Perturbation of Regulatory T cell Subsets in HIV Infected Children

Khaitan A[1,2], Mwamzuka M[6], Marshed F[6], Kravietz A[1,3], Ilmet T[1,2], Said S[6], Ahmed A[6], Borkowsky W[1,2], Unutmaz D[1,3,4,5]

[1]New York University School of Medicine, New York, USA; [2]Department of Pediatrics, Division of Infectious Diseases and Immunology; [3]Department of Microbiology; [4]Department of Pathology; [5]Department of Medicine; [6]Bomu Hospital, Mombasa, Kenya

Introduction

Regulatory T cells (Tregs) mediate immune tolerance during autoimmune disease and chronic infections by suppressing the functions of effectorT cells (1). During HIV infection, Tregs may act either beneficially to 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 compared to controls (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 (5,6,7,8). Concomitant expression of FOXP3 and Helios has been suggested as a highly specific marker of "bona fide" Tregs (9,10). We evaluated identification o of Tregs with traditional markers CD25 and FOXP3 or Helios and FOXP3 in vertically infected HIV positive children. Next we compared bona fide Treg levels 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 naïve (HIV+ Naive), and HIV positive on 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 then run on BD LSRII flow cytometer. Data was analyzed using FlowJo software (Treestar) and gated on live cells based on fixable viability dye (eBioscience). All statistical analysis was performed with GraphPad Prism software using Mann-Whitney or Spearman's correlation tests.

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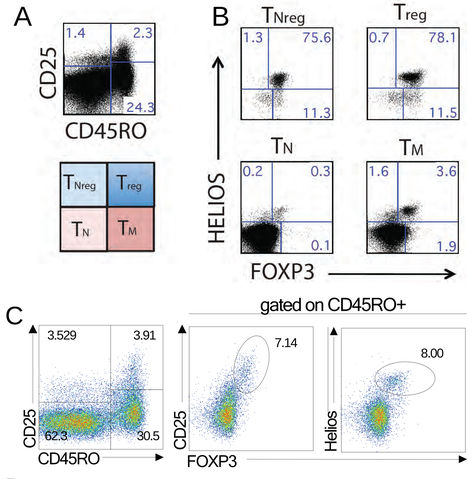
Results

Table 1: Subject Characteristics

	HIV-	HIV+ Naive	HIV+ ART	p
n	19	21	20	ns
Age*	8.5 (5.7-11.1)	8.9 (6.9-9.5)	7.8 (4.9-10)	ns
%CD4*	39 (33-44)	25 (18-29)	34 (30-41	p<0.0001

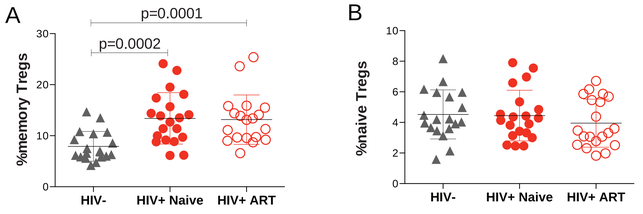
*Median values with upper and lower quartile range

Figure 1. Identification of regulatory T cells



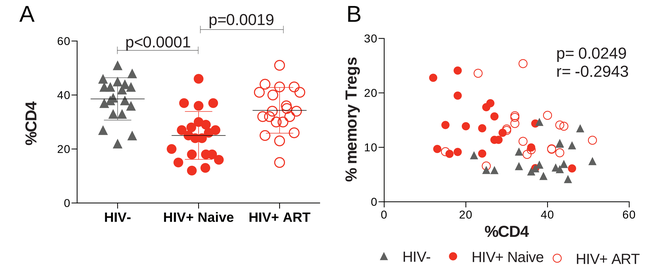
(A) PBMCs were stained with CD3, CD4, CD25 and CD45RO to identify naïve and memory conventional and regulatory T cells in the following subsets: TN (CD25-CD45RO-), TM (CD25-CD45RO+), Treg (CD25+CD45RO+), and TNreg (CD25+CD45RO-). (B) Above PBMCs were permeabilzed then stained with FOXP3 and Helios, identifying a significant portion of Tregs within the TM subset. (C) Shown is Treg gating strategies, using markers CD25, CD45RO, and transcription factors FOXP3 and Helios. (D) Mean values of Tregs as identified by FOXP3+CD25+ or FOXP3+Helios+ cells within memory CD4 T cells in the study cohort of HIV- and HIV+ children. ** and *** represent p<0.01 and p<0.001 respectively.

Figure 2. Elevated Memory Tregs in HIV Infected Children



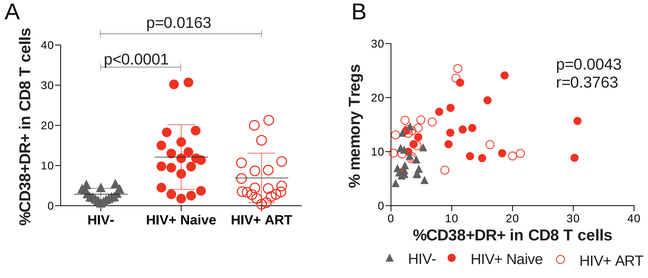
PBMC were stained for surface markers CD3, CD4 and CD45RO then permeabilized and stained for transcription factors FOXP3 and Helios. (A) Memory Tregs identified by FOXP3+Helios+ cells within CD45RO+ CD4 T cells. (B) Naïve Tregs identified by FOXP3+Helios+ cells within CD45RO- CD4 T cells.

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



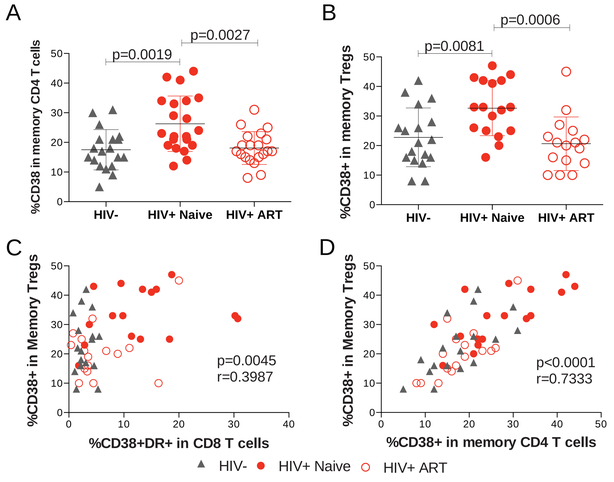
(A) %CD4 T cells in HIV- and HIV+ children. (B) Memory Tregs inversely correlate with %CD4 T cells.

Figure 4. Immune Activation Correlates with Memory Tregs



(A) PBMC were stained for surface markers CD3, CD4, CD8, and HLADR. CD38+HLA-DR+ levels were determined in the CD3+CD8+ population to identify chronically activated CD8 T cells. (B) Activated CD8 T cells positively correlate with memory Tregs.

Figure 5. Activated Phenotype in Memory Tregs



(A) PBMCs were stained for surface markers CD3, CD4, CD45RO and CD38. Memory CD4 T cells were gated on the CD38+ population to identify activated CD4 T cells. (B) HIV+ children who were ART naïve exhibit an activated phenotype identified by CD38 expression within memory Tregs. CD38 levels in memory Tregs correlate with generalized immune activation in (C) CD8 and (D) memory CD4 T cells.

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 Tregs in untreated 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.

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