Plasma concentrations of transforming growth factor beta 1 in non-progressive HIV-1 infection correlates with markers of disease progression

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1. Background

The pathogenesis of Human Immunodeficiency Virus (HIV) is known for its extremely heterogeneous manifestations. HIV infection is characterized by gradual loss of CD4⁺ T cells, which leads to the loss of immune competence, susceptibility to opportunistic infections and persistent systemic immune activation resulting in Acquired Immune Deficiency Syndrome (AIDS) [1]. In most HIV infected individuals, clinical and immunological deficits develop within a few years after infection and these individuals are referred to as typical progressors (TPs). However, a small proportion of infected individuals described as long-term non-progressors (LTNPs), remain healthy and immunologically stable for a long period of time after infection, usually more than ten years [2]. The absence of any apparent disease progression in LTNPs for a long period of time is particularly intriguing and factors governing the maintenance of this condition are not fully explored. However, some important points have been recently addressed to shed light on the ability of LTNPs to control human immunodeficiency virus type 1 (HIV-1) infection. LTNPs are characterized by low plasma viral loads, strong neutralizing antibody response and vigorous inhibitory CD8⁺ lymphocytes [2]. Although the balance between inflammatory mediators and their counter-regulatory molecules may be crucial for progression of HIV-1 infection, little is known about the role these mediators play in HIV-1 non-progressive disease. While cytokine interactions during HIV-1 infection has not received much attention, induction of certain cytokines, including IFN- γ , TGF β 1 and TNF- α , with viral replication inhibitory activities have been suggested [3]. A beneficial role of regulatory T-cells and related cytokines such as IL-10 and TGF β 1 has also been suggested. These cytokines limit immune activation, thereby controlling the availability of HIV-1 targets as well as preventing immunopathology [4]. Although the presence of regulatory cytokines including TGF β 1 and IL-10 have been analyzed for their role in regulating immune responses to a variety of pathogens [5–8], their importance in HIV-1 infection remains under explored.

TGF β 1 is known to inhibit several events in T-cell activation [9] and function, culminating in low production and/or functionally defective forms of cytokines important in host immune response [10]. Contribution of TGF β 1 immunosuppression and association with viral infections including cytomegalovirus [5,8] hepatitis B [11] and Epstein-Barr virus [7] have been suggested. Evidence suggests that TGF β 1 is also involved in several aspects of HIV-1 infection, including macrophage deactivation and CD4⁺ T cell apoptosis [3] through multiple distinct mechanisms. It has been reported that patients with HIV-1 have increased production of TGF β 1 from peripheral blood mononuclear cells (PBMC), and this has been suggested as being responsible for the inability of these individuals to mount effective inflammatory responses [12]. Production of TGF β 1 following macrophage infection with HIV-1 has been suggested to contribute to the replication of the virus within these cells [13], and this increases the infectivity of HIV-1 isolates for macrophages [14]. Further,

TGF β 1, being a potent macrophage deactivating molecule [15], disarms the very effector cells that contain invading pathogens.

Although plasma levels of TGF β 1 isoforms have been measured in several diseases [5,7,8], the significance of circulating levels of this profibrotic factor in HIV-1 disease progression remains unclear. TGF β 1 requires liberation from its inactive latent forms and small latent TGF β complex to its biologically active forms in order to act on its receptors. Although total levels of TGF β 1 in HIV-1 infection have previously been suggested, discrimination of active TGF β 1 levels in HIV-1 infection in relation to disease progression has not been reported. Measuring active TGF β 1 levels in individuals with progressive and non-progressive HIV-1 infection would inform whether this cytokine, together with other cytokines with antiviral properties such as TNF- α , IL-1 β and IL-12p70, is an important mediator in HIV-1 infection and its association with disease progression. In this study, we measured plasma levels of active TGF β 1 in well-characterized patients with progressive and non-progressive HIV-1 infection. We also determined the interplay between Th1 and Th2 cytokine profiles in relation to the progression of HIV-1 infection. Our aim was to investigate, as suggested by *in vitro* observations, the correlation(s) of immunosuppressive, Th1 and Th2 cytokine levels with variables known to associate with disease progression as well as HIV-1 disease status under *in vivo* conditions. We report that plasma levels of Th1 cytokines TNF- α , IL-1 β and IL-12p70 are elevated in patients with non-progressive infection while levels of immunosuppressive cytokines TGF β 1 and IL-10 are decreased compared to individuals with progressive HIV-1 infection. Additionally, elevated plasma levels of active TGF β 1 inversely correlates with variables known to associate with disease progression including CD4⁺ T-cell counts and CD4:CD8 ratios. These findings suggest that active TGF β 1 may be vital in pathogenesis of HIV-1 infection, findings that may have implications for interventions including development of vaccines and systemic TGF β 1-therapies in HIV-1 infection. Moreover, elevated plasma levels of active TGF β 1 in HIV-1 infection may identify individuals likely to progress fast with HIV-1 infection where CD4 T-cell count and viral loads data are unavailable.

2. Materials and Methods

2.1 Study subjects

Patients were recruited over a 12-months period in two hospitals that provide support and care to HIV infected patients in Ghana. Fourteen non-progressive and 47 progressive HIV-1 infected patients along with 12 healthy controls were enrolled into the study. Non-progressive HIV-1 infected patients were defined as having been infected with HIV-1 for over 10 years, without any sign/symptoms of HIV disease progression, had maintained a stable CD4⁺ T-cell counts (> 500 cells/mL) over the period of infection and ART naive. On the other hand, progressive HIV-1 infected patients included individuals who within three years after infection had their immune system severely compromised as indicated by low CD4⁺ T-cell count (< 200 cells/mL), had clinical HIV-1 disease progression, and ART exposed. The Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research approved the study and all subjects provided written informed consent to participate. Patients were categorized according to clinical status, HIV-1 disease progression and antiretroviral treatment (ART) history, as having progressive or non-progressive HIV-1 infection. Baseline characteristics of these patients are shown in Table 1.

2.2 Patients inclusion criteria

HIV-1 infected patients who provided written informed consent and satisfied the group-based inclusion criteria were included in the study. They included men and women between 18 and 65 years old.

2.3 Patients exclusion criteria

HIV-1 infected patients with a history of high blood pressure, coronary heart disease, diabetes, stroke, tumor, history of inflammatory bowel disease, having any active inflammatory conditions or chronic infection/disease,

showing any signs and symptoms of opportunistic infections, history of alcohol or tobacco abuse, on immune modulators or anti-inflammatory drugs, pregnant women and those who refused to provide written informed consent were excluded from the study. Patients below 18 and above 65 years were also excluded from the study.

2.4 Absolute T-cell counts enumeration

Four-color flow cytometry was performed on fresh peripheral blood using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) with data acquisition using CellQuest software. Absolute CD4⁺ and CD8⁺ T-cell counts were determined using Multitest CD3/CD4/CD8/CD45 kit (BD Biosciences) following the manufacturer's instructions. Conjugated antibodies were as follows: CD4-allophycocyanin (APC), CD8-phycoerythrin (PE), CD3–fluorescein isothiocyanate (FITC) and CD45-peridinin chlorophyll protein (PerCP) (BD Biosciences).

2.5 Determination of HIV-1 infection

Plasma was separated from patients' blood collected in EDTA tubes. HIV-1 infection was confirmed by detection of HIV-1 antibodies in patients' plasma using INNO-LIA™ HIV I/II kit (Fujirebio Europe N.V) following manufacturer's instructions.

2.6 Quantification of plasma HIV-1 RNA

Peripheral blood plasma viral loads were determined using the automated Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0. Viral RNA was extracted from plasma samples and quantified by HIV-1 TaqMan assay with a lower limit of detection of 20 copies/ml, strictly following the manufacturer's instructions. RNA was extracted from the plasma samples and the reverse-transcriptase polymerase chain reaction (RT-PCR) was performed with the GeneAmp kit (Roche Diagnostics, Nutley, N.J.) The CobasAmpliprep/CobasTaqMan 48 analyzer (Roche Diagnostics, Ltd., Rotkreuz, Switzerland) was used for automated extraction, amplification, and quantification following the Roche manufacturer's standard guidelines.

2.7 Cytokine assays

TGF β 1 levels in plasma samples were measured using the MILLIPLEX® MAP TGF β 1 single plex magnetic bead kit (EMD Millipore Corporation) on LuminexMAP®200 system following manufacturer's instructions. The assay specifically measures active TGF β 1 forms. Briefly, to measure total TGF β 1 present in plasma samples, biologically latent TGF β 1 was activated by acid-treatment. For this purpose, plasma samples were diluted with four volumes of latent TGF β buffer (EMD Millipore Corporation). Samples were then incubated for 1h at room temperature in the presence of 0.02 vol of 1 N HCl, then neutralized with equal volume of 1N NaOH. Analysis was performed in 96-well plates following the instructions of the manufacturer (EMD Millipore Corporation). Plasma levels of IL-10, IL-12p70, IL-13, IL-1β, IFN-γ and TNF-α were measured using a premixed MILLIPLEX® MAP Human Th17 magnetic bead panel kit (EMD Millipore Corporation) on LuminexMAP® 200 system following manufacturer's instructions. To minimize inter-plate variability, each subject's samples were prepared on the same plate.

2.8 Statistical analysis

All statistical analysis was performed using the GraphPad Prism 6 statistical package. Significance of differences between study groups was calculated using non-parametric unpaired t-test. For correlation analyses, the Pearson correlation was used. A p-value of <0.05 was considered significant.

3. Results

3.1 Patients' characteristics

HIV-1 infection was confirmed based on positive HIV-1 antibodies in patients' serum as discriminated by INNOLIA HIV I/II immunoassay (Fujirebio Europe N.V). All patients underwent clinical and biochemical examinations and were excluded from the study if found positive for any condition(s) stated under exclusion criteria. Medical history of each patient under the study was retrospectively assessed and those found to have underlying chronic infections/diseases excluded from the study. All patients were asymptomatic and none of the studied patients exhibited symptomatic signs of infection or concomitant acute opportunistic infections. Forty seven patients had progressive HIV-1 infection and all were on antiretroviral therapy (ART) with variable baseline CD4⁺ T-cell counts. Fourteen of these ART-experienced patients had been switched to second line ART on account of treatment failure. Patients with progressive HIV-1 infection had been infected for a mean of 6.4 (1-12) years, and on ART for 4.6 (0.1-10) years. Fourteen patients were with non-progressive HIV-1 infection and had been infected for a mean of 11.2 years, were ART-naïve and had maintained CD4⁺ T-cell counts above 500 cell/ml over the period of infection (Table 1).

Table 1. Demographic and immunological characteristics of study subjects

Group	N	CD4 ⁺	ART	Mean age in years (ranges)	Mean years (ranges) on ART	Mean years (ranges) infected
NPI	47	>500	Naïve	47.1 (23-65)	N/A	11.2 (10-15)
PI	14	Variable	Exposed	43.8 (22-60)	4.6 (0.1-10)	6.4 (1-12)
Control	12	>500	N/A	35.7 (24-64)	N/A	N/A

- NPI; Non-progressive HIV-1 infection
- PI; Progressive HIV-1 infection
- Control; HIV uninfected
- N/A; Not Applicable
- ART; Antiretroviral therapy
- N; Number of subjects

3.2 Absolute CD4⁺ T-cell counts and CD4:CD8 ratio

Low CD4⁺ T-cell counts and CD4:CD8 ratios less than one are known markers of progressive HIV-1 infection. To determine the correlation of these markers to HIV-1 infection and plasma TGF β 1 levels, CD4⁺ and CD8⁺ T-cell counts were measured in non-progressive and progressive HIV-1 infected patients. Absolute CD4⁺ T-cell counts obtained in this study were highly variable and 52% of patients with progressive infection had absolute CD4⁺ T-cell counts less than 500 cells/mL. However, all patients with non-progressive infection had absolute CD4⁺ T-cell counts greater than 500 cells/mL. The range in non-progressives (543-936) was not as variable as those found in the progressives (51-949) (Figure 1). There was no significant difference in CD4⁺ T-cell count between HIV-1 uninfected controls and patients with non-progressive infection (p=0.1056). CD4⁺ T-cell count in patients with non-progressive infection was significantly higher than those with progressive infection (p<0.0027), Figure 1. In patients with progressive infection, absolute CD4⁺ T-cell count was significantly lower (P<0.0001) compared to the healthy control group. All HIV uninfected controls had CD8⁺ T-cell count below 635 cells/mL as reported elsewhere [16]. Interestingly, despite normal CD4⁺ T-cell count (>500) in all patients with non-progressive infection, a significantly elevated CD8⁺ T-cell count above 1000 cells/mL (p<0.0001) was observed as compared to levels in healthy controls (Figure 1). Eighty one percent of patients with progressive infection had a higher CD8⁺ T-cell count above 635 cells/mL compared to the healthy control group (p<0.0001). CD4:CD8 ratios were normal (greater than one) in all HIV uninfected controls and significantly reduced in patients with non-progressive and progressive infection (p<0.0001). Compared to patients with progressive infection, there was no significant difference in CD4:CD8 ratios from patients with non-progressive infection (p=0.1712).

FIG. 1. Individual and mean ($\pm$ SEM) absolute T-cell counts. CD4⁺ and CD8⁺ T-cell counts were enumerated among non-progressive and progressive HIV-1 infected patients. HIV-1 uninfected healthy individuals are controls. (A); Absolute CD4⁺ T-cell counts, (B); Absolute CD8⁺ T-cell counts (C); CD4:CD8 ratios are shown.

3.3 Plasma HIV-1 RNA

To assess the clinical importance of plasma HIV-1 viral loads and progression of HIV-1 infection, Plasma viral loads (HIV-1 RNA) was determined in patients with non-progressive or progressive HIV-1 infection. Individual and mean plasma viral loads, (Figure 2), were determined in each study group.

FIG. 2. HIV-1 RNA levels in the study population (A); HIV-1 RNA in patients with progressive and non-progressive HIV-1 infection, (B); TGF β 1 levels in ART exposed patients with undetectable HIV RNA, HIV RNA below the limit of detection and patients with detectable HIV RNA. LOD; L limit of detection

Plasma viral loads were detectable in 79% (11/14) of patients with non-progressive HIV-1 infection while plasma viral loads were detectable in 74.5% (35/47) of patients with progressive HIV-1 infection. Viral loads were below the limit of detection (20 cps/mL) in 17% (6/35) of the progressors. An additional 25.5% (12/47) of the progressors had undetectable viral loads (Figure 2A). Overall, patients with non-progressive HIV-1

infection had significantly higher plasma viral loads than those with progressive infection ($p=0.0127$), possibly related to the progressives being on ART. No HIV-1 RNA was detected in all the negative control subjects.

3.4 Active Plasma TGF β 1 levels

Through its profound and broad inhibitory effects on different antiviral immune effector mechanisms, TGF β 1 may facilitate more rapid progression of HIV-1 infection. We sought to determine plasma levels of this pleiotropic cytokine in both patients with non-progressive and progressive HIV-1 infection. Differences in active plasma TGF β 1 levels among study subjects are shown in Figure 2 and Figure 3. Compared to HIV uninfected healthy controls, while active plasma TGF β 1 levels in patients with non-progressive and progressive HIV-1 infection in this study were significantly higher ($p=0.0194$ and $p=0.0002$ respectively), levels obtained in progressives, were higher compared to non-progressives ($p=0.003$) (Figure 3). Furthermore, in the progressives, plasma concentrations of active TGF β 1 inversely correlated with $CD4^+$ T- cell counts, $CD8^+$ T-cell counts, and $CD4:CD8$ ratios (Figures 1 and 3) but no correlation was observed between TGF β 1 levels and HIV-1 RNA (Figure 2B) or ART exposure Figure 3C. While all the 14 subjects from the non-progressive HIV-1 infection group had TGF β 1 levels below 16,135 pg/mL, almost half of the progressors, 45%, (21/47), had higher TGF β 1 levels, above 16,137 pg/mL (Figure 3), with the highest levels at 50,118 pg/mL, recorded in two subjects. The lowest active plasma TGF β 1 levels seen in patients with non-progressive HIV-1 infection was 2,904 pg/mL while the lowest in patients with progressive HIV-1 infection was 5,155 pg/mL (Figure 3). The HIV uninfected control group had a mean TGF β 1 levels of 4,361 pg/mL comparable to the levels reported elsewhere [15].

FIG. 3. Mean ($\pm$ SEM) plasma concentrations of immunosuppressive cytokines (A).Transforming growth factor- β 1, (B). IL-10, and (C). Plasma transforming growth factor- β 1 concentrations in ART exposed patients . Control group (HIV-1 uninfected), Patients with non-progressive HIV- 1 infection (NPI) and patients with progressive HIV-1 infection (PI).

3.5 Plasma cytokine concentrations

The immunopathology of HIV-1 infection includes immune defects in T-cell cytokine secretion. To profile the dynamics of cytokine levels in relation to HIV-1 disease progression, plasma concentrations of pro-inflammatory cytokines, IL-1 β ,IL-12p70, IFN- γ , TNF- α , immunosuppressive cytokine IL-10 and T-cell homeostatic cytokine, IL-13 were analyzed in non-progressive and progressive HIV-1 infected subjects and compared them to levels in healthy uninfected controls. Results demonstrated a significant low plasma concentration of IFN- γ ($p=0.0072$) and IL-10 ($p=0.0381$) in patients with non-progressive HIV-1 infection than those with progressive HIV-1 infection. In the non-progressors, the low plasma concentrations of IFN- γ had an inverse correlation with $CD4^+$ T-cell counts, $CD8^+$ T-cell counts, $CD4:CD8$ ratio, IL-12p70, TNF- α , IL1 β

(Figures 1 and 4). In the progressors, the high plasma concentrations of IFN- γ also had a inverse correlation with CD4⁺ T-cell counts, CD8⁺ T-cell counts, CD4:CD8 ratio, IL-12p70, TNF- α , IL1 β (Figures 1 and 4). Compared to uninfected controls, median IFN- γ levels were slightly higher in patients with non-progressive infection, but difference did not reach significant levels (Figure 4). Concentration of TNF- α and IL-12p70 was significantly higher in non-progressive HIV-1 infection than progressive infection (p=0.0073 and p=0.0243 respectively) (Figure 4).

FIG. 4. Mean ($\pm$ SEM) plasma concentrations of inflammatory cytokines (A). IFN- γ , (B). IL-13, (C). IL-1 β , (D). IL-12p70 and (E). TNF- α , in control group (HIV-1 uninfected), patients with non-progressive HIV-1 infection (NPI) and patients with progressive HIV-1 infection (PI).

4. Discussion

The importance of cytokine regulation in progressive and non-progressive HIV-1 infection is not well understood. We sought to examine whether plasma pro-inflammatory and immunosuppressive cytokine concentrations, in addition to CD4⁺ T-cell counts and viral loads would explain immunologic competence in patients with non-progressive HIV-1 infection. We also looked for correlations between cytokine concentrations, viral loads, CD4⁺ and CD8⁺ T-cell counts and CD4:CD8 ratios to identify cytokine profiles associated with non-progressive HIV-1 infection as well as markers of disease progression. Plasma concentrations of immunosuppressive cytokines TGF β 1 and IL-10, Th2 cytokine IL-13, and Th1 cytokines IFN- γ , TNF- α , IL-1 β and IL-12p70 were quantified in plasma from patients with progressive or non-progressive HIV-1 infection.

Antiretroviral-naïve patients with non-progressive HIV-1 infection in this study had significantly high HIV-1 RNA, CD4⁺ and CD8⁺ T-cell counts than patients with progressive infection. Having higher CD8⁺ T-cells in the non-progressive infection group than in the progressive infection group observed in this study could signal the involvement of HIV-1 specific-CD8⁺ T-cell mediated immunity, which is helping to maintain the non-progressive HIV-1 infection state. Interestingly, most ART exposed patients with progressive HIV-1 infection in this study had low CD4⁺ T-cell counts, high CD8⁺ T-cell counts but low HIV-1 RNA. Effective ART has been shown to result in reduced viral replication and eventual replenishment of CD4⁺ T-cell counts [17]. While the benefit of ART may account for low HIV-1 RNA, low CD4⁺ T-cell counts in ART exposed patients with progressive HIV-1 infection in this study may point to a possible deficiency in the regeneration of central CD4⁺ T-cells even when viral loads are low or below the limit of detection. Factors responsible for maintenance of high CD4⁺ T-cell in non-progressive HIV infection despite high plasma HIV RNA, and low CD4⁺ T-cell in progressive HIV infection despite ART administration is not well understood.

Although cytokines are important for inducing T-cell maturation, proliferation, and survival, correlation of cytokines and markers of HIV-1 disease progression, including low CD4⁺ T-cell counts, inverted CD4:CD8 ratios and high viral loads, is not known. Transforming growth factor beta 1 (TGF β 1) has been implicated in the pathogenesis of HIV-1 [3,12,18], and as a CD4⁺ T- lymphocyte pro-apoptosis factor [19]. In this study we observed increased active plasma TGF β 1 levels in ART exposed patient with progressive HIV-1 infection relative to uninfected healthy controls ($p=0.0002$) and patients with non-progressive HIV-1 infection ($p=0.003$). This observation may suggest a role of TGF β 1 in facilitating more rapid progression of HIV-1 infection as has been shown in other viral infections [7,5,8,11]. Also, patients with non-progressive HIV-1 infection, in this study, had significantly lower levels of immunosuppressive cytokines IL-10 ($p=0.0381$) than patients with progressive HIV-1 infection. Plasma TGF β 1 and IL-10 levels from patients with non-progressive infection in this study inversely correlated with CD4⁺ T-cell counts and CD4:CD8 ratios. This observation suggests a possible role for these cytokines in determining progression of HIV-1 infection, through their effect on CD⁺ T-cells. It is hypothesized that effector T-cells succumb to an immunosuppressive environment, especially the increased expression of TGF β 1 and IL-10 [20]. Moreover, TGF β 1 is a well-known CD4⁺ T-lymphocytes pro-apoptosis factor [19], emphasizing the importance of this cytokine in progressive loss of immune function and eventual failure of T-cell responses in chronic HIV-1 infection. We also noticed a significantly lower CD4⁺ T-cell counts and variable HIV-1 RNA levels in progressive HIV-1 infection ($p=0.0027$) despite antiretroviral therapy. This observation, points to the heterogeneous nature of patients on ART and the need for continuous monitoring to identify patients with treatment failure. Most ART exposed patients with detectable HIV-1 RNA presented an almost two fold increase of plasma TGF β 1 concentrations in comparison to those with undetectable HIV-1 RNA and healthy controls. Although most patients who had ART exposure for less than three year had higher plasma concentrations of TGF β 1 compared to those exposed for more than three years, we did not observe any relationship between TGF β 1 and ART exposure or HIV-1 RNA. It should be borne in mind that patients included in this study were not stratified due to immune or viral response to ART and hence plasma cytokines concentrations in relation to ART exposure cannot be clearly ascertained. As expected, healthy participants had significantly higher CD4⁺ T-cell counts than patients with progressive HIV-1 infection. However, CD4⁺ T-cell counts in patients with non-progressive HIV-1 infection was not significantly different from HIV uninfected healthy control ($p=0.1056$). Together, these findings suggest that increased immunosuppressive environment due to cytokines TGF β 1 and IL-10 may contribute to decreased CD4⁺ T-cell counts and eventual HIV-1 progressive state observed in patients with progressive HIV-1 infection in this study.

Also, ART exposure seem not to affect plasma TGF β 1 as no correlation between TGF β 1 and ART exposure was observed in ART exposed patients in this study.

Cytokines produced by Th1 are known to suppress apoptosis of CD4⁺ T-lymphocytes, whereas those produced by Th2 accelerate the process of apoptosis of CD4⁺ T-lymphocytes [21]. All patients with progressive HIV-1 infection in this study showed significantly high plasma concentration of Th2 cytokines IL-13 ($p=0.0032$) compared to healthy controls. IL-13 concentration in patients with non-progressive infection was low but not significantly different from patients with progressive infection. The lack of statistical significance between patients with progressive infection and those with non-progressive infection for IL-13 levels may have been due to the relatively small numbers of patients with non-progressive infection in the current study. Antiviral effects of both IL-13 and IFN- γ on HIV-1 macrophage infections have been demonstrated [22] and are associated with improved disease status. Compared to patients with progressive HIV-1 infection, patients with non-progressive infection had significantly lower plasma concentration of IFN- γ ($p=0.0072$). In patients with non-progressive HIV-1 infection, decreased plasma concentration of IFN- γ positively correlated with plasma HIV-1 RNA. IFN- γ has previously been demonstrated to have direct antiviral effects on HIV-1 macrophage infections [23] and to enhance antigen presenting function [24]. Our study demonstrated a significant low plasma concentration of IFN- γ ($p=0.0072$) in patients with non-progressive HIV-1 infection than those with progressive HIV-1 infection. In the non-progressors, the low plasma concentrations of IFN- γ had an inverse correlation with CD4⁺ T-cell counts, CD8⁺ T-cell counts, CD4:CD8 ratio. Demonstration of IFN- γ association with improved disease status (increased CD4⁺ T-cell counts and CD4:CD8 ratios) in patients with non-progressive HIV-1 infection in this study suggest a contribution by these cytokines in maintaining immune function following HIV-1 infection. Moreover, IFN- γ has previously been identified as a correlate of better disease prognosis in HIV-1 infection, and was positively associated with CD8⁺ T-cell counts [25]., confirming the prognosis values of IFN- γ in HIV-1 infection. Production of IL-12p70 and IFN- γ is partly regulated by a positive feedback loop, with IL-12p70 and IFN- γ promoting IL-12p70 production and IL-12p70 in turn stimulating IFN- γ [26]. IL-12p70 and IFN- γ promote Th1 differentiation, favoring cell-mediated immunity and inhibiting Th2 responses [27]. We also showed that plasma Th1 pro-inflammatory cytokines IL-12p70 and TNF- α were significantly increased in patients with non-progressive HIV-1 infection relative to patients with progressive infection ($p=0.0243$ and $p=0.0073$ respectively). This implies the central role of the Th1 response in conferring a non-progressive HIV-1 infection status. Taken together, these findings suggest that a Th1 skewed environment may be necessary for effective control of the virus in HIV-1 non-progressives, while the regulatory cytokines TGF β 1 and IL-10 most likely down-regulate the Th1 response in HIV-1 progressive infection, resulting in the virus having a survival advantage. Therefore, plasma pro-inflammatory cytokine response and CD8⁺ T-cell counts may be a useful determinant of the rates of progression of HIV-1 infection.

Because CD4⁺ T-lymphocyte is a prime marker of immune health in HIV-1 infection, the correlation of cytokines and the state of CD4⁺ T-lymphocyte shown in this study provide insight into the role of cytokines in both progressive and non-progressive HIV-1 infection. However, it should be noted that we were limited to comparing systemic plasma cytokine levels in ART exposed progressive HIV-1 infected patients with ART naïve non-progressive HIV-1 infected patients, which would result in statistical bias. It should also be borne in mind that plasma level of cytokine following ART is unlikely to compare directly with cytokine levels in plasma from ART naïve patients or healthy controls. Nevertheless, these findings suggest a contribution by immunosuppressive cytokines in maintaining immune function in non-progressive HIV-1 infection. Future studies would explore whether high levels of virus suppress production of pro-inflammatory cytokines and/or that the virus survives better when production of these cytokines are limited. Also, it would be prudent if a cohort of HIV-1 infected patients are stratified due to immune and viral response to ART and followed to establish changes in TGF β 1 levels after initiation of ART.

5. Conclusion

This study provides insight in to the role of cytokines in modulation of HIV-1 disease progression. Given the correlations observed in cytokines profiles and HIV-1 disease progression, this study speculates to a possible target for immune-based therapy for HIV-1 infection.

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Competing interest

The authors declare that they have no competing interests.

Author's contribution

EKM was responsible for experimental design, execution of laboratory experiments, data analysis and manuscript writing. EB, MS, ML and WKA assisted in the study design, revising the manuscript as well as offering expert advice. All authors read and agreed to the submission of the final manuscript for publication.

References

- [1] M. Stevenson, HIV-1 Pathogenesis, *Nat. Med.* 9 (2003) 853 – 860.
- [2] J.F. Okulicz, Elite Controllers and Long-term Nonprogressors: Models for HIV Vaccine Development?, *J. AIDS Clin. Res.* 01 (2012) 8–11. doi:10.4172/2155-6113.S8-005.
- [3] M. Lotz, P. Seth, TGF β and HIV infection, in: *Ann. N. Y. Acad. Sci.*, 1993: pp. 501–511. <http://www.scopus.com/inward/record.url?eid=2-s2.0-0027184884&partnerID=tZOtx3y1>.
- [4] M.-A. Jenabian, P. Ancuta, N. Gilmore, J.-P. Routy, Regulatory T cells in HIV infection: can immunotherapy regulate the regulator?, *Clin. Dev. Immunol.* 2012 (2012) 908314. doi:10.1155/2012/908314.
- [5] S. Michelson, J. Alcamí, S.J. Kim, D. Danielpour, F. Bachelierie, L. Picard, et al., Human cytomegalovirus infection induces transcription and secretion of transforming growth factor beta 1., *J. Virol.* 68 (1994) 5730–5737.
- [6] N.L. McCartney-Francis, M. Frazier-Jessen, S.M. Wahl, TGF- β : A balancing act, *Int. Rev. Immunol.* 16 (1998) 553–580. <http://www.scopus.com/inward/record.url?eid=2-s2.0-0031831154&partnerID=tZOtx3y1>.
- [7] S.G. Reed, TGF- β in infections and infectious diseases, *Microbes Infect.* 1 (1999) 1313–1325.
- [8] et al. Blobe GC, Role of transforming growth factor beta in human disease, *N Engl J Med.* 342 (2000) 135–1358.
- [9] Z. Toossi, J.J. Ellner, SHORT ANALYTICAL REVIEW The Role of TGF b in the Pathogenesis of Human Tuberculosis, 87 (1998) 107–114.
- [10] R.L. Furler, C.H. Uittenbogaart, GLI2 Regulates TGF- b 1 in Human CD4 + T Cells : Implications in Cancer and HIV Pathogenesis, 7 (2012). doi:10.1371/journal.pone.0040874.

- [11] Y. Do Yoo, H. Ueda, K. Park, K.C. Flanders, Y.I. Lee, G. Jay, et al., Regulation of transforming growth factor- β 1 expression by the hepatitis B virus (HBV) X transactivator: Role in HBV pathogenesis, *J. Clin. Invest.* 97 (1996) 388–395. <http://www.scopus.com/inward/record.url?eid=2-s2.0-0030032374&partnerID=tZOtx3y1>.
- [12] J. Kekow, W. Wachsman, J. Allen McCutchan, W.L. Gross, M. Zachariah, D.A. Carson, et al., Transforming growth factor- β and suppression of humoral immune responses in HIV infection, *J. Clin. Invest.* 87 (1991) 1010–1016. <http://www.scopus.com/inward/record.url?eid=2-s2.0-0025734832&partnerID=tZOtx3y1>.
- [13] V. Navikas, J. Link, Increased levels of interferon-gamma (IFN- γ), IL-4 and transforming growth factor-beta (TGF- β) mRNA expressing blood mononuclear cells in human HIV infection, (1994) 59–63.
- [14] J.K. Lazdins, T. Klimkait, K. Woods-Cook, M. Walker, E. Alteri, D. Cox, et al., In vitro effect of transforming growth factor- β on progression of HIV-1 infection in primary mononuclear phagocytes, *J. Immunol.* 147 (1991) 1201–1207. <http://www.scopus.com/inward/record.url?eid=2-s2.0-0025838110&partnerID=tZOtx3y1>.
- [15] A. Wiercińska-Drapalo, R. Flisiak, J. Jaroszewicz, D. Prokopowicz, Increased plasma transforming growth factor-beta1 is associated with disease progression in HIV-1-infected patients., *Viral Immunol.* 17 (2004) 109–113.
- [16] S. Serrano-Villar, T. Sainz, S. a. Lee, P.W. Hunt, E. Sinclair, B.L. Shacklett, et al., HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality, *PLoS Pathog.* 10 (2014). doi:10.1371/journal.ppat.1004078.
- [17] B. Emu, W.J. Moretto, R. Hoh, M. Krone, J.N. Martin, D.F. Nixon, et al., Composition and Function of T Cell Subpopulations Are Slow to Change Despite Effective Antiretroviral Treatment of HIV Disease., *PLoS One.* 9 (2014) e85613. doi:10.1371/journal.pone.0085613.
- [18] V. Navikas, J. Link, B. Wahren, C. Persson, H. Link, Increased levels of interferon-gamma (IFN- γ), IL-4 and transforming growth factor-beta (TGF- β) mRNA expressing blood mononuclear cells in human HIV infection, *Clin. Exp. Immunol.* 96 (1994) 59–63. <http://www.scopus.com/inward/record.url?eid=2-s2.0-0028275847&partnerID=tZOtx3y1>.
- [19] J. Wang, E. Guan, G. Roderiquez, M. a Norcross, Synergistic induction of apoptosis in primary CD4(+) T cells by macrophage-tropic HIV-1 and TGF-beta1., *J. Immunol.* 167 (2001) 3360–3366. doi:10.4049/jimmunol.167.6.3360.
- [20] M. Elrefaei, C.M. Burke, C. a R. Baker, N.G. Jones, S. Bousheri, D.R. Bangsberg, et al., TGF- β and IL-10 production by HIV-specific CD8+ T cells is regulated by CTLA-4 signaling on CD4+ T cells, *PLoS One.* 4 (2009) 4–10. doi:10.1371/journal.pone.0008194.
- [21] T.M. Ross, Using death to one ' s advantage : HIV modulation of apoptosis, (2001) 332–341.
- [22] R.T. Bailer, a Holloway, J. Sun, J.B. Margolick, M. Martin, J. Kostman, et al., IL-13 and IFN-gamma secretion by activated T cells in HIV-1 infection associated with viral suppression and a lack of disease progression., *J. Immunol.* 162 (1999) 7534–7542.

- [23] R.S. Kornbluth, P.S. Oh, J.R. Munis, C. P. H., R. D. D., Interferons and bacterial lipopolysaccharide protect macrophages from productive infection by human immunodeficiency virus in vitro., *J. Exp. Med.* (1989) 1137.
- [24] R. de Waal Malefyt, C.G. Figdor, R. Huijbens, S. Mohan-Peterson, B. Bennett, J. Culpepper, et al., Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes: comparison with IL-4 and modulation by IFN- γ or IL-10., *J. Immunol.* (1993) 6370.
- [25] H. Ullum, A. Cozzi Lepri, K. Bendtzen, J. Victor, P. Gøtzsche, A. Phillips, et al., Low production of interferon gamma is related to disease progression in HIV infection: evidence from a cohort of 347 HIV-infected individuals., *AIDS*. 13 (1997) 1039–1046.
- [26] C.S. Tripp, S.F. Wolf, E.R. Unanue, Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist., *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 3725–3729.
- [27] R. Manetti, F. Gerosa, M.G. Giudizi, R. Biagiotti, P. Parronchi, M.P. Piccinini, et al., Interleukin 12 induces stable priming for interferon gamma (IFN-gamma) production during differentiation of human T helper (Th) cells and transient IFN-gamma production in established Th2 cell clones., *J. Exp. Med.* 179 (1994) 1273–1283. doi:10.1084/jem.179.4.1273.