Disruption of Innate-Like Unconventional T-Cell Subsets in HIV-Infected Children

Khaitan A[1,2], Kravietz A[1,3], Ilmet T[1,2], Mwamzuka M[6], Marshed F[6], Said S[6], Tastan C[1,3], 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

Innate immunity plays a critical role in HIV pathogenesis. A portion of non-conventional T cells exhibit features of innate cells such as invariant T cell receptors and recognition of non-peptide antigens, and have been termed innate-like T cells. There are three subsets of innate-like T cells: natural killer T cells (NKT), mucosal associated invariant T cells (MAIT), and γδ T cells.

NKT cells express the semi-invariant Vα24 TCR and recognize non-peptide glycolipid antigens presented by the MHC class I-like molecule CD1d[1,2]. NKT cells mediate immune responses in autoimmune, oncologic, and infectious diseases via potent cytotoxic activity and secretion of pro-inflammatory Th1, Th2, and Th17 cytokines[1]. During HIV infection, NKT cells are preferentially infected and depleted in early stages of disease[3,4].

MAIT cells are MR-1 restricted innate-like lymphocytes, primarily found in CD8 T cells. The semi-invariant antigen receptor, Vα7.2, on MAIT cells recognizes riboflavin metabolites derived from bacteria or fungi[5,6]. MAIT cells preferentially migrate to mucosal surfaces and play an important role in mediating tolerance to commensal organisms and immune responses to invading pathogens[5]. MAIT cells also have cytotoxic activity and secrete Th1 and Th17 cytokines[5,6]. In HIV infected adults, MAIT cells are depleted in both the peripheral blood and gastrointestinal mucosa[7-9].

γδ T cells respond rapidly to numerous stess-induced ligands recognized by their γδ TCR, natural killer receptors, and toll-like receptors[10]. These cells have a tropism for epithelial cells, and home to the liver, skin, and mucosal sites[10]. Like NKT cells, γδ T cells exhibit cytotoxic activity and secrete Th1, Th2, or Th17 cytokines to mediate pathogen clearance, antitumor responses, or inflammation[11]. In HIV infection, a subclass of γδ T cells, Vγ1 cells, show an early and persistent expansion[11].

While studies In HIV+ adults have shown disruption of innate-like T cells, with lower MAIT and NKT cells and higher γδ T cells in the peripheral blood, little is known about these T cell subsets in HIV+ children. We sought to determine whether innate-like T cells are disrupted in treated and untreated perinatally infected HIV+ children.

Methods

We evaluated peripheral blood samples of 76 perinatally-infected HIV+ and 40 unexposed HIV negative (HIV-) children between 3-12 years old from Bomu Hospital in Mombasa, Kenya. The HIV+ cohort included 39 antiretroviral therapy (ART) naïve (ART-) and 37 children treated with ART (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 with surface antibodies CD3, CD4 and CD8 with Vα7.2 and CD161 (MAIT), γδ TCR, and Vα24Jα18 TCR (NKT) then run on BD LSRII flow cytometer. Data was analyzed using FlowJo software (Treestar). Plasma sCD14 was measured by ELISA (R&D). Statistical analysis was performed with GraphPad Prism software using Mann-Whitney or Spearman's correlation tests.

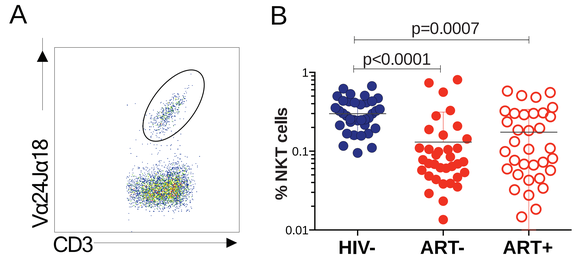
Table 1: Subject Characteristics

	HIV-	ART-	ART+	p
n	40	39	36	ns
Age*	7.6 (3.9-10.9)	8.7 (6-10.6)	6.6 (3.6-9.3)	ns
%CD4*	37 (30-42)	25 (13-33)	34 (29-41)	p<0.0001
log HIV copies/ml*	4.8 (3.9-5.3)	2 (2-2.5)		p<0.0001

Median values with upper and lower quartile range

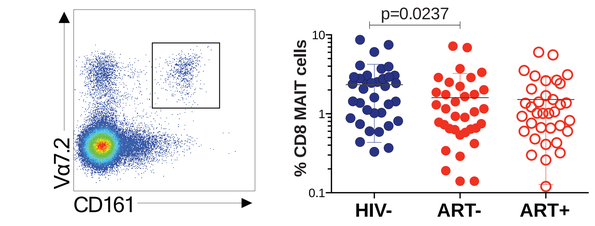
Results

Fig. 1: Decreased NKT Cells in HIV+ children



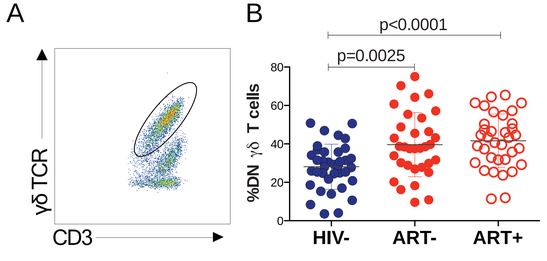
PBMC were stained for surface markers CD3, CD4, CD8, and Vα24Jα18 TCR then analyzed with flow cytometry. (A) NKT cells were identified as the Vα24Jα18+ T cells. (B) Percent of NKT cells in T cells in HIV-, ART-, and ART+ children.

Fig. 2: Decreased CD8 MAIT Cells in HIV+ children



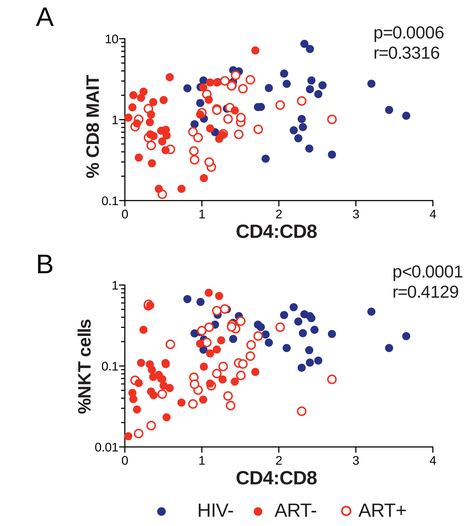
PBMC were stained for surface markers CD3, CD4, CD8, CD161, and Vα7.2 then analyzed with flow cyometry. (A) MAIT cells were identified as the Vα7.2+CD161+ population within CD8 gated T cells. (B) Percent of MAIT cells in CD8 T cells in HIV-, ART-, and ART+ children.

Fig. 3: Increased DN γδT cells in HIV+ children



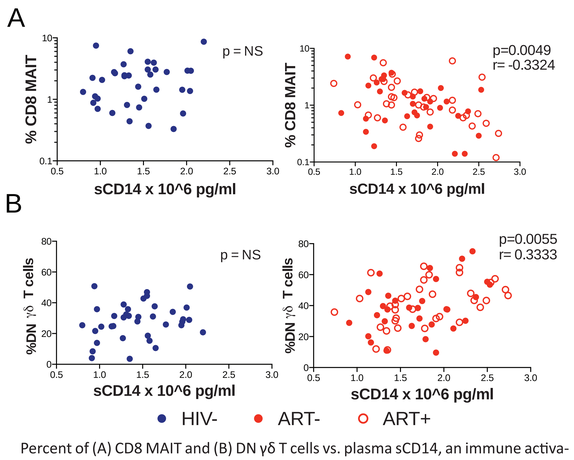
PBMC were stained for surface markers CD3, CD4, CD8, and γδ TCR then analyzed with flow cytometry. (A) γδ T cells were identified as γδ TCR+ cells in CD4-CD8- (DN) gated T cells. (B) Percent of γδ T cells in DN T cells in HIV-, ART-, and ART+ children.

Fig. 4: CD8 MAIT and NKT cells correlate with immunolgic status



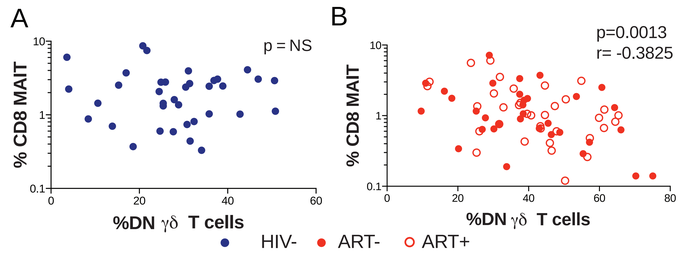
Percent of (A) CD8 MAIT and (B) NKT cells graphed vs. CD4:CD8 ratios in HIV-, ART-, and ART+ children. Correlations were computed using Spearman's rank test.

Fig. 5: MAIT, γδ T cells, & Immune Activation



Percent of (A) CD8 MAIT and (B) DN γδ T cells vs. plasma sCD14, an immune activation marker, in HIV- and HIV+ children.

Fig. 6: CD8 MAIT cells inversely correlate with DN γδ T cells in HIV infected children



Percent of CD8 MAIT cells vs. percent of DN γδ T cells in (A) HIV- and (B) HIV infected children.

Conclusions

CD8+ MAIT and NKT cells are relatively lower in HIV+ children despite ART compared to HIV- children. This decline in CD8 MAIT and NKT cells worsens with deteriorating immune status, marked by CD4:CD8 ratios. In HIV infected children, sCD14, a marker that reflects immune activation triggered by microbial translocation, inversely correlates with CD8 MAIT cells and positively correlates with DN γδ T cells. Loss of MAIT cells from the periphery may reflect migration to mucosal tissues in response to microbial translocation. Furthermore, in HIV+ children a decrease in CD8 MAIT cells correlates with increased DN γδ T cells. Expansion of DN γδ T cells may be a compensatory response to diminished CD8 MAIT cells in order to maintain innate-like functions in the peripheral blood of HIV infected children. Overall, disruptions of innate-like T cells in HIV infected children parallel those reported in the adult population.

References

1. Li D, Xu XN. NKT cells in HIV-1 infection. Cell research 2008;18:817-22.
2. Unutmaz D. NKT cells and HIV infection. Microbes and infection / Institut Pasteur 2003;5:1041-7.
3. Fernandez CS, Kelleher AD, Finlayson R, Godfrey DI, Kent SJ. NKT cell depletion in humans during early HIV infection. Immunology and cell biology 2014;92:578-90.
4. Motsinger A, Haas DW, Stanic AK, Van Kaer L, Joyce S, Unutmaz D. CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. The Journal of experimental medicine 2002;195:869-79.
5. Gold MC, Lewinsohn DM. Co-dependents: MR1-restricted MAIT cells and their antimicrobial function. Nature reviews Microbiology 2013;11:14-9.
6. Ussher JE, Klenerman P, Willberg CB. Mucosal-associated invariant T-cells: new players in anti-bacterial immunity. Frontiers in immunology 2014;5:450.
7. Cosgrove C, Ussher JE, Rauch A, et al. Early and nonreversible decrease of CD161++ /MAIT cells in HIV infection. Blood 2013;121:951-61.
8. Eberhard JM, Hartjen P, Kummer S, et al. CD161+ MAIT cells are severely reduced in peripheral blood and lymph nodes of HIV-infected individuals independently of disease progression. PloS one 2014;9:e111323.
9. Greathead L, Metcalf R, Gazzard B, Gotch F, Steel A, Kelleher P. CD8+/CD161++ mucosal-associated invariant T-cell levels in the colon are restored on long-term antiretroviral therapy and correlate with CD8+ T-cell immune activation. Aids 2014;28:1690-2.
10. Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. Nature reviews Immunology 2010;10:467-78.
11. Pauza CD, Poonia B, Li H, Cairo C, Chaudhry S. gammadelta T Cells in HIV Disease: Past, Present, and Future. Frontiers in immunology 2014;5:687.

NYU School of Medicine
NYU LANGONE MEDICAL CENTER