Anti-mycobacterial activity is enhanced by blocking the Tim3-Galectin 9 interaction in HIV patients

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Background: T cell immunoglobulin and mucin-(Tim)-3 domain is an inhibitory molecule involved in immune tolerance, autoimmune responses, and antiviral immune evasion. CD4+ and CD8+ T cells express it at variable levels. High expression of Tim3 on T cells from HIV patients has been associated with an exhausted immune phenotype. However, it has also been demonstrated that the Tim3 interaction with its ligand Galectin-9 (Gal9) induces macrophage activation that results in killing of *Mycobacterium tuberculosis* (M.tb). We speculate that manipulation of the Tim3-Gal9 pathway can restore lymphocyte function to eliminate intracellular Mtb-infection.

Methods: We included 20 HIV patients and 20 healthy controls (HC). All samples were obtained in accordance with the Institutional Review Board's protocol. Tim3 and Gal9 expression were analyzed by flow cytometry. Monocyte-derived macrophages (MDM) were infected with Mtb (H37Rv; MOI=10:1). After infection, T cells or blocking antibodies were added. Four days after infection, cells were lysed and mycobacteria counted. Cytokines were analyzed by FlexSet.

Results: We identified that basal expression of Tim3 was elevated on CD8+ T cells from HIV patients (26±9.2%) *vs.* HC (14.8±6.9%) (p=0.001). No changes were observed in Gal expression. Suppression of intracellular bacterial growth was normalized to the amount of bacterial growth in the absence of T cells (= 100%). T cells from HIV patients led to a 59.9% reduction in bacterial growth vs. 70.6% on HC. Control of bacterial growth in HIV patients increased to 100% based on the antibody blocking studies. The opposite phenomenon was observed in HC. We identified that the antimicrobial effect was partially dependent on the production of pro-inflammatory cytokines critical to limit bacterial growth.

Conclusion: Our results established that modulation of the Tim3-Gal9 pathway upon treatment with specific blocking antibodies might recover cytokine production by macrophages and T cells accelerating antimicrobial immunity against M.tb.