Two-Year Clinical and Immune Outcomes in Human Immunodeficiency Virus–Infected Children Who Reconstitute CD4 T Cells Without Control of Viral Replication After Combination Antiretroviral Therapy

Guity Ghaffari, PhD*; Dominick J. Passalacqua, MSc‡; Jennifer L. Caicedo, MD*; Maureen M. Goodenow, PhD*‡§; and John W. Sleasman, MD‖

ABSTRACT. *Objective.* **To evaluate 96-week clinical and immune outcomes to protease inhibitor–containing antiretroviral therapy.**

Methods. **A prospective study was conducted of 40 human immunodeficiency virus (HIV)-infected children who displayed viral suppression (VS) with successful immune reconstitution (IS), failure to suppress virus (VF) or develop immune reconstitution (IF), or discordant immune and viral responses (VF/IS) at 24 weeks posttherapy. All children enrolled had viral RNA >4.0 $\log_{10}$ copies per mL and were Centers for Disease Control ad Prevention immune stage 2 or 3. Clinical, viral, and immune outcomes were assessed during the subsequent 72 weeks.**

Results. **VS/IS and VF/IS groups displayed similar sustained increases in CD4 T cells, although viral levels rebounded by 48 and 96 weeks posttherapy to pretherapy levels in the discordant group. The VF/IS outcome group had significant increases in height and weight *z* scores compared with entry and were similar to the VS/IS group. After treatment, antigen-specific responses after tetanus immunization were similar in the VF/IS and VS/IS groups. Prevalence of HIV-associated illnesses decreased in both VS/IS and VF/IS but not in VF/IF response groups.**

Conclusions. **The findings indicate that viral replication under the selective pressure of protease inhibitors fails to exhibit the same deleterious impact on T-cell immunity as pretherapy viruses. CD4 T-cell counts may be a better predictor of disease progression and improvement in growth than viral burden in HIV-infected children who receive a protease inhibitor as part of a highly active antiretroviral therapy regimen. *Pediatrics* 2004; 114:e604–e611. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2004-0274; *children, HIV, immune reconstitution, protease inhibitors, immunization, growth.***

From the *Department of Pediatrics, Division of Immunology and Infectious Diseases, and ‡Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, Florida; §University of Florida Shands Cancer Center, Gainesville, Florida; and ‖Department of Pediatrics, Division of Allergy, Immunology, and Rheumatology, College of Medicine, University of South Florida, and All Children's Hospital, St Petersburg, Florida.

Accepted for publication Jun 9, 2004.
doi:10.1542/peds.2004-0274

Reprint requests to (J.W.S.) Department of Pediatrics, Division of Allergy, Immunology, and Rheumatology, College of Medicine, University of South Florida, Box 9350, 801 6th St South, St Petersburg, FL 33701-4899. E-mail: jsleasma@hsc.usf.edu

ABBREVIATIONS. AIDS, acquired immune deficiency syndrome; HIV-1, human immune deficiency virus type 1; VF/IS, viral failure/immune success; VS/IS, viral success/immune success; VF/IF, viral failure/immune failure; CDC, Centers for Disease Control and Prevention; NRTI, nucleoside reverse transcriptase inhibitor; RTV, ritonavir; NFV, nelfinavir; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; γ-IFN, γ-interferon; ANOVA, analysis of variance.

The goal of combination antiretroviral therapy is to delay progression to acquired immune deficiency syndrome (AIDS) by controlling human immune deficiency virus type 1 (HIV-1) replication and preventing or reversing immunodeficiency.[1–3] A major therapeutic challenge for the management of HIV-infected children and adults is to optimize complex and toxic drug combinations to control viral replication and at the same time improve CD4 T-cell counts. The parameters of viral load and CD4 T-cell counts are the most universally used intermediate prognostic measures of long-term therapy outcome.[4,5] On the basis of these parameters, antiretroviral therapy that suppresses viral replication to undetectable levels provides the best opportunity to delay progression to AIDS and to prolong life. As a result, measurements of plasma viral load and CD4 T-cell counts form the basis of the current principles and guidelines for the use of antiretroviral agents in HIV-infected adults and children.[1,6]

Unfortunately, a high proportion of HIV-infected children and adults who receive combination therapy develop viral breakthrough.[7] Paradoxically, a significant proportion of treated individuals who fail to suppress virus experience CD4 T-cell reconstitution, although viral burdens would predict disease progression.[7–11] Discordant viral and immune responses seem to occur more frequently in children than adults. Factors that contribute to discordant viral and immune responses to therapy are unknown but might include host and viral factors.[7,9,10,12] Children who develop immune reconstitution despite viral rebound have clinical disease progression rates that are lower than those of historical control groups with similar viral levels.[9,10,12,13] We studied the long-term outcomes of growth, prevalence of HIV-associated illnesses, and immune function among children who display discordant immune and viral responses to combination antiretroviral therapy (viral failure/immune success [VF/IS]) compared with children

who have similar pretherapy viral burden and CD4 T-cell counts and have optimal viral and immune response to combination antiretroviral therapy (viral success/immune success [VS/IS]) as well as with those who failed therapy (viral failure/immune failure [VF/IF]).

METHODS

Study Design

This was a prospectively accrued cohort study of HIV-infected children and adolescents between the ages of 2 and 18 years. Patients were recruited according to a protocol approved by the Institutional Review Boards of the University of Florida and the University of South Florida. Informed consent was obtained from the legal guardians for all children, and assent was obtained from children who were older than 7 years. The study was limited to HIV-infected children who had plasma viral loads >4.0 $\log_{10}$ copies per mL (Amplicor 1.0 assay with a lower detection limit of <400 copies per mL; Roche Molecular Systems, Pleasanton, CA), were Centers for Disease Control and Prevention (CDC) immune stage 2 or 3,[14] and were receiving a protease inhibitor for the first time. Previous therapy with nucleoside reverse transcriptase inhibitors (NRTI) was permitted, but those with previous therapy with a nonnucleoside reverse transcriptase inhibitor were excluded. Therapy consisted of 2 NRTIs (at least 1 of which was a new NRTI) plus a protease inhibitor. Protease inhibitors were limited to indinavir, ritonavir (RTV), or nelfinavir (NFV). The protease inhibitors were selected by the patient's primary physician on the basis of the child's ability to tolerate a particular protease inhibitor or to swallow capsules. Optimal drug dosing was based on pharmacokinetic studies of protease inhibitors for children.[15–18] Adherence was monitored at each study visit by measurement of returned medications and interviews with the patient and/or the family. Adherence was defined as evidence that the patient received >90% of the prescribed medication doses.[19] Growth parameters, assessment of adherence, prevalence of HIV-associated illnesses, and measurements of viral load and T-cell subset analysis were assessed for all response groups at screening; entry and 4, 8, 12, and 16 weeks after the initiation of treatment; and 8-week intervals between 16 and 96 weeks. Growth, HIV-associated illnesses, T-cell subsets, and viral load were assessed retrospectively for the 48-week period before the initiation of therapy.

After 24 weeks of treatment, patients were classified on the basis of viral and immune outcomes. VS was defined as posttherapy plasma viral load <400 copies per mL, whereas detectable plasma virus levels were classified as VF. IS was defined as an increase in CD4 T-cell counts (absolute number or percentage) by at least 1 CDC immune stage,[14] whereas failure to achieve success was IF. Patients who were classified as VS/IS or VF/IS continued on their initial antiretroviral treatment for an additional 72 weeks, unless CD4 T-cell counts declined >50% from peak values or a new AIDS-defining illness developed, which were study endpoints. Patients who were classified as VF/IF were changed to a new treatment regimen at 24 weeks but remained on study for ongoing monitoring.

Clinical and Laboratory Outcomes

T-cell subset analysis was performed using flow cytometry assessment as previously described.[11] HIV-associated illnesses were defined using the 1994 CDC Category A, B, and C conditions for pediatric HIV-1 infection.[14] The number of A, B, and C conditions for each patient for the 48 weeks before and 96 weeks after start of combination therapy was totaled and calculated as a prevalence of HIV-associated illnesses per patient before and after therapy.

Height and weight measurements were converted to age- and gender-adjusted z scores, using the SAS software program (www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm) that includes height, weight, gender, and age for children from the 2000 CDC growth chart data (www.cdc.gov/growthcharts). A z score of 0 corresponds to the 50th percentile, which is exactly the age-/gender-appropriate median, whereas a z score of ± 1 indicates that height or weight is 1 standard deviation above or below the gender-specific median height or weight for the normal population.[20–22]

Assessment of Functional Immunity to Recall Antigen

All HIV-infected patients who had not received a tetanus immunization during the 2-year period before enrollment were enrolled in a companion study entitled The Impact of Age on Immune Reconstitution Following HAART. After providing informed consent, enrollees were given an intramuscular booster tetanus immunization (Imovax IM; Connaught Laboratories, Inc, Swiftwater, PA) at 48 weeks after initiation of treatment. Age-matched healthy children were recruited from the pediatric clinics of the University of Florida under the same protocol. After obtaining consent from the patient's guardian, these children were immunized as control subjects.

Blood samples were obtained immediately before and 30 days after immunization. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density centrifugation and cryopreserved in liquid nitrogen as described.[23,24] Lymphocyte proliferation assays were performed in triplicate using 10^5 cells per well in 96-well round-bottom microtiter plates (Costar; Corning Inc, Corning, NY). Cells were stimulated with either phytohemagglutinin (PHA; 10 μg/mL; Sigma Chemical Company; St Louis, MO) for 3 days or tetanus toxoid (8 μg/mL; Wyeth Ayerst Pharmaceuticals; Marietta, PA) for 6 days in a humidified 37°C incubator with 5% CO_2. Cells that were incubated in media alone served as unstimulated controls to determine background cellular proliferation for both PHA and tetanus stimulation. After incubation, cells were pulsed with 1 μCi/mL ^{3}[H]thymidine (Amersham Pharmacia Biotech; Buckinghamshire, England), cultured for an additional 18 hours, and harvested using a PHD cell harvester (Cambridge Technology Inc, Cambridge, MA). Incorporation of ^{3}[H]thymidine was determined using an LS-250 scintillation counter (Beckman Instruments, Inc, Van Nuys, CA). The stimulation index for each time point was calculated as average counts per minute for each set of triplicate wells divided by counts per minute from unstimulated PBMCs.

Tetanus-induced γ-interferon (γ-IFN) production was measured using 3×10^6 cells per mL cultured in 24-well plates with 8 μg/mL tetanus toxoid, 10 μg/mL PHA, or media alone for 1, 2, 3, or 5 days. Supernatant γ-IFN was measured using ELISA (R&D Systems, Minneapolis, MN) with a lower limit of detection of 15.6 pg/mL. γ-IFN levels <15.6 pg/mL indicated no response and were recorded as 14 pg/mL.

Statistical Analysis

Analysis was performed using SigmaStat 3.0 Software (Jandel Scientific Corp, San Rafael, CA). Analysis of pre- and posttherapy height and weight z scores or changes in lymphocyte proliferation or γ-IFN production before and after immunization within response groups were evaluated by paired t test. Proliferation and antigen-specific γ-IFN responses to mitogen and recall antigen among outcome groups were compared using both Kruskal-Wallis 1-way analysis of variance (ANOVA) and a 1-way ANOVA. The Bonferroni method was applied to control for type I errors. Comparisons of pretherapy characteristics of the study population and prevalence of HIV-1–associated illnesses pre- and posttreatment among the 3 response groups were evaluated using χ^2 analysis or the Kruskal-Wallis 1-way ANOVA on ranks with pairwise multiple comparisons (Dunn's method). An effect by treatment on prevalence of HIV-1–associated illnesses was evaluated among different response groups pre- and posttherapy using Wilcoxon signed rank test. Values are expressed as mean ± SEM or as medians with interquartile ranges. $P < .05$ was considered significant.

RESULTS

Baseline Characteristics of the Outcome Groups

Forty patients were recruited from 1999 to 2001 and successfully completed the study. Baseline characteristics of the study cohort are shown in Table 1. Among the cohort, 3 of 40 were previously treated with NRTI. The median age was 7.1 years with no significant differences among the cohort with respect

TABLE 1. Baseline Clinical, Immune, and Viral Characteristics of the Study Group

Variable	Patients			
	All	VS/IS	VF/IS	VF/IF
n	40	18	17	5
Age, y*†	7.1	6.9	5.7	9.5
Gender†				
Male	24	10	13	1
Female	16	8	4	4
Race†				
Black	27	13	11	3
White	10	3	5	2
Hispanic	2	2	0	0
Asian	1	0	1	0
Mode of infection†				
Sexual	1	0	0	1
Vertical	34	16	14	4
Blood products	5	2	3	0
Treatment‡				
Indinavir	9	3	4	2
NFV	13	10	3	0
RTV	18	5	10	3
Immune stage				
2	11	9	2	0
3	29	9	15	5
CD4%* (25th–75th quartile)	13 (3–28)	24 (11–32)	8 (3–18)	1 (1–7)
CD4 T cells per μL* (25th–75th quartile)	256 (45–733)	672 (265–903)	205 (33–407)	25 (5.7–57)
Log_{10} viral RNA copies per mL* (25th–75th quartile)	5.1 (4.2–5.5)	4.3 (4.0–5.4)	5.1 (4.4–5.6)	5.4 (5.3–5.5)
Disease stage				
A	10	6	4	0
B	14	8	6	0
C	16	4	7	5

* Median.
† *P* values: .12 for age, .07 for gender, .54 for ethnicity, and .09 for mode of infection (χ^2).
‡ *P* value: .014 between NFV and non-NFV (χ^2).

to gender, clinical disease stage, or the type of protease inhibitor administered. The majority (85%) of the patients were infected through maternal transmission, and 68% were black. By design, the cohort had advanced disease and high viral loads. The median CD4 T-cell counts were 13% (absolute 256 cells per μL), median viral load was 5.1 log_{10} copies per mL, and 73% were CDC immune stage 3.

A majority (57.5%) of the 40 patients experienced viral rebound and were classified as VF by 24 weeks. On the basis of classification by both viral and immune outcomes, 18 (45%), 17 (42.5%), and 5 (12.5%) were VS/IS, VF/IS, and VF/IF, respectively (Table 1). None of the children fulfilled the criteria for VS/IF. Outcome groups were similar with respect to age, gender, ethnicity, and mode of infection. VS/IS patients more frequently received NFV as their protease inhibitor, whereas VF/IS patients more commonly received RTV. Overall, viral outcome among children who received NFV was better among children who received another protease inhibitor (P = .014, χ^2 analysis). Viral outcome among children who received NFV was better than among children who received RTV (P = .01, Fisher exact test) but similar to children who received indinavir (P = .08, Fisher exact test). Among 7 treatment-naïve patients 3, 3, and 1 were VS/IS, VF/IS, and VF/IF, respectively, indicating no significant relationship between treatment-naïve and pretreated patients (Fisher exact test).

Although entry criteria selected for patients with elevated viral loads and suppressed CD4 T-cell counts, baseline CD4 T-cell counts were lower and viral levels were higher among children who had VF (VF/IS and VF/IF) compared with children who maintained VS (VS/IS; P = .03 and .02, respectively; Table 1). At entry, 17 of 22 VF children but only 8 of 18 VS children had CD4 T-cell counts that were <15%. VS/IS responders had higher percentages and absolute CD4 T-cell counts compared with VF/IS responders at entry (P = .03 and .04, respectively; Table 1). Twelve (55%) of 22 children who experienced VF were CDC clinical stage C compared with 4 (22%) of 18 of the VS group (P = .05). All VF/IF patients were CDC disease stage C3, whereas VS/IS and VF/IS groups were similar with respect to entry clinical disease stage (P = .4).

Changes in Viral Load and CD4 T-Cell Counts After Treatment

Viral load in the VS/IS group declined from pretherapy levels of 4.6 (±0.2 SEM) to 2.7 (±0.16 SEM) log_{10} copies per mL by 4 weeks (P = .001) and 2.3 (±0.1 SEM) log_{10} copies/mL by 24 weeks (P = .001; Fig 1A). Average viral levels in the VS/IS group remained suppressed for the duration of the 96-week study. Both VF groups demonstrated significant declines in viral burden between entry and 4 weeks on study (Fig 1 B and C). VF/IF patients rebounded to pretherapy levels by 12 weeks of therapy and changed to new antiretroviral regimens at 24 weeks. Average viral burden in the VF/IS group declined

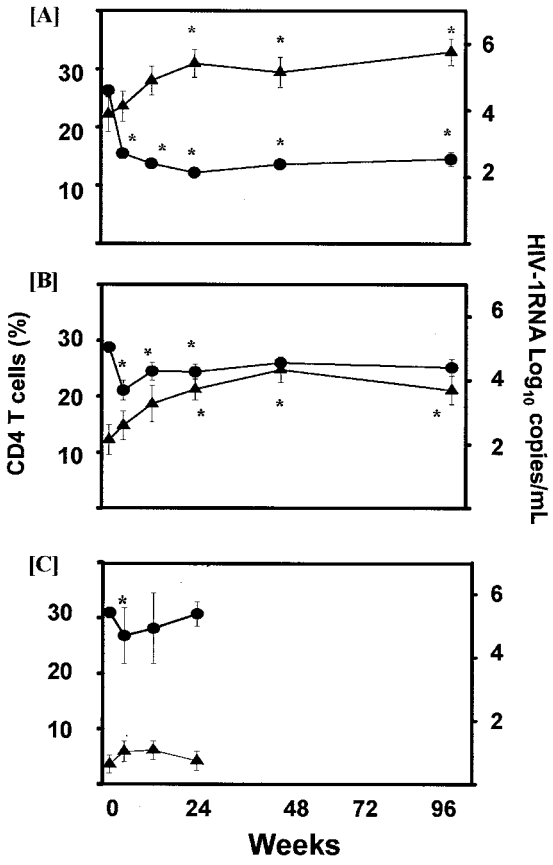

Fig 1. Therapy-induced changes in viral loads and CD4 T-cell percentages among therapy response groups. A, VS/IS outcome group. B, VF/IS outcome group. C, VF/IF outcome group. Mean Log_{10} HIV RNA copies per mL (●) is indicated on the right axis. Average CD4 T cell% (▲) are indicated on the left axis. Vertical bars show ±SEM. Weeks on therapy are shown on the x-axis. Significant differences between the mean baseline and post-therapy time point are indicated by * ($P < .05$, paired t test). The VF/IF group changed their regimens at 24 weeks of therapy; therefore, viral load and CD4 data are not shown after 24 weeks in this outcome group.

from 5.0 (±0.2 SEM) at entry to 4.2 (±0.3 SEM) log_{10} HIV RNA copies per mL by 24 weeks of treatment ($P = .001$) and then increased to 4.6 (±0.2 SEM) by 48 weeks and 4.4 (±0.2 SEM) log_{10} by 96 weeks ($P = .06$ and .05 relative to baseline, respectively).

By study design, CD4 T-cell percentage or absolute number in the VF/IF group failed to increase during the initial 24 weeks of treatment (Fig 1C). In contrast, mean CD4 T-cell percentages in the VS/IS group increased from 22% (±3.0% SEM) at entry to 32% (±2.4% SEM) at 24 weeks ($P = .001$), which represented an absolute increase from 580 to 829 CD4 T cells per μL ($P = .001$; Fig 1A). After 96 weeks of study, CD4 T-cell percentages were 33% (±2.3% SEM) and absolute counts were 801 cells per μL ($P = .23$ and $P = .5$, respectively, when comparing 24 and 96 weeks).

The VF/IS group showed a mean increase in CD4% from 13% (±2.8% SEM) to 21% (±2.0% SEM) and in absolute CD4 T-cell number from 378 to 766 cells per μL during the first 24 weeks of treatment ($P = .001$; Fig 1B). CD4 increases were sustained over 96 weeks with no significant declines in either percentages or absolute numbers between 24 and 96 weeks ($P = .9$ and $P = .6$, respectively). Although the VF/IS group had lower CD4 T-cell counts than the VS/IS group at entry, the VF/IS group experienced significant increases in CD4 T-cell percentages and absolute numbers during 24 weeks of treatment, which were sustained over 96 weeks, similar to the VS/IS group (Fig 1A and B). The VS/IS group had higher percentages of CD4 T cells than the VF/IS group at 96 weeks ($P = .001$), although absolute numbers of CD4 T cells were not significantly different between VS/IS and VF/IS response groups ($P = .9$).

Changes in Growth Parameters Among Outcome Groups

For all patients, pretherapy height z scores (mean: −0.87 ± 0.24 SEM) were more suppressed than weight z scores (mean: −0.31 ± 0.28 SEM), although the difference failed to achieve statistical significance ($P = .08$). Entry growth parameters among the 3 response groups were similar ($P = .54$ for height and $P = .66$ for weight).

Although VF/IF patients underwent therapy changes at 24 weeks, height and weight parameters continued to be monitored. Mean pretreatment height z score of −0.9 decreased to −1.6 by 96 weeks ($P = .2$), whereas average weight z score changed from −0.06 to −0.9 ($P = .8$; Table 2). The VS/IS group displayed relatively normal pretreatment height and weight z scores that improved minimally during treatment for 96 weeks (Table 2). In contrast to VF/IF or VS/IS response groups, the discordant VF/IS group improved in both weight and height over the course of therapy for 96 weeks. Weight z score increased from −0.5 to −0.1 ($P = .07$), and height z score increased significantly from a mean of −1.2 to −0.5 by 96 weeks of treatment ($P = .003$). Improvement in height-for-age z score in the VF/IS response group was achieved as early as 48 weeks of therapy (data not shown) and was stable between 48 and 96 weeks of study ($P = .5$). Overall height and weight z scores at 48 and 96 weeks posttherapy were similar between the VF/IS and VS/IS groups ($P = .6$ and .5 for height, $P = .7$ and .6 for weight).

TABLE 2. Comparison Among Response Groups of Height and Weight z Scores Before and After 96 Weeks of Therapy

Outcome Group	Height z Score			Weight z Score		
	Before, Mean (±SEM)	After, Mean (±SEM)	P Value	Before, Mean (±SEM)	After, Mean (±SEM)	P Value
VS/IS	−0.2 (0.4)	0.1 (0.3)	0.3	0.3 (0.3)	0.3 (0.3)	0.9
VF/IS	−1.2 (0.3)	−0.5 (0.3)	0.003	−0.5 (0.4)	−0.1 (0.5)	0.07
VF/IF	−0.9 (0.2)	−1.6 (0.2)	0.2	−0.06 (0.9)	−0.9 (0.1)	0.8

In Vitro Response to Recall Antigen Among Outcome Groups

Twenty (9 VS/IS, 8 VF/IS, and 3 VF/IF) of the 40 patients enrolled had not received recent tetanus immunizations and qualified for evaluation of responses to recall antigen. These patients received tetanus immunization 48 weeks after study entry. Response to immunization was measured by in vitro lymphocyte proliferation and γ-IFN production to tetanus toxoid. Before immunization, all patients exhibited lymphocyte proliferation responses to PHA. VS/IS, VF/IS, and healthy children demonstrated similar response to PHA stimulation (SI >20; $P = .6$, 1-way ANOVA), whereas the response to PHA stimulation by VF/IF children (SI of 7) was significantly less ($P < .05$, 1-way ANOVA; data not shown). Postimmunization tetanus-specific proliferation responses were significantly increased compared with preimmunization levels in VS/IS, VF/IS, and healthy control patients ($P = .03$, .002, and .001, paired t test; Fig 2A). In contrast, VF/IF patients failed to demonstrate lymphocyte proliferation to tetanus before or after immunization. The postimmunization responses were highest in the healthy children at 6.8 (±1.1 SEM) compared with 4.7 (±1.2 SEM) in VS/IS and 5.3 (±1.3 SEM) in VF/IS patients. However, the differences were not significant ($P = .4$, 1-way ANOVA).

Although a capacity to produce γ-IFN after PHA stimulation was detected in the VF/IF group (376 ± 142 pg/mL SEM; data not shown), tetanus-specific γ-IFN production was undetectable before or after immunization in the VF/IF group (Fig 2B). In contrast, VS/IS, VF/IS, and healthy control subjects showed postimmunization significant tetanus-specific increases in γ-IFN production ($P = .004$, .05, and .001, respectively, paired t test; Fig 2B). Before immunization, production of γ-IFN was similar among VF/IS, VS/IS, and healthy children ($P = .5$, 1-way ANOVA). Postimmunization, mean γ-IFN production was highest in the healthy children (356 ± 56 pg/mL SEM) compared with 218 (±98 SEM) pg/mL in VS/IS or 179 (±86 SEM) pg/mL in VF/IS. Multiple comparisons for postresponse were performed using the Bonferroni method to control for type I errors. The pairwise comparisons between healthy patients and VS/IS, healthy patients and VF/IS, and VS/IS and VF/IS indicated that there were no significant differences in postimmunization γ-IFN production between healthy control subjects and VS/IS or VS/IS to VF/IS ($P = .02$ and .85, respectively, Kruskal-Wallis 1-way ANOVA by ranks). However, γ-IFN production was significantly less in the VF/IS group compared with healthy children ($P = .009$, Kruskal-Wallis 1-way ANOVA by ranks).

Changes in HIV-1–Associated Illnesses Among Therapy Outcome Groups

The prevalence of CDC A, B, or C category HIV-1–associated illnesses during the 48 weeks before initiation of treatment and during the subsequent 96 weeks after therapy was calculated for each patient. When comparing the 3 response groups before therapy, prevalence of category A and B illnesses was similar ($P = .11$ and $P = .28$, respectively). In contrast, the VS/IS group had significantly fewer CDC category C-defining conditions than the 2 VF groups ($P = .002$). However, after treatment, VS/IS and VF/IS groups displayed similar prevalence of CDC category C-defining illnesses ($P = .5$, Wilcoxon signed rank test), although CDC category B-defining conditions remained significantly higher in the VF/IS group ($P = .03$, Wilcoxon signed rank test).

Change in the prevalence of HIV-associated illnesses was compared within each outcome group before and after therapy (Fig 3). In the VF/IF group, the prevalence of category A, B, and C illnesses failed to decline with therapy, and 1 AIDS-related death occurred. In contrast, the VS/IS outcome groups had significant decreases in category A- and B-defining

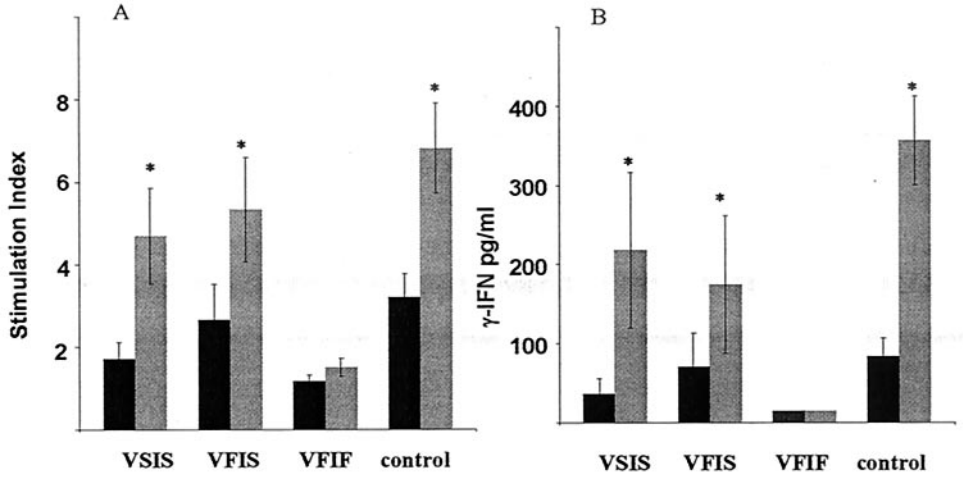

Fig 2. Posttherapy responses to recall antigen. PBMC was obtained before (■) and 4 weeks after (▨) immunization with tetanus toxoid from HIV-infected VS/IS, VF/IS, and VF/IF children who had been on antiretroviral therapy for 48 weeks and from 12 age-matched healthy, uninfected children. A, PBMC proliferation responses, as measured by uptake of tritiated thymidine and reported as the average stimulation index (SI; y-axis) for each group. B, Mean γ-IFN production (pg/mL; y-axis) from PBMC supernatant fluid after in vitro culture of PBMCs with tetanus toxoid. Significant difference between the pre- and postimmunization levels are shown by * ($P < .05$, paired t test).

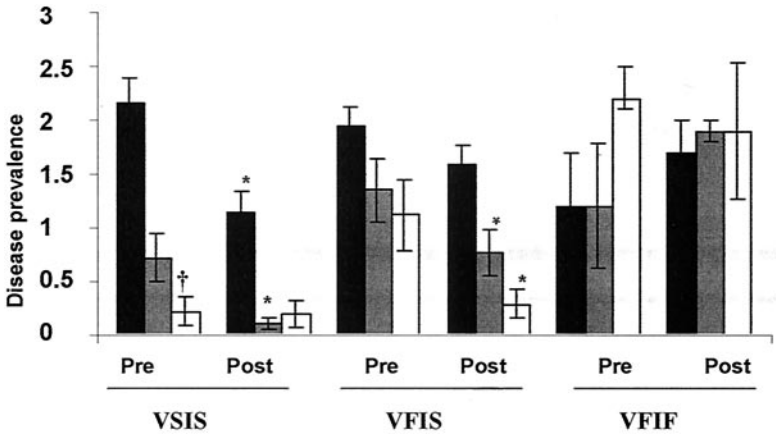

Fig 3. Comparison of the prevalence of HIV-associated illnesses among response groups before and after therapy. The average prevalence per year of CDC category A, B, and C illnesses for each patient (shown on the y-axis) during the year before treatment (pre) and the 2 years after treatment (post). Categories A (■) B (▨), and C (□) are represented for each outcome group, VS/IS, VF/IS, and VF/IF. Significant difference between the pre- and post-CDC category A, B, and C illnesses are shown by * ($P < .05$, Wilcoxon signed rank test). Significant difference in the pretherapy C-defining illnesses among response groups are shown by † ($P = .002$, Kruskal-Wallis 1-way ANOVA on ranks).

illnesses after treatment ($P = .003$ and $P = .008$, respectively), whereas the prevalence of category C illnesses was similar. In the VF/IS group, the prevalence of category A illnesses was unchanged ($P = .1$), whereas category B conditions declined ($P = .02$). In contrast to the VS/IS and VF/IF groups, the prevalence of CDC stage C conditions in the VF/IS group declined by >4-fold after therapy ($P = .02$).

DISCUSSION

By several parameters known to be associated with HIV disease progression, children who are treated with protease inhibitor–containing antiretroviral therapy and reconstitute their CD4 T cells despite viral rebound have similar outcomes to those who optimally suppress viral replication. In our study, discordant viral and immune response outcome groups showed sustained increases in CD4 T-cell counts and displayed growth parameters, prevalence of HIV-associated illnesses, and levels of functional immunity that were equivalent to VS/IS children. The substantial restoration of CD4 T-cell counts in the VS/IS and VF/IS response groups during the initial 24 weeks of treatment was sustained over the subsequent 72 weeks in both outcome groups. The durability of CD4 reconstitution in VF/IS children who were enrolled in our study is in contrast to previous examinations of CD4 T-cell reconstitution in HIV-infected adults in which 30% to 40% of patients who displayed discordant viral and immune responses while receiving a protease inhibitor–containing regimen developed IF within 2 years of viral rebound.[7,25] This indicates that HIV-infected children, when compared with HIV-infected adults, have a greater capacity to sustain immune reconstitution even when viral load is high.

Growth failure in HIV infection in children is well recognized as an indicator of disease progression that often precedes declines in CD4 T-cell counts.[22] Although protease inhibitors can suppress growth in HIV-infected children with intact immunity, change in growth parameters can be applied as a measure of the effectiveness of combination antiretroviral therapy because control of viral replication has a positive effect on height and weight.[22,26] Lack of a significant increase of height and weight parameters in the VS/IS outcome group might reflect a deleterious impact by protease inhibitors or, more likely, the relatively normal pretherapy growth parameters. Our study demonstrates that reconstitution of CD4 T-cell counts is a better correlate of growth than suppression of viral load because growth within the VF/IS outcome group was similar to HIV-infected children who fully suppress viral replication. It is clear that continued replication by posttherapy virus does not have the same deleterious impact on growth as pretherapy viruses.

In addition to a positive effect on growth, combination antiretroviral therapy results in a significant decrease in the prevalence of HIV-associated illnesses among VF/IS and VS/IS response groups. Although a low pretherapy prevalence of CDC category C illnesses in the VS/IS group failed to change significantly with therapy, significant declines in category A and B conditions occurred. Advanced pretherapy disease in the VF/IS group enabled us to observe a significant decline with therapy, not only in the prevalence of category B illnesses but also in C-defining conditions to a level that was similar to VS/IS children. Children with discordant viral and immune outcomes can reconstitute functional immunity to a level that leads to significant clinical improvement.

Our study extends previous studies in adults and children with discordant viral and immune responses to therapy by providing an in vitro assessment of immune function.[27] Although the number of children whose antigen-specific functional immunity was examined was small, overall VS/IS and VF/IS outcome groups exhibited significant improvements in antigen-specific postimmunization immune responses, signifying a limited effect by posttherapy virus replication on functional immunity. All children who restored CD4 T-cell numbers, including children who failed to control viral replication, displayed tetanus proliferation responses that were similar to immunization responses by healthy children. VF/IS patients displayed reduced postimmunization levels of tetanus-specific γ-IFN production compared with healthy age-matched children, suggesting only partial reconstitution of functional immunity. In contrast, in the VS/IS outcome group, postimmunization levels of tetanus-specific γ-IFN production was similar to healthy children. Additional studies are

needed to explore the impact of viral replication on immune function and to develop improved strategies to enhance antigen-specific immunity in HIV-infected children who receive antiretroviral therapy.

In contrast to the children in our study, evaluations of immune reconstitution HIV-infected adults indicate that a small percentage of patients demonstrated restoration of lymphocyte proliferation responses to tetanus toxoid during the first year after treatment. Restoration of functional immunity correlated with increases in the number of naïve T cells, reflecting a critical role for the thymus.[28,29] Other studies in HIV-infected adults, by comparison with children, showed considerably slower reconstitution of the T-cell receptor Vβ repertoire and naïve T cells after the initiation of combination antiretroviral therapy.[11,28–32] In children, kinetics of CD4 T-cell reconstitution is more rapid, and the proportion of VS/IS and VF/IS children who restore functional immunity by 48 weeks on therapy is greater than among HIV-infected adults who had similar pretherapy CD4 T-cell counts.[33,34] Together, these results underscore that by virtue of their intact thymus, children have a greater capacity to restore immunity than adults.

There seem to be several pretherapy clinical and laboratory parameters that characterize the various outcome groups. Before initiation of antiretroviral therapy, advanced clinical disease stage, high viral loads, and severe immune suppression but not age, gender, or ethnicity characterize children who fail to maintain durable viral suppression in response to antiretroviral therapy. Low pretherapy CD4 T-cell counts are predictive of VF and can discriminate between VS/IS and VF/IS outcome groups.[7] In the present study, IS therapy outcome groups (VS/IS and VF/IS) were similar with respect to pretreatment disease stage, viral loads, and weight. In contrast, VF outcome groups (VF/IS and VF/IF) displayed delayed linear growth and lower CD4 T-cell counts when compared with children with VS/IS therapy outcomes. The current Public Health Service guidelines for initiating antiretroviral treatment for HIV-infected adults recommends delaying antiretroviral therapy until CD4 T-cell counts decline or viral burden increases .[1] Similar criteria are proposed for the treatment of asymptomatic HIV-infected children.[6] However, in agreement with others, our study indicates that delaying therapy until advanced CD4 T-cell attrition and clinical disease increases the likelihood of treatment failure for HIV-infected children.[7,9,12]

Our protocol was not designed to evaluate the differences among different protease inhibitors to control viral replication or reconstitute immunity. The particular protease inhibitor administered was selected by the patient's primary physician on the basis of the patient's ability to tolerate the medication or swallow capsules. It was surprising to find that VF/IS children more frequently received RTV, whereas VS/IS patients more commonly received NFV, as their protease inhibitor. To date, no large, randomized, clinical trials have compared directly the efficacy of these protease inhibitors in children; therefore, the reasons for the improved viral outcome in the children who receive NFV is unclear. One possibility for the difference is improved compliance and better gastrointestinal tolerability associated with NFV compared with RTV. However, previous studies show better compliance in children receiving RTV compared with NFV containing treatment regimens.[35] We have previously demonstrated that pretherapy viral protease genotype has limited prognostic value in predicting viral and immune outcomes.[7] Clearly, larger studies are needed to determine the differences among protease inhibitors to control viral replication and provide durable immune reconstitution. Although establishing the rationale for future studies, any conclusions with respect to drug efficacy based on the findings of our study should be made cautiously.

Several studies of adults who fail antiretroviral therapy show that virus quasispecies revert rapidly to wild-type when treatment is discontinued.[36–38] Reversion to pretherapy genotypes results in declines in CD4 T cell count and disease progression. In contrast, viruses replicating under the selective pressure of protease inhibitors have a lower replication potential within the thymus compared with wild type viruses.[38] The critical role of the thymus in CD4 T cell reconstitution and the greater thymic capacity in children provides an explanation as to why VF/IS children have better long-term immune and clinical outcomes compared with HIV-infected adults. The principal disadvantage of continuing antiretroviral therapy in the presence of ongoing viral replication is accumulation of amino acid substitutions that confer cross-resistance to other protease inhibitors.[39,40] However, viruses resistant to protease inhibitors appear to be less virulent, based on studies of replacement rates of CD4 T cell subsets, than wild type viruses.[7,41] Continuing a therapy that fails to suppress viral replication but provides reconstitution of CD4 T cell number and improvement of immune function may be reasonable for HIV-infected children who have limited antiretroviral treatment options. Based on the results of our study, a reasonable next step in the management of HIV-infected children would be the initiation of clinical trials in which changes in antiretroviral medications were based on CD4 T cell decline rather than rebound in viral replication.

ACKNOWLEDGMENTS

This study was supported by Public Health Service Awards RO1 HD32259 and RO1 AI 47723, the National Institutes of Health–sponsored General Clinical Research Center PR0082, an award PG-50956 from the Elizabeth Glaser Pediatric AIDS Foundation, McClamma HIV Foundation, and the University of Florida Interdisciplinary Center for Biological Research. It was also supported by the Pediatric Clinical Research Center of All Children's Hospital and the University of South Florida, and the Maternal and Child Health Bureau, CFDA 93.110 PCRC, United States Health Resources and Services Administration.

We are grateful to Dr Lili Tian, from the Biostatistical core of the University of Florida, Shands Cancer Center, for help with the statistical analysis.

REFERENCES

1. Centers for Disease Control and Prevention. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. Recom-

mendations of the Panel on Clinical Practices for Treatment of HIV. *MMWR Morb Mortal Wkly Rep.* 2002;51:1–55
2. Detels R, Munoz A, McFarlane G, et al. Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. *JAMA.* 1998;280:1497–1503
3. Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J Med.* 1998;338:853–860
4. Mellors JW, Munoz A, Giorgi J, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med.* 1997;126:946–954
5. Palumbo P, Raskino C, Fiscus S, et al. Predictive value of quantitative plasma RNA and CD4+ lymphocyte count in HIV-infected infants and children. *JAMA.* 1998;279:756–761
6. Centers for Disease Control and Prevention. Guidelines for the use of antiretroviral agents in pediatric HIV infection. *MMWR Recomm Rep.* 1998;47:1–44
7. Perez EE, Rose SL, Peyser B, et al. Human immunodeficiency virus type 1 protease genotype predicts immune and viral responses to combination therapy with protease inhibitors (PIs) in PI-naive patients. *J Infect Dis.* 2001;183:579–588
8. Deeks SG, Barbour JD, Martin JN, et al. Sustained CD4+ T cell response after virologic failure of protease inhibitor-based regimens in patients with human immunodeficiency virus infection. *J Infect Dis.* 2000;181:946–953
9. Essajee SM, Kim M, Gonzalez C, et al. Immunologic and virologic responses to HAART in severely immunocompromised HIV-1-infected children. *AIDS.* 1999;13:2523–2532
10. Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study. *Lancet.* 1999;353:863–868
11. Sleasman JW, Nelson RP, Goodenow MM, et al. Immunoreconstitution after ritonavir therapy in children with human immunodeficiency virus infection involves multiple lymphocyte lineages. *J Pediatr.* 1999;134:597–606
12. Jankelevich S, Mueller BU, Mackall CL, et al. Long-term virologic and immunologic responses in human immunodeficiency virus type 1-infected children treated with indinavir, zidovudine, and lamivudine. *J Infect Dis.* 2001;183:1116–1120
13. Peruzzi M, Azzari C, Galli L, et al. Highly active antiretroviral therapy restores in vitro mitogen and antigen-specific T-lymphocyte responses in HIV-1 perinatally infected children despite virological failure. *Clin Exp Immunol.* 2002;128:365–371
14. Centers for Disease Control and Prevention. Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep.* 1994;43:1–12
15. Krogstad P, Wiznia A, Luzuriaga K, et al. Treatment of human immunodeficiency virus 1-infected infants and children with the protease inhibitor nelfinavir mesylate. *Clin Infect Dis.* 1999;28:1109–1118
16. Krogstad P, Lee S, Johnson G, et al. Nucleoside-analogue reverse-transcriptase inhibitors plus nevirapine, nelfinavir, or ritonavir for pretreated children infected with human immunodeficiency virus type 1. *Clin Infect Dis.* 2002;34:991–1001
17. Mueller BU, Nelson RP, Sleasman J, et al. A phase I/II study of the protease inhibitor ritonavir in children with HIV infection. *Pediatrics.* 1998;101:335–343
18. Mueller BU, Sleasman J, Nelson RP, et al. A phase I/II study of the protease inhibitor indinavir in children with HIV infection. *Pediatrics.* 1998;102:101–109
19. Williams A, Friedland F. Adherence, compliance, and HAART. *AIDS Clin Care.* 1997;9:51–54
20. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. *N Engl J Med.* 2001;345:1522–1528
21. Miller TL, Mawn BE, Orav EJ, et al. The effect of protease inhibitor therapy on growth and body composition in human immunodeficiency virus type 1-infected children. *Pediatrics.* 2001;107(5). Available at: www.pediatrics.org/cgi/content/full/107/5/e77
22. Verweel G, van Rossum AM, Hartwig NG, et al. Treatment with highly active antiretroviral therapy in human immunodeficiency virus type 1-infected children is associated with a sustained effect on growth. *Pediatrics.* 2002;109(2). Available at: www.pediatrics.org/cgi/content/full/109/2/e25
23. Ghaffari G, Passalacqua DJ, Bender BS, et al. Human lymphocyte proliferation responses following primary immunization with rabies vaccine as neoantigen. *Clin Diagn Lab Immunol.* 2001;8:880–883
24. Sleasman JW, Leon BH, Aleixo LF, et al. Immunomagnetic selection of purified monocyte and lymphocyte populations from peripheral blood mononuclear cells following cryopreservation. *Clin Diagn Lab Immunol.* 1997;4:653–658
25. Buchacz K, Cervia JS, Lindsey JC, et al. Impact of protease inhibitor-containing combination antiretroviral therapies on height and weight growth in HIV-infected children. *Pediatrics.* 2001;108(4). Available at: www.pediatrics.org/cgi/content/full/108/4/e72
26. Nachman SA, Lindsey JC, Pelton S, et al. Growth in human immunodeficiency virus-infected children receiving ritonavir-containing antiretroviral therapy. *Arch Pediatr. Adolesc Med.* 2002;156:497–503
27. Deeks SG, Barbour JD, Grant RM, et al. Duration and predictors of CD4 T-cell gains in patients who continue combination therapy despite detectable plasma viremia. *AIDS.* 2002;16:201–207
28. Valdez H, Smith KY, Landay A, et al. Response to immunization with recall and neoantigens after prolonged administration of an HIV-1 protease inhibitor-containing regimen. ACTG 375 team. *AIDS Clinical Trials Group. AIDS.* 2000;14:11–21
29. Valdez H. Immune restoration after treatment of HIV-1 infection with highly active antiretroviral therapy (HAART). *AIDS Rev.* 2002;4:157–164
30. Connors M, Kovacs JA, Krevat S, et al. HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. *Nat Med.* 1997;3:533–540
31. Douek DC, Koup RA, McFarland RD. Effect of HIV on thymic function before and after antiretroviral therapy in children. *J Infect Dis.* 2000;181:1479–1482
32. Kou ZC, Puhr JS, Wu SS, et al. Combination antiretroviral therapy results in a rapid increase in t cell receptor variable region beta repertoire diversity within CD45RA CD8 T cells in human immunodeficiency virus-infected children. *J Infect Dis.* 2003;187:385–397
33. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science.* 1997;277:112–116
34. Connick E, Lederman MM, Kotzin BL, et al. Immune reconstitution in the first year of potent antiretroviral therapy and its relationship to virologic response. *J Infect Dis.* 2000;181:358–363
35. Van Dyke RB, Lee S, Johnson GM, et al. Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. *Pediatrics.* 2002;109(4). Available at: www.pediatrics.org/cgi/content/full/109/4/e61
36. Deeks SG, Grant RM, Wrin T, et al. Persistence of drug-resistant HIV-1 after a structured treatment interruption and its impact on treatment response. *AIDS.* 2003;17:361–370
37. Lecossier D, Bouchonnet F, Schneider P, et al. Discordant increases in CD4+ T cells in human immunodeficiency virus-infected patients experiencing virologic treatment failure: role of changes in thymic output and T cell death. *J Infect Dis.* 2001;183:1009–1016
38. Stoddart CA, Liegler TJ, Mammano F, et al. Impaired replication of protease inhibitor-resistant HIV-1 in human thymus. *Nat Med.* 2001;7:712–718
39. Condra JH, Schleif WA, Blahy OM, et al. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature.* 1995;374:569–571
40. Molla A, Korneyeva M, Gao Q, et al. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. *Nat Med.* 1996;2:760–766
41. Deeks SG, Hoh R, Grant RM, et al. CD4+ T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy. *J Infect Dis.* 2002;185:315–323

Two-Year Clinical and Immune Outcomes in Human Immunodeficiency Virus–Infected Children Who Reconstitute CD4 T Cells Without Control of Viral Replication After Combination Antiretroviral Therapy

Guity Ghaffari, Dominick J. Passalacqua, Jennifer L. Caicedo, Maureen M. Goodenow and John W. Sleasman

Pediatrics 2004;114;e604
DOI: 10.1542/peds.2004-0274 originally published online October 18, 2004;

Two-Year Clinical and Immune Outcomes in Human Immunodeficiency Virus–Infected Children Who Reconstitute CD4 T Cells Without Control of Viral Replication After Combination Antiretroviral Therapy

Guity Ghaffari, Dominick J. Passalacqua, Jennifer L. Caicedo, Maureen M. Goodenow and John W. Sleasman

Pediatrics 2004;114;e604
DOI: 10.1542/peds.2004-0274 originally published online October 18, 2004;