Perturbation of Regulatory T Cell Subsets in HIV Infected Children

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Background: Regulatory T cells (Tregs) mediate immune tolerance during autoimmune disease and chronic infections. During HIV infection, Tregs may act either beneficially to curb immune activation or pathologically to suppress HIV-specific immune responses. Previous reports of Tregs during chronic HIV have conflicting results with higher or lower levels compared to controls. Identifying true Tregs with suppressive activity proves challenging during HIV infection, as traditional Treg markers, CD25 and FOXP3, may transiently upregulate expression as a result of immune activation. Helios is a recently identified transcription factor that marks natural Tregs with suppressive activity. Moreover FOXP3+Helios+ CD4 T cells do not produce the cytokine IL-17, and have been called “bona fide” Tregs. We sought to identify these bona fide Tregs in vertically infected HIV positive children.

Methodology: We evaluated Treg levels by flow cytometry in the peripheral blood of 60 children from Bomu Hospital in Mombasa, Kenya. The cohort included age-matched children between 3 to 12 years old in the following categories: HIV negative (HIV-), HIV positive antiretroviral therapy naïve (ART-), and HIV positive on antiretroviral therapy (ART+). Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved from each subject. Thawed PBMCs were stained for surface antibodies CD3, CD4, CD25, CD38, CD45RO, and intracellular transcription factors FOXP3 and Helios. All statistical analysis was performed with GraphPad Prism software using Mann-Whitney or Spearman’s correlation tests.

Results: HIV+ children (ART- and ART+) expressed higher levels of FOXP3 and Helios in CD4 T cells compared with HIV- controls (ART-: p=0.0012, ART+: p=0.0057). FOXP3+Helios+ expression inversely correlated with the percent of CD4 T cells (p<0.0001, r=-0.4883), despite nearly normal CD4 levels in ART+ children. As previously reported, HIV infected children had higher immune activation as measured by CD38+HLA-DR+ expression on CD8+ T cells (ART-: p<0.0001, ART+: p=0.0223). This immune activation (CD38+HLADR+ CD8 T cells) positively correlated with FOXP3+Helios+ Treg levels (p=0.0008, r=0.4301). The ART- group also demonstrated an activated phenotype by increased CD38 expression in memory (p=0.0001) and in FOXP3+Helios+ memory CD4 T cells (p=0.0010) that was not present in the ART+ subjects.

Conclusions: Bona fide Treg levels increase during HIV infection and correlate with waning CD4 T cells and higher immune activation. While antiretroviral therapy decreases the activated state in these bona fide Tregs, it does not restore Treg levels to the homeostatic proportion in HIV negative children. This increase in Helios+ Tregs may act to ameliorate chronic immune activation during HIV infection.