CD4+ T Lymphocyte and Monocyte Activation within One Year After Treatment Completion for Pulmonary Tuberculosis: A Pilot Study

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Background

- Persons with active tuberculosis (TB) have increased immune activation that declines with TB treatment.
- Whether residual immune activation is present after completion of TB treatment is unknown. We conducted immunophenotyping of T cells and monocytes from individuals who had completed TB treatment, and compared them to controls without history of active TB.

Objective

We aimed at characterizing T cell and monocyte immune activation phenotypes in individuals who had recovered from TB within the last year

Methods

Study design and setting:

- Cross-sectional study of HIV-uninfected individuals 40 to 70 years old recruited in Lima, Peru.
- For this analysis, we included 6 individuals who had completed treatment for pulmonary TB within the past year, and 10 healthy controls without history of active TB (6 QuantiFERON®-TB positive; 4 QuantiFERON®-TB negative).
- Participants provided blood for T cell and monocyte immunophenotyping using multi-parameter flow cytometry, including expression of cell surface activation markers.

Flow cytometry assays:

- Cryopreserved peripheral blood mononuclear cells were stained with two flow cytometry panels:
  - T cell panel markers: CD3, CD4, CD8, CD25, CD38, CD69, HLA-DR, ICAM-1, CD127; FoxP3 (intracellular staining).
  - Monocyte panel markers: CD3, CD14, CD16, CD36, CD56, CD69, CD163, CCR2, CX3CR1, TLR4.
  - Data was acquired using BD™ LSR II flow cytometer.
- Data was analyzed using FlowJo v10.

Statistical analyses:

- We used the Mann-Whitney test for group comparisons of continuous and flow cytometry data.
- We used the Fisher exact test for comparisons of categorical data.
- For adjusted analyses, we used multivariable linear regression modeling of log-transformed flow cytometry variables of interest.
- *P* values <0.05 were considered statistically significant.

Results

Study population characteristics

- Data from 6 subjects who completed treatment for pulmonary TB within the past year, and 10 healthy controls were included in this analysis.
- There were no significant differences in terms of comorbidities and clinical metabolic parameters between the two groups (see Table 1).
- All participants were HIV negative.
- Median time from TB treatment completion was 4 months (range, 2 – 9) for the previously treated group.

Table 1. Characteristics of the study population

Characteristics	Prior TB disease (n=6)	No TB disease (n=10)	*p* value
Age in years	49 (47 – 51)	47 (43 – 61)	0.544
Male sex	3 (50%)	5 (50%)	1
Body mass index	25.3 (24 – 25.9)	27.7 (26.8 – 28.9)	0.104
Diabetes mellitus	1 (17%)	1 (10%)	1
Hypertension	1 (17%)	1 (10%)	1
Dyslipidemia	1 (17%)	4 (40%)	0.588
Current tobacco use	0	0	1
Creatinine in mg/dL	0.64 (0.54 – 0.64)	0.67 (0.57 – 0.86)	0.479
Hemoglobin A1c, %	5.6 (5.5 – 5.8)	5.7 (5.3 – 6.3)	0.786
Total cholesterol in mg/dL	215 (196 – 225)	180 (164 – 193)	0.051
HDL cholesterol in mg/dL	55 (44 – 65)	44 (37 – 46)	0.102
LDL cholesterol in mg/dL	129 (119 – 141)	107 (98 – 125)	0.193
Triglycerides in mg/dL	152 (82 – 244)	130 (119 – 202)	0.828

In multivariate analyses, T cell and monocyte activation markers remained associated with previously treated TB

- HLA-DR and CD38 co-expression in CD4+ T cells remained associated with previously treated TB after adjusting for age, sex, history of diabetes mellitus, and hypertension (*b*=0.75; *p*=0.02).
- Increased HLA-DR density on monocytes remained associated with previously treated TB after adjusting for age, sex, history of diabetes mellitus, and hypertension (*b*=0.51; *p*=0.048).
- Persons previously treated for TB showed a trend towards increased CD4+ to CD8+ ratio (*b*=0.42; *p*=0.081) or increased CD4+ T cell expression of ICAM-1 (*b*=0.47; *p*=0.062) in adjusted analysis.

Persons previously treated for TB exhibit increased CD4+ T Cell and Monocyte Activation

Figure 1. Previously treated TB is associated with higher frequency of HLA-DR+ CD38+ cells in CD4+ T cell subsets

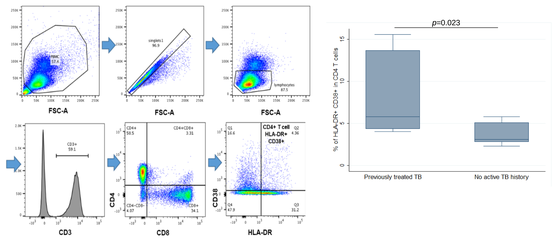
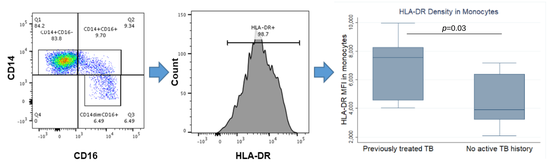


Figure 2. Previously treated TB is associated with increased HLA-DR expression in monocytes



T cell phenotyping:

- Previously treated TB was associated with an increased CD4+ to CD8+ ratio (2.4 vs. 1.3; *p*=0.03).
- There was an increased proportion of CD4+ T cells co-expressing HLA-DR and CD38 activation markers among people with previously treated TB compared to controls without history of active TB (5.8% vs. 3.1%; *p*=0.023; Figure 1).
- Previously treated TB was also associated with increased expression of ICAM-1 in CD4+ T cells, compared controls (56.3% vs. 33.2%; *p*=0.03).
- There were no differences in HLA-DR, CD38, ICAM-1, or CD69 expression in CD8+ T cells between study groups.

Monocyte phenotyping:

- There was a trend toward a higher proportion of inflammatory monocyte subsets in the previously treated TB group compared to controls (8.7% vs. 2.8%; *p*=0.065).
- HLA-DR density on monocytes was increased in the prior TB group compared to the group without TB disease (HLA-DR median fluorescence intensity [MFI]; 7572 vs. 3917; *p*=0.03; Figure 2).
- There were no significant differences in expression of TLR4, CD69, CD36, CD163, CCR2, CX3CR1 on total monocytes and monocyte subsets between the study groups.

Conclusions

- Persons previously treated for TB exhibited increased CD4+ T cell and monocyte activation markers within one year after completion of TB treatment.
- Future longitudinal studies of individuals who recover from TB are warranted to understand the implications of persistent immune activation in the pathogenesis of TB recurrence and inflammation-driven diseases.

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