB co-infection.

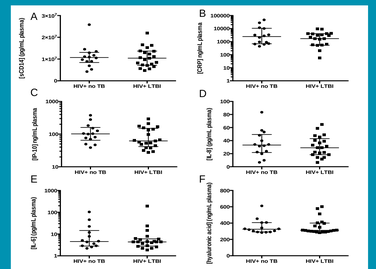

Figure 1: Plasma concentrations of sCD14 (A), CRP (B), IP-10 (C), IL-6 (D), IL-8 (E), and hyaluronic acid (F) in HIV-infected individuals with either no evidence of TB, or latent TB infection.

Characteristic	HIV+ no TB n=13	HIV+ LTBI n=21	P
Age, median years (IQR)	35 (30.5-47)	35 (29-50)	0.95
Female sex, no.(%)	11 (85)	17 (81)	0.99
CD4 T-cell count, median cells/uL (IQR)	425 (322-498)	426 (357.5-541.5)	0.29
HIV RNA, median log10 copies/mL (IQR)	3.7 (3.1-4.1)	4.1 (3.2-4.7)	0.34

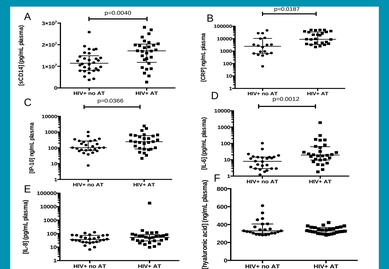

Figure 2: Plasma concentrations of sCD14 (A), CRP (B), IP-10 (C), IL-6 (D), IL-8 (E), and hyaluronic acid (F) in HIV-infected individuals with either no evidence of active TB, or active TB disease.

Characteristic	HIV+ no AT n=30	HIV+ AT n=28	P
Age, median years (IQR)	34 (29-40)	29.5 (25.8-37.3)	0.14
Female sex, no.(%)	22 (73.3)	17 (63.0)	0.4
CD4 T-cell count, median cells/uL (IQR)	289 (104.3-405.3)	166.5 (29.75-391.8)	0.81
HIV RNA, median log10 copies/mL (IQR)	4.3 (3.4-4.8)	4.8 (3.8-5.3)	0.17

Definition of five groups used for CD4-matched comparisons

Group	Cohort	TB status	Mantoux skin test	M.tb RD-1 ELiSpot	Chest X-ray	sputum culture	CD4 count
HIV+ no TB	iThimba	No evidence of latent or active TB infection	<5mm	negative	normal lung parenchyma	negative	>250
HIV+ LTBI	iThimba	Latent TB infection	>5mm	positive	normal lung parenchyma	negative	>250
HIV+ AT	iThimba	Active TB infection, micro confirmed	variable	variable	abnormal lung parenchyma	positive for M.tb	any
HIV+ no AT	Ithimba and Sinikithemba	No evidence of active TB infection	< 5mm or not done	negative or not done	normal or not done	negative or not done	any
HIV+ AT, higher CD4	iThimba and String	Active TB infection, micro confirmed	variable	variable	normal or not done	positive for M.tb	>250

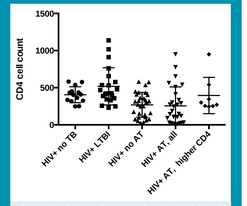

Figure 5: CD4 cell counts for the individuals in each group demonstrate that the HIV+ no TB, HIV+ LTBI and HIV+ AT, higher CD4 groups are well matched on this parameter.

However, both latent and active TB are associated with lymphocyte activation.

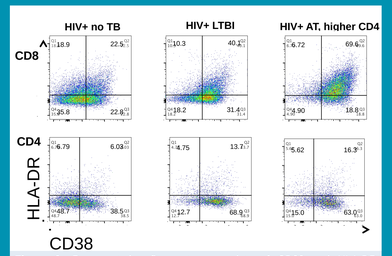

Figure 3: Representative flow cytometry plots of CD38 and HLA-DR expression on CD8+ (top panel) and CD4+ (bottom panel) T-cells from HIV-infected individuals with no evidence of TB, latent TB infection, or active TB. Cells gated on live, lymphocytes (FSC vs. SSC), and CD3+. HLA-DR and CD38 gates were determined by FMO.

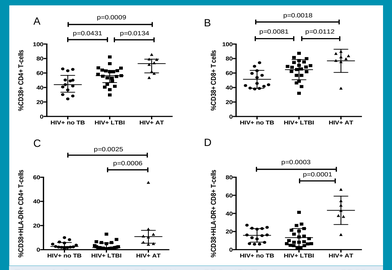

Figure 4: Percentage of CD4+ (A) and CD8+ (B) T-cells expressing CD38 in HIV-infected individuals with no TB, latent TB, and active TB (higher CD4). Percentage of CD4+ (C) and CD8+ (D) T-cells co-expressing CD38 and HLA-DR in HIV-infected individuals with no TB, latent TB, and active TB (higher CD4).

Characteristic	HIV+ no TB n=13	HIV+ LTBI n=21	HIV+ AT higher CD4, n=8	P
Age, median years (IQR)	35 (30.5-47)	35 (29-50)	33 (27-49)	0.95
Female sex, no.(%)	11 (85)	17 (81)	4 (50)	0.16
CD4 T-cell count, median cells/uL (IQR)	425 (322-498)	426 (357.5-541.5)	286 (253.8-492)	0.33
HIV RNA, median log10 copies/mL (IQR)	3.7 (3.1-4.1)	4.1 (3.2-4.7)	4.4 (3.5-5)	0.4

KEY RESULTS

Active TB was associated with elevated plasma levels of sCD14, CRP, IP-10, and IL-6 compared to those with no evidence of active TB. Latent TB was not associated with elevated plasma biomarkers of inflammation. Markers of lymphocyte activation, CD38 and HLA-DR, were highest in those with active TB, but also elevated in individuals with latent TB as compared to those with no evidence of TB infection.

CONCLUSIONS

Both active and latent TB infection were associated with elevated levels of markers of immune activation and inflammation that have previously been shown to predict more rapid disease progression in HIV-infected individuals. These results suggest that in the highly TB- and HIV-endemic settings of Southern Africa, latent TB infection may contribute to HIV disease progression and exacerbate the HIV epidemic. Further work to determine if treatment of latent TB infection improves lymphocyte activation is needed, as this would provide an additional rationale for the use of isoniazid preventative therapy (IPT) in those with HIV and latent TB infection.

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