Increased HLA-DR Expression in Monocytes of Persons with HIV and Latent Tuberculosis Infection

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Background

- Persistent monocyte activation contributes to the increased risk of end-organ complications in persons living with HIV (PLWH)
- Whether *Mycobacterium tuberculosis* (*Mtb*) coinfection has an effect on monocyte activation in PLWH is unknown. We hypothesized that there would be greater monocyte activation phenotypes in PLWH with *Mtb* coinfection

Objective

We aimed to characterize monocyte phenotypes and activation in HIV and latent TB infection

Methods

Study design and setting:

- Cross-sectional study within a cohort of PLWH and HIV-uninfected participants enrolled at the Joint Clinical Research Centre in Kampala, Uganda
- Participants were 45 years of age or older
- PLWH had to be on stable antiretroviral therapy with HIV viral load ≤1,000 copies/mL within the 6 months prior to cohort entry
- HIV-uninfected participants had at least one traditional cardiovascular disease risk factor
- For the nested cross-sectional study, participants completed a TB questionnaire and had a QuantiFERON TB (QFT) test

Definition of TB infection status:

- We defined 3 TB-related categories:
  - **Latent TB infection (LTBI)** was defined by a positive QFT and no TB symptoms
  - **Prior active TB** was defined by self-report and verified by medical records
  - **No TB infection** was defined by a negative QFT, no TB symptoms, and no prior TB

Flow cytometry assays:

- Fresh blood samples were stained with the following antibodies/conjugated fluorochromes: CD14/Pacific blue, CD16/PE, CD62p/PE-Cy5, CD69/PE-Cy7, CX3CR1/APC, HLA-DR/APC-Cy7, and tissue factor/FITC
- Data was acquired using a MACSQuant flow cytometer
- Data was analyzed using FlowJo v10

Statistical analyses:

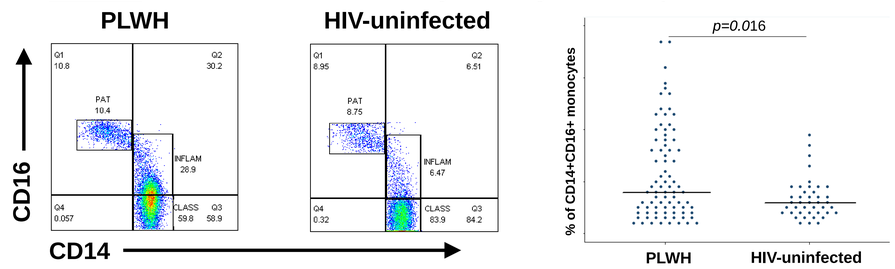
- We used Mann-Whitney and Kruskal-Wallis tests for group comparisons of numeric and flow cytometry data
- We used chi-square for comparisons of categorical data
- Multivariable linear regression modeling of log-transformed flow cytometry variables of interest
- *P* values <0.05 were considered statistically significant

Results

1. *CD14⁺CD16⁺ inflammatory monocytes were expanded in PLWH*

- We included 125 participants (83 PLWH and 42 without HIV) with flow cytometry data and defined TB status in this analysis
- In PLWH, the median CD4⁺ count was 582 cells/uL (interquartile range, 414 – 764); 98% had HIV viral load <1,000 copies/mL
- PLWH had a higher frequency of total monocytes (4.3% vs 3.2%; *p*<0.001) and inflammatory monocyte subset (15.5% vs 11.7%; *p*=0.016; see Figure 1) compared to HIV-uninfected participants
- In multivariable regression, a higher frequency of inflammatory monocytes remained associated with HIV infection after adjusting for TB status, age, sex, diabetes mellitus, and cholesterol levels (log-%, b=0.37; p=0.019)

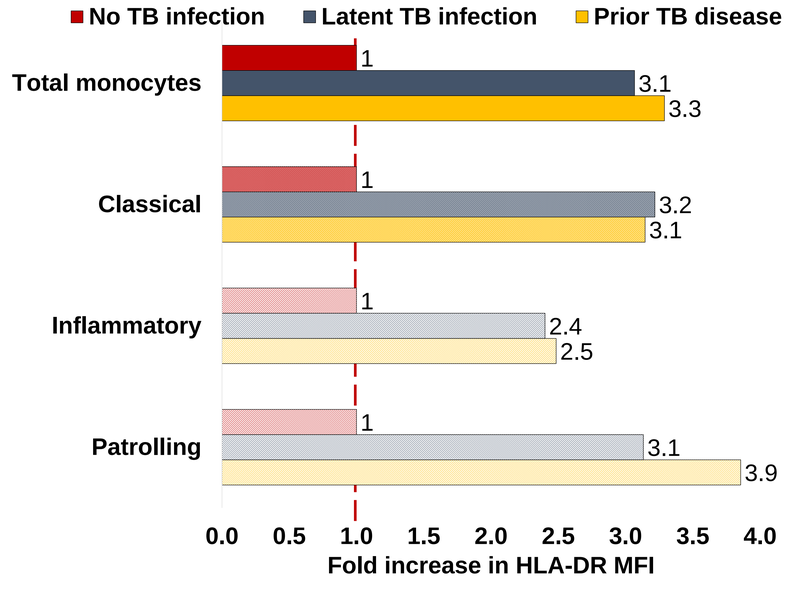
Figure 1. CD14⁺CD16⁺ inflammatory monocytes are expanded in PLWH



2. *Among PLWH, prior TB and LTBI were associated with increased HLA-DR expression in all monocyte subsets*

- Among PLWH, prior TB was associated with increased frequency of total monocytes compared to LTBI (5.1% vs 3.7%; *p*=0.013), but not when compared to PLWH without TB infection
- HLA-DR density on all monocyte subsets was higher in PLWH with LTBI or prior TB compared to PLWH without TB infection (see Figure 2)
- In multivariable regression, a higher density of HLA-DR on monocytes remained associated with LTBI or prior TB (log-MFI; b=1.17; *p*<0.001), after adjusting for age, sex, diabetes mellitus, and cholesterol levels

Figure 2. Compared to PLWH without TB infection, PLWH with LTBI or prior TB disease had increased HLA-DR expression in monocytes



3. *Among HIV-uninfected persons, LTBI was not associated with alterations in monocyte subsets or HLA-DR expression*

- Among HIV-uninfected individuals, no participants with prior TB disease were identified. Therefore only comparisons between participants with and without LTBI were conducted
- There were no differences in the proportion of total monocytes, monocyte subsets, or HLA-DR expression by LTBI status

Discussion

- We found that both LTBI and prior TB disease were associated with increased HLA-DR expression in circulating monocytes of PLWH from a TB-endemic area
- Our findings indicate increased monocyte activation in the setting of HIV and LTBI coinfection
- To our knowledge, this is the first study to characterize monocyte activation phenotypes in PLWH with defined TB coinfection status
- Even after TB disease clinical cure, PLWH have evidence of increased monocyte activation compared to PLWH without TB infection
- Increased monocyte activation in PLWH with LTBI coinfection or prior TB disease may contribute to the pathogenesis of non-communicable diseases observed in PLWH. Future studies of this cohort will specifically examine associations with subclinical coronary artery disease
- Our HIV-uninfected population was relatively small (n=42), which may have limited our ability to detect differences in monocyte activation by LTBI status in this group

Conclusions

- LTBI and prior active TB were associated with increased HLA-DR expression on all monocyte subsets in PLWH
- These results indicate increased monocyte activation in the setting of HIV and TB coinfection, which may contribute to the pathogenesis of non-communicable diseases in PLWH

Acknowledgements

This work was supported in part by a Pilot Award from the University of Cincinnati Department of Internal Medicine, NCATS KL2 TR001426, and NHLBI K23 HL123341

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