Perturbation of Regulatory T Cell Subsets in HIV Infected Children

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Introduction

Regulatory T cells (Tregs) mediate immune tolerance during autoimmune disease and chronic infections by suppressing the functions of effector T cells (1). During HIV infection, Tregs may either beneficially curb immune activation or pathologically to suppress HIV-specific immune responses (2). Previous reports of Tregs during chronic HIV disease have conflicting results with higher or lower levels correlated with disease progression (1,2). 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, which is a hallmark of HIV infection (3,4).

Helios is a recently identified transcription factor that marks natural Tregs with suppressive activity and does not up-regulate expression after activation (5,6,7). Concomitant expression of FOXP3 and Helios has been suggested as a highly specific marker of “ bona fide” Tregs (4,8). We evaluated identification of Tregs with traditional markers CD25 and FOXP3 or Helios and FOXP3 in vertically infected HIV positive children. Next we compared bona fide Tregs in HIV infected and uninfected children and their correlation with markers of HIV disease progression.

Methods

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 naive (HIV+ Naive), and HIV positive antiretroviral therapy (HIV+ ART). Subject characteristics are shown in Table 1. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque gradient (GE Healthcare) and cryopreserved from each subject. thawed PBMCs were stained for surface antibodies CD3, CD4, CD8, CD25, CD38, CD45RO, and HLA-DR and intracellular transcription factors FOXP3 and Helios. All statistical analysis was performed with GraphPad Prism software using Mann-Whitney or Spearman’s correlation tests.

References


Figure 1. Identification of regulatory T cells

Figure 2. Elevated Memory Tregs in HIV Infected Children

Figure 3. Memory Tregs Correlate with Declining %CD4 T cells

Figure 4. Immune Activation Correlates with Memory Tregs

Figure 5. Activated Phenotype in Memory Tregs

Table 1: Subject Characteristics

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Conclusions

Traditional markers of Tregs, CD25 and FOXP3, may under-estimate Treg levels, as a significant portion of memory Tregs are CD25 negative (Figure 1). We identified bona fide memory Tregs in HIV infected children by FOXP3 and Helios co-expression within memory CD4 T cells. Memory Treg levels increase during HIV infection and correlate with falling CD4 T cells and increased chronic immune activation in CD8 T cells. Interestingly, memory HIV+ children also expressed an activated phenotype with CD38 expression. 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. Finally, the activated phenotype within memory Tregs correlates with generalized immune activation in CD8 and memory CD4 T cells. An increase in Helios+ Tregs may act to ameliorate chronic immune activation during HIV infection. The functional effects of Treg activation are unknown and a question for future studies.