Profile of T Cell Immune Responses in HIV Infected Children from Uganda
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ABSTRACT
HIV immunopathogenesis in children remains poorly understood. We assessed T cell immune activation and HIV-specific responses in antiretroviral (ART) naive children in Uganda (N=154). Increased CD4+ and CD8+ T cell activation strongly correlated with decreased CD4 cell percentage (r=0.417, p<0.001, r=0.427, p=0.004, respectively). Interestingly, no correlation between plasma HIV RNA and T cell activation was observed after controlling for CD4 count (p=0.05), in contrast to findings observed in HIV positive adults. HIV-specific T cells were readily detectable and the presence of Gag-specific CD4+ T help in children was associated with increased HIV-specific effector CD8+ T cells. Our study is the first to report the T cell profile in HIV infected children in Uganda. Understanding the balance between immune activation and T cell immunity in HIV infected children may provide further insights into the mechanisms leading to effective immune control.

METHODS
Samples were obtained from the study cohort of the children with HIV and Malaria Project (CHAMP), an ongoing prospective observational study investigating interactions between HIV and malaria coinfections in children. Only antiretroviral therapy (ART)-naive volunteers were evaluated for this immunological study. Demographic information, CD4 T cell status (percentage and absolute count), and HIV load were obtained at the time of blood draw. For comparison, samples from HIV+ positive adult volunteers (n=111) were obtained from existing cohort studies in Uganda. Demographic information, CD4 T cell counts, and plasma HIV RNA levels for the adults were also obtained at the time of enrollment and blood draw. All study participants gave written informed consent. Parents or legal guardians consented on behalf of the children.

Immune Activation
Activation status was performed by incubating fresh blood samples with the following antibodies: CD3 APC, CD8 Perp and PE, HLA-DR FITC, and CD16 PE. CD8 and CD4 activation was defined as the percentage of CD3+ CD8+ HLA-DR+ or CD3+ CD4+ HLA-DR+ lymphocytes, respectively. A minimum of 30,000 CD3+ cells per sample were acquired using a 4-color flow cytometers (FACS Calibur, BD Biosciences). Analysis was performed by FLOWJO software (TreeStar).

Measurement of HIV-specific T-cell responses
Depletion assay was performed as previously described. Cytotoxic CD8+ T cell function has been shown to be correlated directly with T cell degranulation, a prerequisite process of perforin granzyme mediated lytic function, which can be measured by increased expression of surface CD107. Briefly, PBMC (3 x 10^6) were incubated with 10 ng/ml of anti CD49D and anti-CD165s and anti-CD107a/b PE (BD 740704) and Granzyme-penta-s, B-6.6.3 of final volume 20 % for 1 h in the presence of Golgi stop. Sphingosine and antimonial B use (1 mg/ml) and media alone were used as positive and negative controls respectively. Cells were then stained with the following antibodies: IFN-, PE, CD3 PerCP Cy5, and CD8 APC. A minimum of 30,000 CD3+ cells per sample were acquired using a 4-color flow cytometers and analysis was performed by FLOWJO software. Results were expressed as Percent CD107a/b+, IFN+ positive CD3+CD8+ T cells (Percent positivity = % antigen-specific + % negative control). CD8 T cell responses were not assessed separately and analyzed as CD1+CD8+ T cell responses. Responses greater than or equal to 0.1% and 2 times the background were considered positive. All volunteers demonstrated significant CD107a/b+ and IFN+ expression following stimulation. Background expression was <0.1%.

Statistical Analysis
Groups were compared using the Mann-Whitney U test and analysis was performed with Prism software version 4.02 GraphPad, San Diego, CA. Spearman’s correlation coefficient was used to determine the correlation between two variables, and linear leastsquares regression model was used in multivariate analysis. Statistical significance was defined as P<0.05.

RESULTS

Immune Activation: Immunophenotyping strategy

Association between CD8+ T cell immune activation and CD4 count or HIV RNA levels in HIV infected Ugandan children

Figure 2: Correlation between the CD8 count in plasma HIV RNA and T cell activation in Children. The percentage of CD8+ T cell activation is plotted against the CD8+ T cell count in Ugandan children. The correlation coefficient between CD8+ T cell activation and plasma HIV RNA is 0.163 (P<0.05).

Figure 3: Correlation between the CD4 count in plasma HIV RNA and T cell activation in Children. The correlation coefficient between CD4+ T cell activation and plasma HIV RNA is 0.417 (P<0.001).

SUMMARY AND CONCLUSIONS
CD4+ and CD8+ T cell activation in Ugandan HIV positive children correlated significantly with CD4 status (percent or absolute counts), but not viral load.

Presence of HIV specific T helper response was associated with higher HIV+ and CD107+ CD8 effector T cell response.

Presence of CD4 T helper HIV specific response was significantly associated with higher CD4+ and CD8+ T cell activation.