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Plasma Cytokine Predictors of Tuberculosis Recurrence in Antiretroviral-Treated Human Immunodeficiency Virus-infected Individuals from Durban, South Africa

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Background. Immune correlates of tuberculosis (TB) risk in populations infected with human immunodeficiency virus (HIV) remain understudied, despite HIV being associated with a high burden of TB disease. Here we describe plasma cytokine correlates of TB recurrence in a well-characterized cohort of HIV-infected individuals on antiretroviral therapy (ART) with a history of prior TB cure.

Methods. Study participants were drawn from a prospective cohort study initiated at the conclusion of a randomized clinical trial in which individuals presented with untreated HIV infection and active pulmonary TB. At baseline, ART was initiated, and TB successfully cured. Participants were screened for TB recurrence quarterly for up to 4 years. TB recurrent cases (n = 63) were matched to controls (n = 123) on sex, study arm assignment in the original trial, and month of enrollment with a subset of cases sampled longitudinally at several time-points.

Results. Three cytokines were associated with increased rates of TB recurrence in univariate models: interleukin 6 (IL6) (odds ratio [OR] 2.66, 95% confidence interval [CI] 1.34–5.28, $P = .005$), IP10 (OR 4.62, 95% CI 1.69–12.65, $P = .003$), monokine induced by IFN-γ (MIG) (OR 3.11, 95% CI 1.10–8.82, $P = .034$). Conversely, interferon β (IFNβ) was associated with decreased TB risk (OR 0.34, 95% CI 0.13–0.87, $P = .025$). Following multivariate analyses adjusting for covariates IL6, interleukin 1β (IL1β), and interleukin 1Rα (IL1Rα) were associated with increased risk and IFNβ with decreased TB risk. Longitudinal analysis showed that levels of many TB-associated markers, including IL6, IP10, sCD14, and interferon γ (IFNγ) are reduced following TB treatment.

Conclusion. These data show that TB recurrence, in HIV-infected individuals on ART is predicted by biomarkers of systemic inflammation, many of which are implicated in more rapid HIV disease progression.

Clinical Trials Registration. NCT 01539005

Keywords. Tuberculosis; HIV; ART; cytokine; chemokine.

South Africa has the highest burdens of both human immunodeficiency virus (HIV) and tuberculosis (TB) infections globally; these infections are frequently concomitant, suggesting the need for joint solutions to improve the health in this population. An estimated 1.2 million of the 9.6 million people that developed TB worldwide were HIV infected, with Africa accounting for 74% of coinfection cases. Given the scale of the HIV-associated TB epidemic, more effective TB prevention and control strategies, especially in those with HIV-related immune deficiency, are urgently needed.

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The interaction between *Mycobacterium tuberculosis* (MTB) and the host evokes innate and adaptive immune responses during both quiescent and active replicating phases of the pathogen [1]. Although the immune mechanisms that drive the transition of TB from quiescence to active disease remain unclear, 2 of the main known risk factors for activation of latent TB infection (LTBI) are HIV infection and treatment with tumor necrosis factor (TNF) inhibitors [2–4]. Natural history studies suggest that most people infected by MTB do not develop TB disease, with an estimated 10% lifetime odds of reactivation. Risk increases dramatically to 10% annually in the context of HIV infection and is more pronounced as HIV disease progresses, with CD4 T-cell count being inversely associated with the incidence of TB disease [5]. Treatment with antiretroviral therapy (ART) and the associated immunological recovery are associated with a decreased TB incidence; however, despite ART treatment, the overall TB incidence rate for HIV-infected individuals remains 10-fold higher compared to individuals

without HIV [6]. Although it has been established that the host immune system is critical to both containment and cure of TB infection, a greater understanding of the balance between protective and detrimental immune responses to MTB is needed, especially in the context of HIV coinfection.

Both TB and HIV infections have been associated with changes in the expression of various soluble plasma proteins, which either directly or indirectly contribute to the morbidity and/or mortality associated with these diseases. Several markers of systemic immune activation, including interleukin 6 (IL6), D-dimer, sCD14, and sCD163, have been associated with progression of HIV disease and an increased risk of HIV-related all-cause mortality while on HIV treatment [7–9]. Although immune activation and inflammation are elevated during untreated HIV infection, presumably due to increased levels of viral replication, most innate and adaptive immune responses remain dysfunctional despite effective ART and HIV viral suppression. Recent papers suggest this could be due to viral replication in distal tissues during ART [10, 11]. Independently of HIV infection, active TB infection has been associated with elevated levels of immune activation and inflammation, including elevated levels of tumor necrosis factor α (TNFα) and interleukin 17 (IL17) [12], interleukin 18 (IL18) [13, 14] and sCD14 [15]. As with HIV, inflammatory responses act as a double-edged sword in TB infection; although beneficial for containment of MTB and HIV in early infection, inflammation contributes to immune mediated pathology if left unregulated. Immune activation and systemic inflammation during HIV/TB coinfection have not been fully characterized; however, elevated T-cell activation was observed in HIV-infected individuals with both latent and active TB, whereas elevated levels of plasma sCD14, CRP, IL6, and IP10 were observed only in patients with active TB [16].

There is a continuing need for prospective studies of HIV-infected TB patients in order to better characterize the immune factors that predict the risk of TB recurrence in coinfected patients. Despite a large number of studies focusing on the relationship between markers of immune activation in HIV and TB only a few have assessed the impact of MTB infection and treatment completion on immune activation and microbial translocation in HIV-infected individuals on ART [16, 17]. Here we hypothesized that markers of inflammation would be predictive of active TB disease. We sought to characterize plasma cytokine/chemokine correlates of TB recurrence among HIV-infected patients on ART who had their previous episode of TB treated successfully.

METHODS

Study Group

Participants were enrolled from the CAPRISA 005 TB Recurrence upon Treatment with HAART (TRuTH) cohort, a prospective study, based at the CAPRISA eThekwini clinic in Durban, KwaZulu-Natal, South Africa. All TRuTH participants were previously enrolled in a CAPRISA 003 SAPiT trial investigating the timing of ART initiation during treatment for pulmonary TB [18, 19], and entry into TRuTH included a confirmed TB-negative status at the conclusion of TB treatment. The TRuTH study maintained participants on ART while screening quarterly for a maximum of 4 years for TB recurrence, defined as the first microbiologic confirmation of MTB by TB smear or culture. We conducted a nested case-control study with cases defined by TB recurrence with an available pre-recurrence sample matched 1:2 to individuals with no evidence of TB recurrence during the follow-up period. Written informed consent was obtained from all study participants prior to enrolment and the University of KwaZulu-Natal Biomedical Research Ethics Committee approved the study (ref: BF 051/09; Clinicaltrials.gov number NCT 01539005).

Case-control matching was carried out on the basis of study arm in the SAPiT trial, month of enrollment in that study, and sex. This ensured that cases and controls were on ART for a similar duration at sample time-point. Cases were sampled at a minimum of 3 and maximum of 9 months prior to TB recurrence, and controls at comparable time-points, ensuring no difference in length of sample cryopreservation between groups. A subset of 16 cases was followed longitudinally at following time points: Baseline (at the time of initial TB diagnosis in the SAPiT trial); Cure1 (following successful treatment of the first TB episode occurring 6 months post- SAPiT baseline); PreTB2 (a time point 18 to 6 months before TB recurrence); Recurrence/TB2 (2-month window before or after TB recurrence); Cure2 (capturing a 2-month window before and 3-month window after recurrent TB treatment completion date).

Sample Collection and Processing

Peripheral blood was collected in acid citrate dextrose (ACD) tubes. Following centrifugation plasma was collected and cryopreserved at −80°C for further analysis.

Cytokine/Chemokine Measurement

Cytokine/chemokine levels were measured using the Bio-RAD multiplex assays (Human Inflammation Panel and Human Cytokine/Chemokine Panel I and Panel III) and analyzed on a BioPlex-200 (Bio-Rad, Mississauga, ON, Canada). The Human Inflammation Panel includes following cytokines: IFNα2, IFNβ, IFNγ, IL8, IL12 (p40), IL12 (p70), IL27 (p28), IL28A/IFNλ2, IL29/IFNλ1. The Human Cytokine/Chemokine panel I included: (IL1ß, IL1Rα, IL2, IL6, IL7, IL10, IL15, IP10, TNFα). The Human Cytokine/Chemokine Panel III included: IL1α, IL18, and monokine induced by IFN-γ (MIG). Soluble CD14 (sCD14) levels were measured using the Human CD14 Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN). I-FABP levels were

measured using the Human FABP2/I-FABP DuoSet ELISA (R&D Systems Inc., Minneapolis, MN). All assays were performed following manufacturer's instructions. Samples with values outside the range of the standard curve were assigned the value half the limit of detection in pg/mL, LOD/2.

Statistical Analysis

Wilcoxon signed ranks test, McNemar test, and Bowker test of symmetry were used to compare baseline characteristics of cases and controls. Data were modeled using univariate and multivariate conditional logistic regression to account for matching. *P*-values are reported and discussed as observed, and no corrections for the multiple comparisons were made [20].

All specimens from matched cases and controls were analyzed blinded to clinical status with longitudinal specimens from the same individual and strata ran on the same multiplex plate. In the primary comparison, TB recurrence status was used as the dependent variable, and each cytokine modeled individually as an independent variable. All biomarkers with more than 60% of samples above the limit of detection were analyzed as continuous variables and log-transformed to adjust for skewness (IL12P40, IFNβ, IFNγ, IFNα2, IL6, IL1α, IL10, IL1β, TNFα, IL7, IL1Rα, MIG, IP10, IL18, sCD14); those below 60% detectability were analyzed binary variables (IL15, IFNλ1, IL2, IL27, IL8, IFNλ2, IL12P70, I-FAB). Multivariate analyses adjusted for a wide range of clinical and demographic covariates at the sample time point, including: age, body mass index, CD4 count, and viral load (VL). In addition, baseline covariates included lung cavities (both vs. no and one vs. no), previous history of TB (yes vs. no), history of IRIS, World Health Organization (WHO) stage (4 vs. 3). Longitudinal analyses were performed using paired *t*-tests. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Graphs were made using the GraphPad Prism (V 5, La Jolla, CA).

RESULTS

Study Participants Characteristic

The original study design included 63 and 126 controls; however, the main analysis included 63 cases and 123 controls, with 3 controls missing due to sample unavailability. The median age was 32 (interquartile range [IQR] 29–38) years for cases and 35 (IQR 28–41) years for controls ($P = .719$). The median CD4 counts were 145 cells/mm^3 (IQR 57–234) for cases and a median of 151 cells/mm^3 (IQR 77–247) for controls ($P = .159$). Despite all participants being maintained on ART and a high rate of treatment success, viral loads were detectable in 40% of cases and 22.5% of controls. Only 3 cases and 1 control had a new AIDS-defining illness within 3 months of sample time point. Additional clinical and demographic cohort characteristics are listed in Table 1.

Plasma Cytokine/Chemokine Predictors of Tuberculosis Recurrence

To define the plasma cytokine/chemokine expression patterns that correlate with TB recurrence, we examined the expression of 21 cytokines/chemokines known to be important in immune activation and IFN signaling. Because immune activation in HIV is believed to be driven by microbial translocation, we further studied an intestinal fatty-acid binding protein (I-FABP/FABP2), a marker of gut damage, and soluble sCD14 (sCD14), a marker of lipopolysaccharide (LPS) bioactivity and monocyte activation. Univariate conditional logistic regression analysis revealed that 3 cytokines were associated with increased rates of TB recurrence, including IL6 (OR 2.66, 95% CI 1.34–5.27, $P = .005$), IP10 (OR 4.62, 95% CI 1.69–12.65, $P = .003$), MIG (OR 3.11, 95% CI 1.10–8.82, $P = .034$), whereas IFNβ was associated with decreased risk (OR 0.34, 95% CI 0.13–0.87, $P = .025$) of TB recurrence (Figure 1. and Table 2). IL1β (OR 1.89, 95% CI 0.97–3.68, $P = .064$) and IL1Rα (OR 1.52, 95% CI 0.95–2.45, $P = .084$) showed statistical trends toward increased rates of TB recurrence (Figure 1 and Table 2). Multivariate analysis, correcting for age, body mass index (BMI), CD4 count, VL, lung cavities, previous TB, history of IRIS, WHO stage confirmed the results for IL6 (adjusted odds ratio [aOR] 4.79, 95% CI 1.66–13.81, $P = .004$, Table 2). IL1β and IL1Rα that showed a trend toward significance in the univariate model were significant in the multivariate model (IL1β: aOR 3.41, 95% CI 1.26–9.24, $P = .016$; IL1Rα: aOR 2.04, 95% CI 1.04–3.98, $P = .038$). The interferon gamma-induced chemokines IP10 and MIG were no longer significantly associated with TB outcome following adjustments for covariates (IP10: aOR 3.74, 95% CI .90–15.53, $P = .069$; MIG: aOR 2.92, 95% CI .81–10.57, $P = .103$), likely due to the fact that they are influenced by HIV viral load, as described previously (Table 2) [21]. IFNβ (aOR 0.27, 95% CI .07–.99, $P = .049$) remained significantly associated with decreased risk of TB recurrence.

Longitudinal Changes in Plasma Cytokine/Chemokine Expression

To determine the impact of TB treatment on cytokine markers, we included a small subset of cases (n = 16) who were followed longitudinally, starting with the first TB episode until the end of treatment of the second TB episode (Sup. Table 2). Expression levels of 2 analytes were decreased following completion of treatment after the first TB episode: IP10 (mean difference [MD] 0.27, 95% CI 0.062–0.480, $P = .015$) and sCD14 (MD 0.085, 95% CI 0.019–0.151, $P = .016$), (Figure 2A). Expression levels of 4 analytes were decreased after completion of treatment following TB reoccurrence including IP10 (MD 0.281, 95% CI −0.0167 to 0.5788, $P = .063$), sCD14 (MD 0.129, 95% CI 0.0249–0.2328, $P = .019$), IL6 (MD 0.535, 95% CI 0.191–0.879, $P = .005$) and IFNγ (MD 0.682, 95% CI 0.131–1.233, $P = .019$) (Figure 2B). Second TB episode occurred a median of 26 (IQR 11.7–44.3) months following the ART initiation. There was a significant increase in sCD14 between PreTB2 (time point

Table 1. Clinical and Demographic Cohort Characteristics

Variable	TB recurrence (N = 65)	No TB recurrence (N = 130)	*P*-Value
SAPiT enrollment			
Randomization arm, n (%)			
Early integrated arm	17 (26.1)	34 (26.1)	
Late integrated arm	25 (38.5)	50 (38.5)	
Sequential arm	23 (35.4)	46 (35.4)	
Age (y), median (IQR)	32 (29–38)	35 (28–41)	.719
Body mass index (kg/m^2), median (IQR)	22.35 (20.2–24.7)	22 (19.7–24.7)	.235
CD4 count (cells/mm^3), median (IQR)	145 (57–234)	151 (77–247)	.159
Viral load (log copies/mL), mean (SD)[a]	5.1 (0.8)	5.0 (0.8)	.201
Sex, n (%)			
Male	30 (46.2)	60 (46.2)	-
Female	35 (53.8)	70 (53.8)	
WHO stage, n (%)[b]			
3	59 (93.7)	116 (96.7)	.180
4	4 (6.3)	4 (3.3)	
History of TB, n (%)			
Yes	28 (43.1)	39 (30.0)	.024
No	37 (56.9)	91 (70.0)	
Lung cavities, n (%)[c]			
Both	8 (13.3)	11 (9.4)	.635
No	33 (55.0)	70 (59.8)	
One	19 (31.7)	36 (30.8)	
SAPiT ART initiation			
CD4 count (cells/mm^3), median (IQR)	119 (51–249)	172 (88–264)	.159
Viral load (log copies/ml), mean (SD)[d]	5.0 (0.8)	4.9 (0.9)	.272
End of TB treatment in SAPiT			
CD4 count (cells/mm^3), median (IQR)	212.5 (101.5–445.5)	233.5 (127.5–353.5)	.634
Viral load, n (%)[e]			
Detectable	22 (37.9)	53 (44.9)	.096
Undetectable	36 (62.1)	65 (55.1)	
Variables at sample collection			
Age (y), median (IQR)	34 (31–40)	37 (31–43)	.789
CD4 count (cells/mm^3), median (IQR)	344 (127–551)	402 (257–547)	.011
Viral load, n (%)[f]			
Detectable	26 (40.0)	29 (22.5)	.03
Undetectable	39 (60.0)	100 (77.5)	
History of IRIS, n (%)			
IRIS	12 (18.5)	17 (13.1)	.281
No IRIS	53 (81.5)	113 (86.9)	

Missing data: [a]8, [b]12, [c]18, [d]2, [e]19, [f]1.

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome; SD, standard deviation; TB, tuberculosis; WHO, World Health Organization.

18–6 months before TB recurrence) and the second TB episode (MD −0.101, 95% CI −0.154 to −0.048, $P = .001$, Sup. Table 1.), suggesting sCD14 is the biomarker of active TB, as has been previously described [15, 16]. There was a significant increase in IFNγ between completion of treatment for the first TB episode and TB recurrence (MD −0.8999, 95% CI −1.661 to −0.138, $P = .024$), likely reflecting immune response to the increase in bacterial load. Additionally, there was a significant decrease in IL10 (MD 0.214, 95% CI 0.021–0.407, $P = .0321$), IP10 (MD 0.419, 95% CI 0.186–0.653, $P = .002$) and IFNα (MD = 0.529, 95% CI 0.089–0.969, $P = .0218$) between the baseline TB and recurrent TB episode (Sup. Table 1), potentially reflecting the effect of ART duration, as the expression of all 3 cytokines is known to decrease with ART [22, 23].

DISCUSSION

There is an urgent need for the identification and validation of TB diagnostic markers in HIV-positive individuals as this is key for timely patient management and for the development of new therapeutics and vaccines. Because all HIV-infected individuals now qualify for ART, but ART does not completely ameliorate HIV-associated TB risk, the population for this study is important for defining TB risk factors. Significantly higher plasma levels of IL6, IL1β and sIL1Rα were associated with increased

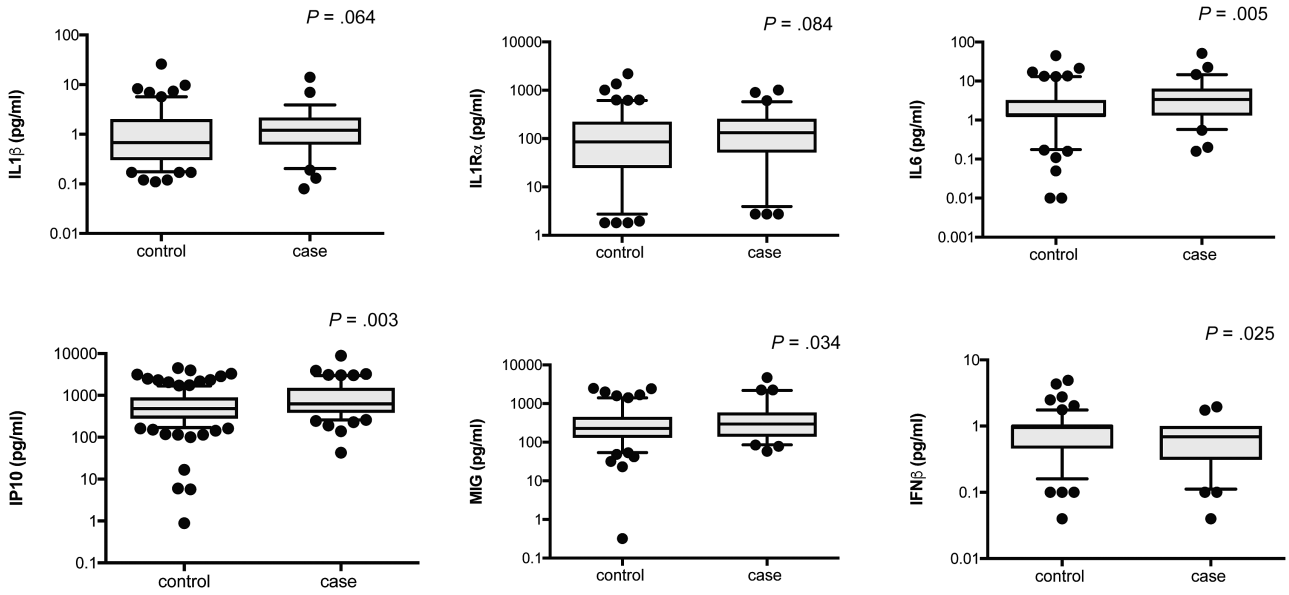

Figure 1. Cytokines/chemokines differentially expressed between controls (n = 123) and cases (n = 63) by univariate conditional logistic regression. Five cytokines were associated with increased rates of TB recurrence, including IL6 (OR 2.66, 95% CI 1.34–5.27, *P* = .005), IP10 (OR 4.62, 95% CI 1.69–12.65, *P* = .003), MIG (OR 3.11, 95% CI 1.10–8.82, *P* = .034) and IL1β (OR 1.89, 95% CI 0.97–3.68, *P* = .064) and IL1Rα (OR 1.52, 95% CI 0.95–2.45, *P* = .084, whereas IFNβ was associated with decreased risk (OR 0.34, 95% CI 0.13–0.87, *P* = .025). Cytokines were plotted on log scale (Log 10), Box and Whiskers (5–95%). *P*-values indicated in the figures are result of a univariate conditional logistic regression (Table 1). Abbreviations: CI, confidence interval; IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; MIG, monokine induced by IFN-γ; OR, odds ratio; TB, tuberculosis.

Table 2. Influence of Different Cytokines on Tuberculosis Recurrence

	Univariate		Multivariate[b]	
Cytokine	OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value
IL1β	1.89 (0.97–3.68)	.064	3.41 (1.26–9.24)	**.016**
IL1Rα	1.52 (0.95–2.45)	.084	2.04 (1.04–3.98)	**.038**
IL6	2.66 (1.34–5.27)	**.005**	4.79 (1.66–13.81)	**.004**
IL7	1.27 (0.53–3.03)	.591	1.73 (0.48–6.31)	.403
IL10	1.08 (0.58–2.01)	.814	1.61 (0.63–4.12)	.317
IP10	4.62 (1.69–12.65)	**.003**	3.74 (0.90–15.53)	.069
TNFα	0.98 (0.57–1.67)	.934	1.04 (0.48–2.27)	.916
IL1α	0.95 (0.33–2.69)	.916	1.26 (0.32–4.97)	.739
MIG	3.11 (1.10–8.82)	**.034**	2.92 (0.81–10.57)	.103
IFNβ	0.34 (0.13–0.87)	**.025**	0.27 (0.07–0.99)	**.049**
IFNα2	0.99 (0.48–2.02)	.967	0.59 (0.19–1.84)	.369
IFNγ	0.83 (0.57–1.22)	.349	0.69 (0.42–1.16)	.167
IL18	1.38 (0.80–2.38)	.249	1.53 (0.75–3.11)	.241
IL12P40	1.83 (0.66–5.09)	.244	3.44 (0.79–15.02)	.101
sCD14	0.90 (0.08–10.42)	.931	0.83 (0.04–17.41)	.902
IL15[a]	0.20 (0.02–1.64)	.133	0.39 (0.03–4.69)	.460
IFNλ1[a]	0.80 (0.26–2.51)	.706	0.56 (0.14–2.29)	.419
IL2[a]	0.73 (0.29–1.84)	.500	0.64 (0.16–2.52)	.519
IL27[a]	0.64 (0.28–1.46)	.288	0.72 (0.22–2.31)	.580
IL8[a]	1.10 (0.53–2.28)	.803	1.14 (0.45–2.88)	.780
IFNλ2[a]	1.49 (0.72–3.11)	.284	1.69 (0.60–4.72)	.319
IL12P7[a]	1.15 (0.56–2.35)	.713	1.72 (0.62–4.78)	.298
I-FAB[a]	1.27 (0.66–2.44)	.476	1.00 (0.44–2.27)	.993

Abbreviations: CI, confidence interval; IFN, interferon; IL, interleukin; IP, interferon gamma induced protein; MIG, monokine induced by IFN-γ; OR, odds ratio; sCD, soluble CD; TNF, tumor necrosis factor.

[a]Analyzed as binary variables.

[b]Multivariate analyses adjusted for WHO stage of the disease, body mass index, lung cavities, age, CD4+ count, viral load, previous history of TB, history of IRIS. Viral load closer to sample collection was predictive of TB recurrence in all multivariate models.

risk of TB recurrence. Interestingly, plasma IFNβ levels were associated with a decreased risk of TB recurrence. Active TB correlated with markers of systemic immune activation (IL6 and sCD14), known to correlate with more rapid HIV disease progression and mortality [8, 24, 25].

Significantly higher plasma IL6 and IL1β levels, known to exert pro-inflammatory effects, were found to be the strongest predictors of TB recurrence in both univariate and multivariate models. IL6 was previously described as a strong predictor of active TB irrespective of HIV status [16, 26]. TB treatment completion in the longitudinal subanalysis was associated with decrease in IL6 expression, further suggesting that IL6 could be a useful biomarker of both active TB disease and treatment success [26]. IL1β-induced immune responses have the contrasting potential to both clear bacterial infection [27] and to promote disease by inflicting tissue damage [28]. Here we show that increased plasma IL1β levels associate with TB disease recurrence. TB treatment did not have any effect on IL1β plasma levels suggesting that IL1β likely reflects the sub-clinical TB effects and could be used as a biomarker of latent TB. In addition to IL6 and IL1β, the interleukin 1 receptor antagonist (IL1Rα) was found to be significantly associated with TB recurrence independent of CD4 count, VL, and other adjusted covariates. Increase in serum sIL1Rα levels was previously associated with TB disease activity [29]. Soluble IL1Rα binds to IL1 receptors but does not induce any intracellular response, thereby acting as a regulatory mechanism for IL1-induced responses. Both IL6 and IL1β are known to be potent inducers of sIL1Rα and could be driving the increased sIL1Rα levels observed here [30, 31].

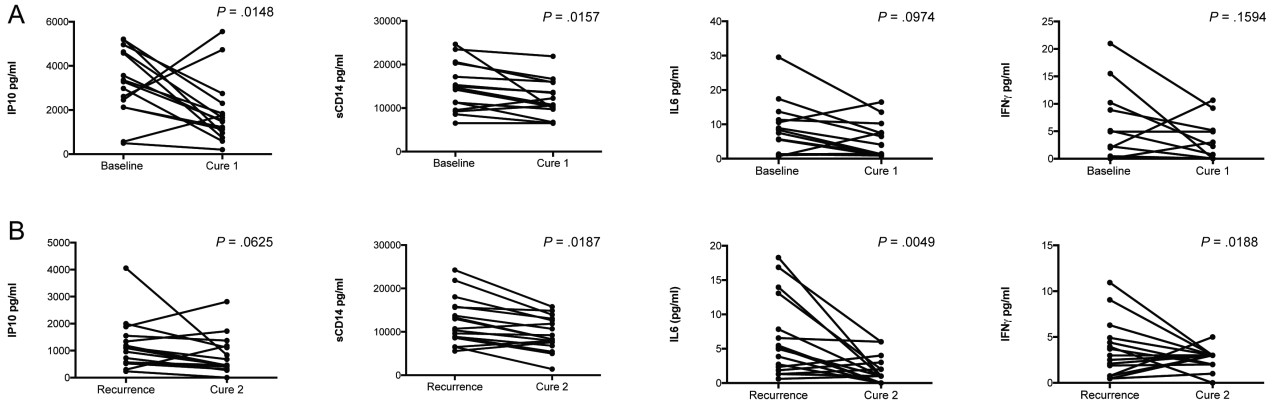

Figure 2. Effect of treatment completion on cytokine/chemokine expression following active TB (n = 15). *A*, Effect of treatment following the first TB episode, during early ART. Expression of 2 analytes was decreased IP10 (mean difference (MD) 0.271, 95% CI 0.0618 to 0.4803, *P* = .0148), sCD14 (MD = 0.0847, 95% CI 0.0186–0.1508, *P* = .0157), whereas IL6 (MD = 0.222, 95% CI –0.0461 to 0.4909, *P* = .097) and IFNγ (MD = 0.4687, 95% CI –0.2097 to 1.1472. *P* = .159) expression was not significantly affected. *B*, Effect of treatment following the recurrent TB episode. Expression of 4 analytes was decreased following TB treatment completion, including IP10 (MD = 0.281, 95% CI –0.0167 to 0.5788 *P* = .0625), sCD14 (MD = 0.1289, 95% CI 0.0249–0.2328, *P* = .0187), IL6 (MD = 0.5352, 95% CI 0.1913–0.8791, *P* = .0049), IFNγ (MD = 0.6821, 95% CI 0.1313–1.233, *P* = .0188). Paired *t*-test was used to evaluate changes in analyte expression between different time points. (Cytokines significantly affected at either of the time-points are shown here. Information for the rest of the measured cytokines can be found in Sup. Table 1). Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TB, tuberculosis.

As with IL1β, TB treatment had no effect on the sIL1Rα levels, further indicating that sIL1Rα may directly reflect the IL1β cytokine response.

A previous study performed in the TRuTH cohort showed that elevated production of IL1β from monocytes following TLR-2, TLR-4, and TLR-7/8 stimulation was associated with reduced odds of TB recurrence, whereas production of IL-1β from both monocytes and mDCs following Bacillus Calmette-Guérin (BCG) stimulation was associated with increased odds of TB recurrence [32]. Here we report that increased IL1β plasma levels are associated with higher risk of TB recurrence. Although increased IL1β production is required for proper development of antimicrobial adaptive immunity during initial MTB infection, if left uncontrolled, chronic IL1β production can be detrimental in MTB immunity. As seen with systemic pDC IFNα production during chronic HIV infection, increased, chronic plasma cytokine levels correlate with decreased ability of cells to produce the same cytokine upon further stimulation [33, 34], reflecting the anergy that can be associated with chronic infection.

Induction of type I interferons has been associated with bacterial virulence and TB pathogenesis in a number of previous studies [35, 36]. We did not observe any difference in IFNα2 expression; however, IFNβ was the only examined cytokine shown to be protective against TB reactivation in this study. Despite sharing the type I IFN receptor (IFNAR1), the responses induced by IFNα and IFNβ differ significantly, likely due to differences in binding affinity for IFNAR1, where IFNβ out-competes IFNα2 at >30-fold affinity [37]. One hypothesis that could explain how IFNβ could provide protection against TB is that it averts the negative effects of IFNα2 signaling by blocking receptor affinity. Furthermore, IL1 and type I IFN pathways counter-regulate each other [38], and IFNβ is known to limit pro-IL1β and pro-IL18 transcription and has opposing effects on NLRP3- and AIM2- inflammosome activation [39]. Validation and further examination of potential mechanisms that explain this observation could have important implications for managing TB infection in HIV coinfected individuals.

Results from the longitudinal analysis suggest that sCD14 is the biomarker of active TB, as has been previously described [15, 16]. Completion of treatment after baseline and recurrent TB led to significant decreases in sCD14 expression, and there was a significant increase in sCD14 between 18 and 6 months before TB recurrence and active TB. We observed no differences between cases and controls when it came to I-FAB, a marker of intestinal epithelial barrier damage, further indicating that the observed changes in sCD14 are due to active TB and monocyte activation and are unlikely to be the result of HIV-associated microbial translocation and the gastrointestinal tissue damage [40].

Observed differences in IP10 and MIG, previously characterized as markers of TB disease [41, 42], were found to be primarily driven by HIV viral load in the context of HIV/TB coinfection. Expression of IP10, IL10, and IFNα decreased between the two TB disease episodes likely due to the duration of ART and the associated immune-reconstitution.

As strain typing was not available we were unable to delineate new infections from relapse in the context of TB recurrence. Future studies should address this issue as immunological mechanisms in these 2 scenarios are likely to differ. Here we show that in HIV-infected individuals on ART, TB recurrence is predicted by biomarkers of soluble, systemic inflammation, many of which are also implicated in more rapid HIV disease progression and non-AIDS mortality in the context of effective HIV treatment.

Some of the identified markers decreased following TB treatment completion, implicating these as surrogates of subclinical TB disease activity and associated immune responses.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Authors' contributions. A. S., L. R. M., and K. N. designed the project. A. S. performed the experiments and wrote the manuscript with critical review from all the authors. Data analysis was done by A. S., L. R. M., and N. Y.; K. N. provided clinical and technical advice, and S. G. and N. S. assisted in subject recruitment and characterization. S. S. A. K. and K. N. provided oversight to study implementation.

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