Profile of T Cell Immune Responses in HIV-Infected Children from Uganda

Isaac Ssewanyana,1,* Mohamed Elrefaei,1,3* Grant Dorsey,4 Theodore Ruel,1 Norman G. Jones,3 Anne Gasasira,2 Moses Kamya,2 Justine Nakwala,2 Jane Achan,2 Edwin Charlebois,4 Diane Havlir,4 and Huyen Cao2

1Joint Clinical Research Centre and 2Makerere University, Kampala, Uganda; 3California Department of Health Services, Richmond, and 4University of California, San Francisco

Human immunodeficiency virus (HIV) immunopathogenesis in children remains poorly understood. We assessed T cell immune activation in antiretroviral therapy–naive children in Uganda (n = 154). Increased CD4+ and CD8+ T cell activation strongly correlated with decreased CD4+ T cell percentage. Interestingly, no correlation between plasma HIV RNA level and T cell activation was observed after controlling for CD4+ T cell count. In addition, the presence of Gag-specific CD4+ T helper responses was associated with increased HIV-specific CD8+ T cells. 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.

The AIDS epidemic has severely affected child mortality in regions with high HIV seroprevalence. To date, few studies have investigated the cellular immune profile in HIV-infected children from sub-Saharan Africa. In adults, T cell immune activation in sub-Saharan Africa appears to be higher than in industrialized countries [1]. Distinct regional HIV subtypes may also lead to more-rapid disease progression [2]. CD8+ T cell responses represent an important arm of antiviral immune responses, and the presence of HIV-specific CD4+ T helper responses is associated with lower immune activation, better immunological profile, and less-advanced disease in HIV-infected adults [3, 4]. In the present study, we evaluated the T cell immune activation and HIV-specific T cell immune profile in HIV-1–infected Ugandan children and compared the results with those in a cohort of HIV-positive Ugandan adults.

Subjects, materials, and 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 malarial coinfection in children. CHAMP has enrolled 300 HIV-infected children (ages 1–10 years) from the Pediatric Infectious Disease Clinic at Mulago Hospital, in Kampala, Uganda. 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-negative children (n = 43) and HIV-1–positive adult volunteers (n = 81) were obtained from existing cohort studies in Uganda [1, 5]. 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.

Institutional review board approval was obtained from the California Department of Health Services; the University of California, San Francisco; the Makerere University Research and Ethics Committee; the Ugandan National Council of Science and Technology; and the Joint Clinical Research Centre Institutional Review Board. All study participants gave written informed consent. Parents or legal guardians consented on behalf of the children.

Results. The median age, CD4+ T cell percentage, and plasma HIV-1 RNA level of the pediatric study population were 6 years (range, 1–10 years), 23% (range, 49%–71%), and 79,993 copies/mL (range, 400–750,000 copies/mL), respectively. The median age, CD4+ T cell count, and plasma HIV-1 RNA level of the adult study population were 37 years (range, 23–60 years), 344 cells/mm³ (range, 9–1,095 cells/mm³), and 22,282 copies/mL (range, 400–750,000 copies/mL), respectively.

Heightened CD8+ T cell immune activation has been shown to be associated with rapid disease progression as well as an independent predictor of viral load and CD4+ T cell count in adults. The prognostic value of CD8+ T cell immune activation in HIV-infected children has also been suggested [6]. We assessed CD8+ T cell immune activation in a population of Ugandan children. Activation staining was performed by incubating fresh blood samples as described elsewhere [1]. CD8+ and CD4+ T cell activation was defined as the percentage of CD3+CD8+CD38+HLA-DR+ or CD3+CD8+CD38+HLA-DR+ lymphocytes, respectively. Immune activation in HIV-positive...
children was significantly higher than in HIV-negative Ugandan children (figure 1A). Because there was no significant correlation between the level of immune activation and the age of children ($P = .97$ and $P = .41$ for CD4$^+$ and CD8$^+$ T cells, respectively; data not shown), data from children of all ages were grouped together for analysis.

We found a significant negative correlation between CD8$^+$ T cell immune activation and CD4$^+$ T cell percentage ($r = -0.279; P = .0004$) (table 1). Although we included CD4$^+$ T cell percentage instead of absolute count for correlation analyses in the pediatric population as a more clinically relevant measure of CD4$^+$ T cell status, the significance of correlation analyses remained comparable when using absolute CD4$^+$ T cell count ($r = -0.38; P < .0001$; data not shown). Interestingly, CD8$^+$ T cell immune activation in children did not correlate with plasma HIV RNA level ($P = .28$; table 1). This is in contrast to a significant positive correlation between CD8$^+$ T cell immune activation and plasma HIV RNA level in a cohort of Ugandan adults ($r = 0.46; P < .0001$) (table 1). The level of CD8$^+$ T cell activation was significantly lower in children than in ART-naive HIV-infected Ugandan adults ($P = .005$; data not shown), despite higher plasma HIV RNA levels in children ($P < .0001$; data not shown).

Chronic HIV immune activation is believed to be a critical factor leading to CD4$^+$ T cell depletion. T cell activation could be a decisive factor in the depletion and recovery of CD4$^+$ T cell count in chronically infected children with virological suppression [7]. We report a significant negative correlation between CD4$^+$ T cell immune activation and CD4$^+$ T cell percentage in our pediatric population ($r = -0.617; P < .0001$) (table 1). Correlation analysis also remained significant when using absolute CD4$^+$ cell count ($r = -0.27; P = .0004$; data not shown). Correlation between CD4$^+$ T cell immune activation and plasma HIV RNA level was also observed ($r = 0.163; P = .042$) (table 1). However, this correlation was not significant in subsequent multivariate regression analysis after controlling for CD4$^+$ T cell percentage ($P > .05$) (table 1). In contrast, correlation between CD4$^+$ T cell immune activation and plasma HIV RNA level in adults remained significant after multivariate analyses ($P < .0001$) (table 1). The level of CD4$^+$ T cell activation was, as with CD8$^+$ activation, significantly lower in children than in ART-naive HIV-infected Ugandan adults ($P = .03$; data not shown).

Cytolytic CD8$^+$ T cells play a critical role in the antiviral effect of HIV-specific CD8$^+$ T cells, and the gradual loss of cytolytic function has been implicated in HIV-associated immune dysregulation. The importance of CD4$^+$ T helper responses in the maintenance of functional CD8$^+$ T cells during chronic viral infections, including with HIV, has long been appreciated [8]. The exact relationship between HIV-specific responses and clinical disease remain unresolved, particularly in HIV-infected children. We evaluated HIV-specific T cell responses, as measured by interferon (IFN)–γ secretion and CD107 degranulation, by use of previously described methodologies [9]. Because of sample volume limitations, only 65 individuals were evaluated. There were no significant differences in age, CD4$^+$ T cell percentage, or plasma HIV RNA level between this subgroup and the larger group of children ($P = .74$, $P = .41$, and $P = .28$, respectively; data not shown). HIV-specific responses were readily detectable, and no significant association between these T cell responses and either CD4$^+$ T cell percentage or plasma HIV RNA level was observed ($P > .05$; data not shown).

The presence of HIV-specific T helper responses was associated with increased number of IFN-γ–CD8$^+$ and CD107$^+$–CD8$^+$ effector T cells ($P = .002$ and $P = .03$, respectively) (figure 1B). Interestingly, the presence of HIV-specific T helper responses was also associated with higher T cell immune activation ($P = .013$ and $P = .005$ for CD4$^+$ and CD8$^+$ T cells, respectively) (figure 1C), despite there being no significant differences in plasma HIV RNA levels or CD4$^+$ T cell percentage between children with and those without HIV-specific T helper responses ($P > .05$; data not shown). In contrast, T cell immune activation in Ugandan adults was significantly elevated, with significant correlation with CD4$^+$ T cell count and plasma HIV RNA level (table 1). The magnitude of CD8$^+$ T cell responses was higher in adults than in children ($P < .0001$ for both IFN-γ and CD107) (figure 1D), and the presence of HIV-specific T helper responses did not correlate with the presence of CD8$^+$ effector T cells in adults ($P > .05$; data not shown).

Discussion. Despite the severity and high prevalence of HIV/AIDS among children living in sub-Saharan Africa, a limited number of immune response studies have been conducted in this population. Chronic activation of the immune system facilitates HIV replication, increases plasma HIV load, and accelerates the progression of HIV infection to AIDS. True correlates of immune protection for HIV infection have remained unclear, but HIV–1–specific T cell responses likely play an important role in viral control. We report here the pattern of immune activation and effector T cell responses in a population of HIV-infected Ugandan children.

Elevated immune activation has been previously described in pediatric HIV-1 infection, in association with reduced numbers of naïve T cells, increased apoptosis, and accelerated T cell differentiation [10, 11]. The present data show that CD4$^+$ and CD8$^+$ T cell activation in Ugandan children correlates significantly with CD4$^+$ T cell status (whether percentage or absolute count) but not with viral load. This is in contrast to a strong correlation with HIV RNA load observed in adults [1]. The lack of association with viral load may indicate that a specific threshold of HIV antigen exposure and duration is required before activation of T cells can occur in children. Alternatively, it may simply reflect the rapidly changing kinetics of HIV load during the first few years after birth [12]. In adults, immune activation–induced cell death has been implicated as one major mechanism for CD4$^+$ T cell loss. In children, however, HIV-associated thymic dysfunction is thought to play a more-direct role
Figure 1. A, Immune activation in HIV-uninfected and HIV-infected children and adults in Uganda. Whole blood was stained with anti-HLA-DR–fluorescein isothiocyanate (FITC), anti-CD38–phycocerythin (PE), anti-CD3–peridinin-chlorophyll-protein (PerCP)–CY5.5, and anti-CD8–allophycocyanin (APC) and was analyzed by flow cytometry. Samples were first gated on the CD3+ and CD3–CD8+ lymphocyte populations, and then the percentage of CD8+ and HLA-DR+ cells were determined. Immune activation was defined as the percentage of CD4+ or CD8+ T cells expressing HLA-DR and CD38. Horizontal lines are medians and interquartile ranges. T cell immune activation was measured in a population of HIV-uninfected children (1–10 years old; n = 33) as well as in antiretroviral therapy–naive HIV-infected Ugandan children (1–10 years old; n = 154) and adults (23–60 years old; n = 81), and it was observed that the level of T cell immune activation was significantly lower in HIV-uninfected than in HIV-infected children.

In addition, T cell immune activation was significantly lower in HIV-infected children than adults, despite there being higher plasma HIV RNA levels in children (P < .0001). B, Association between the presence of a HIV-specific CD4+ T cell response and higher CD8+ T cell responses in children. Peripheral blood mononuclear cells (PBMCs) were stimulated with Gag peptides in the presence of anti-CD107a/b–FITC; stained with anti–interferon (IFN)–γ–PE, anti-CD3–PerCP–CY5.5, and anti-CD8–APC; and analyzed by flow cytometry. Samples were first gated on the CD3+CD8+ or CD3–CD8+CD4+ lymphocyte population, and then the percentage of CD107a/b+ and IFN-γ+ cells were determined. Staphylococcus enterotoxin B (SEB; 1 µg/mL; Sigma) and medium alone were used as positive and negative controls, respectively. Responses >0.1% and 2 times the background were considered positive. All volunteers demonstrated significant CD107a/b and IFN-γ expression after SEB stimulation. Background expression was <0.1%. Results are expressed as the percentage of Gag-specific CD8+ or CD4+ T cells expressing CD107a/b or IFN-γ after subtraction of the background values and are reported as mean ± SE of CD8+ T cell responses in individuals with detectable (n = 16) or undetectable (n = 49) CD4+ T cell IFN-γ responses. The presence of CD4+ T cell responses was associated with significantly higher IFN-γ and CD107a/b+ CD8+ T cell responses (P = .002 and P = .03 for IFN-γ and CD107a/b, respectively). C, Association between the presence of an HIV-specific CD4+ T cell response and higher T cell activation in children. Whole blood was stained with anti-HLA-DR–FITC, anti-CD38–PE, anti-CD3–PerCP–CY5.5, and anti-CD8–APC and was analyzed by flow cytometry. Samples were first gated on the CD3+CD8+ and CD3–CD8+CD4+ lymphocyte populations, and then the percentage of CD38+ and HLA-DR+ cells were determined. Immune activation was defined as the percentage of CD4+ or CD8+ T cells expressing HLA-DR and CD38. Horizontal lines are medians and interquartile ranges in children with detectable (n = 16) or undetectable (n = 49) Gag-specific IFN-γ+CD4+ T cell responses. The presence of CD4+ T cell responses was associated with significantly higher CD4+ and CD8+ T cell immune activation (P = .013 and P = .005 for CD4+ T cells and CD8+ T cells, respectively). D, Lower HIV-specific T cell responses in children. PBMCs from children (n = 65) and adults (n = 51) were stimulated with Gag peptides as described in panel A. CD8+ T cell responses were significantly higher in adults than in children (P < .0001 and P < .0001 for IFN-γ and CD107a/b, respectively). Groups were compared using the Mann-Whitney U test, and analysis was performed with PRISM software (version 4.02; GraphPad). Statistical significance was defined as P < .05.
in immune destruction [13, 14]. Thus, differential thymic production may influence the activation profile in our pediatric study, although more-complex factors likely contribute, including a variable level of T cell maturity.

The dogma in pediatric immunology has been that children have an incompetent immune system. Prior studies have suggested that HIV-specific responses in children (as measured by IFN-γ production) are lower in magnitude and lack breadth [15], compared with those in adults. We reported Gag-specific CD4+ helper responses. Interestingly, the presence of HIV-specific CD8+ T cell responses in Ugandan adults has several limitations. The cross-sectional nature of our study precludes the ability to fully assess the impact of HIV on T cell development and maturation in this population of HIV-infected children.

Our study is the first to report the T cell profile in HIV-infected children in Uganda and has several limitations. The cross-sectional design does not permit definitive analysis of the predictive value of immune activation in clinical disease. Furthermore, our measurement of immune activation did not reflect the differences in activation between naive and memory T cells in children. Further analysis of immune activation in children would ideally assess activation in specific subpopulations of T cells. Clearly, more studies are needed to fully assess the impact of HIV on T cell development and maturation in this population of HIV-infected children.

References


Table 1. Associations between clinical factors and immune activation.

<table>
<thead>
<tr>
<th>Dependent variable, independent variable</th>
<th>Univariate coefficient (P value) in</th>
<th>Multivariate coefficient (95% CI) in</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+HLA-DR+CD38+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+</td>
<td>−0.617 (.0001)</td>
<td>−0.64 (.0001)</td>
</tr>
<tr>
<td>CD4+</td>
<td>0.163 (.042)</td>
<td>0.62 (.0001)</td>
</tr>
<tr>
<td>CD8+HLA-DR+CD38+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+</td>
<td>−0.279 (.0004)</td>
<td>−0.38 (.0003)</td>
</tr>
<tr>
<td>CD4+</td>
<td>0.087 (.28)</td>
<td>0.46 (.0001)</td>
</tr>
<tr>
<td>CD8+</td>
<td>1.89 (.0001)</td>
<td>5.7 (.13 to 10.2)</td>
</tr>
</tbody>
</table>

a Spearman’s correlation at 95% confidence interval (CI).
b $R^2$ using linear least-square model for CD4+ and CD8+ T cell activation (0.35 and 0.11, respectively).
c $R^2$ using linear least-square model for CD4+ and CD8+ T cell activation (0.42 and 0.154, respectively).
d CD4+ is expressed as percentage and absolute count for children and adults, respectively.
e Significance is defined as $P < .05$.
f Viral loads are log transformed.