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Structured Treatment Interruptions (STI) in HIV-1 Infected Pediatric Populations Increases Interferon gamma Production and Reduces Viremia

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Abstract

Background—To test if intermittent treatment interruption in pediatric patients with HIV-1 infection, with progressively longer periods off drug, will induce immunity that controls viremia.

Methods—A prospective multicenter study of aviremic pediatric patients that underwent progressively longer antiretroviral structured treatment interruptions (STIs) starting with 3 days, increasing by 2 days in length each cycle. Antiretrovirals were resumed at the end of a cycle resulting in viremia to reinduce aviremia before starting the next cycle. HIV-specific responses were measured.

Results—Eight individuals became viremic and reached Cycle13 with an STI of 27 days. HIV-specific gamma-interferon production to inactivated HIV and vaccinia vectors expressing gag, env, nef, and pol increased (>10-fold) from baseline in 6 of 8 subjects. Median plasma RNA levels peaked @ Cycle7 and declined to levels <10⁴ cp/ml after Cycle10. In a subset of 5 who reached Cycle 17, HIV-specific IFN-gamma frequencies were 4–30-fold higher and median RNA levels were 0.32–2.10 (median 1.3) log lower than at comparable days off treatment at Cycle 8.

Conclusions—Increased HIV-specific immune responses and decreased HIV RNA were seen in those who have had >13 interruptions, with STI intervals that exceeded 27 days.

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We assessed the effect of progressively longer antiretroviral structured treatment interruptions (STIs) starting with 3 days, increasing by 2 days in length each cycle on HIV-specific immune responses. As well, we correlated these responses with control of HIV viremia. Eight individuals became viremic and reached Cycle13 with an STI of 27 days. HIV-specific gamma-interferon production to inactivated HIV and vaccinia vectors expressing gag, env, nef, and pol increased (>10-fold) in 6 of 8 subjects. Plasma RNA levels peaked @ Cycle7 and declined to levels <10⁴ cp/ml after Cycle10. In a subset of 5 who reached Cycle 17, HIV-specific IFN-gamma frequencies were 4–30-fold higher and median RNA levels were 0.32–2.10 (median 1.3) log lower than at comparable days off drugs.

Keywords

HIV; Pediatric; STI

INTRODUCTION

Immunologic control of HIV viremia is a desired outcome of chronic infection. We hypothesized that controlled limited exposure to autologous HIV would result in increased HIV-specific immune responses. We also hypothesized that such increases would result in improved virologic control. One approach to accomplish this is to allow incremental exposures to autologous virus that will act as a vaccination. However, exposure to high levels of virus may result in apoptosis of the immune cells. Therefore, we planned for a cautious approach, perhaps by starting with low level viremia and then allowing incremental increases in virus for relatively short periods of time..

Therapeutic HIV vaccination has succeeded in increasing HIV-specific responses to some extent but the effectiveness of any particular vaccine is likely to be hindered by marked heterogeneity of HIV strains between individuals(1–3). Andrieu achieved remarkable control of SIV infection by immunizing with inactivated whole virus identical to the infecting virus (4). This strategy would be impractical since each infected individual has a heterologous virus population with a background of multiple quasispecies. As an alternative approach to “immunize” with autologous viral populations, an infected individual could undergo a process of structured treatment interruption (STI) that allows for the replication of endogenous viral species acting as an immune stimulant after suppression by highly active antiretroviral drug therapy (HAART). Several investigators have studied this STI during chronic HIV infection using a variety of interruption schedules and a varied number of interruptions intended to stimulate HIV-specific immunity. STI effects were usually assessed by surrogate immunologic studies or by indefinitely suspending antiretrovirals and determining viral setpoint change. This has met with limited success, usually a delay in the rebound of virus after several interruptions (5–12). A study of STI performed in individuals treated with HAART soon after primary infection found better CD4 T cell-mediated anti-HIV activity and longevity and reduced viral set point (13) but also met with limited success over time (14).

We have attempted to use intermittent exposures of increasing duration to autologous virus as a vaccination to increase HIV-specific immune responses. We started with an antiretroviral interruption of 3 days at Cycle 1 with increases of 2 days at successive cycles. We postulated that exposure to high levels of virus may result in apoptosis of the immune cells but increases in HIV-specific immune responses, after multiple exposures to low levels of autologous virus, would result in more effective control of HIV replication when HAART was stopped. This cautious approach that would result in initial exposure to low level viremia with gradual increasing in the length of the STI's after enhanced HIV-specific

immune responses were induced. Our proof of concept study supports our hypothesis in a subset of the children and adolescents.

METHODOLOGY

STUDY DESIGN

Children and adolescents age between 4 and 21 years of age, receiving HAART (three drugs including a protease inhibitor and excluding non-nucleoside reverse transcriptase inhibitors) and with undetectable plasma virus (<400 copies (cp)/ml) for >12 months prior to entry were enrolled. The STI group participated in a treatment interruption protocol while a comparison group continued their HAART. The non-cycling group consisted of those subjects not willing to volunteer for STI. The STI group participated in cycles that alternate the time on antiviral medication with time off of the medication. Cycle 1 began with 18 days of highly active antiretrovirals (HAART) and a 3-day STI. Drug interruptions lasted for $2x + 1$ days, where x = cycle number. At the end of the STI, the subjects' viral load was tested. Subjects resumed their HAART therapy while waiting for the viral load results (cycle A). If the plasma HIV was below the level of detection (i.e. <50 cp/ml), they began the next cycle. With plasma virus ≤ 50 cp/ml at the end of the STI, HAART was continued and viral load determined after 28 days on treatment (cycles B, C, and D). Plasma virus <50cp/ml at that point qualified the subject to continue to the next cycle. Persistent detectable viremia required additional days of HAART with viral testing serially at 28 day intervals with a viral load <50cp/ml qualifying the subject to enter the next cycle. Failure to suppress virus to <50cp/ml by day 112 (cycle D) terminated the study for that individual. Beginning with Cycle 16 (i.e. a 33 day interruption), an additional visit to measure viremia occurred at approximately mid-point (day 17) during the drug interruption. HIV RNA values measured at the midpoint of later cycles with long interruptions were compared to values measured at the identical number of days off treatment during earlier cycles.

A variety of safety criteria were established for the cycling subjects. The selection of new primary resistance mutations (17) in plasma virus (determined each time virus was >500 cp/ml), defined by mutations associated with current HAART, and not detected in the PBMC from study entry, would cause subjects to discontinue STI. Also, subjects ceased STI when viral replication could not be suppressed by current HAART (i.e., < 50 cp/ml of plasma) within 112 days (four 28-day intervals) of resuming treatment.

IMMUNOLOGIC STUDIES

HIV-specific cell-mediated immune activity was measured in cycling subjects at entry, at the end of each 28 day treatment interval as well as at the end of every third STI: in Cycles 3, 6, 9 etc. It was measured at entry and every 12 weeks in the control subjects.

HIV Lymphoproliferative Assay

Peripheral blood mononuclear cells (PBMC) were cultured in 96 microwell plates for 6 days in the absence or presence of zinc-finger inactivated whole HIV (WHIV) (5mcg/ml). The cells were processed as described in a previous manuscript (18).

HIV gamma– Interferon ELISPOT Assay

Fresh PBMC were stimulated with WHIV (5mcg/ml) or recombinant HIV/vaccinia vectors (HIV/vv) and appropriate controls at 5 and 2×10^5 cells/well, respectively, in duplicate wells pre-coated with anti-IFN-gamma mAb by a method adapted from Larsson et al. (19). After 40 hours, spots were developed with AEC, counted and expressed as the number of HIV-specific spot-forming cells (SFC)/ 10^6 PBMC. SFC were measured separately for each

HIV/vv (Gag (vp1287), Env (vp1174), RT (vp1288), or Nef (vp1218)) and then summed (vv-sum). Responses to WHIV and HIV/vv are predominantly mediated by CD4+ and CD8+ T cells, respectively (Larsson et al. and unpublished data).

VIROLOGIC STUDIES

HIV-1 plasma RNA levels were quantified using the Amplicor HIV-1 Monitor™ Test version 1 and 1.5 (Roche Diagnostics, Basel, Switzerland). Drug-resistant genotypes (17) were evaluated by three methods: (1) end-point dilution PCR of PBMC from study entry; (2) consensus sequencing of rebounding plasma virus from the end of each STI using the ViroSeq Kit (Celera Diagnostics, Alameda, Calif.); and (3) estimates of relative size of mutant & wild-type viral populations over the course of multiple STI using an oligonucleotide ligation assay (19,20) applied to HIV-1 PBMC DNA collected at end of a STI which generated a plasma RNA greater than 500 cp/ml.

RESULTS

Cycling and continuous therapy cohorts enrolled 14 and 21 individuals, respectively. At baseline, the cycling and continuous therapy cohorts had median age of 7 and 10 years (range 4–19 and 4–18) and median CD4% 41 and 37, respectively. The median duration on study for all subjects was 1030 days (14 cycles) and 922 days for the continuous therapy group.

The analysis cohort for the cycling group was restricted to the eight subjects who completed 13 or more cycles. The analysis cohort includes two of the individuals who met a study endpoint after Cycle 13 (new resistance mutations). Excluded are the one subject who lacked detectable viremia at any STI (completed Cycle 14 with 30 days off treatment at study closure) and five subjects who withdrew well before Cycle 13 (two for study endpoints, three for personal reasons). The viral RNA profile for the cycling patients who did not reach Cycle 13 was indistinguishable from these 8 who did (data not shown).

Shown in figure 1 is the plasma RNA levels achieved at the end of drug interruptions (cycles 1...n) and at the end of each month of antiretroviral therapy (cycles A, B, C, D). The “past” values, shown near the y-axis, represent the last plasma RNA determined prior to starting the HAART regimen that resulted in viral suppression, perhaps representative of a “setpoint”. All 8 individuals had detectable HIV plasma RNA by Cycle 5 (median 3.5). Median end-of-interruption plasma RNA level peaked at 26,667 RNA cp/ml at Cycle 7 and declined to below 10,000 cp/ml after Cycle 10, despite a longer interval off treatment. In the 5 subjects who have reached Cycle 17, median RNA at the end of STI was 2,348 cp/ml vs 11,906 cp/ml at Cycle 7. In the continuous therapy cohort, 11 of 20 manifested at least one episode of detectable viremia over the course of the study. In eight cases this was transient and/or low level (i.e. <2,400 RNA cp/ml) viremia. In 3 cases, the viremia was greater (i.e. peaks of 7,200, 24,966, 13,607 cp/ml) and more sustained in time (24–48 weeks).

Greater than 10-fold increases from baseline in HIV-specific immune responses measured as interferon-gamma (IFN-gamma) spot forming cells (SFC) were seen in six of the eight. Overall, the increases were ≥ 6.4 fold. Shown in figure 2 is the kinetics of the change in HIV-specific CD4 and CD8 T cell responses as a function of the number of times that the individuals were exposed to viremia. Interferon gamma production to WHIV and vv-sum (the sum of the responses to HIV env, RT, gag and nef) was seen to increase substantially after 8 exposures to virus (median increases of 461 and 1175 SFC/10⁶ PBMC respectively over baseline (n=8) and continued to increase with additional exposures. The continuous treatment group showed low level increases in cell-mediated immune responses beginning at

the end of their first year after enrollment, coinciding with detection of viremia in some of them.

HIV-specific lymphoproliferative response as measure by the median stimulation index (SI) increased in the treatment group from 1 at baseline to 16, 12, 4, and 3 at cycles 7, 10, 13, and 17, respectively. The lymphoproliferative SI response in the control group was 6 at baseline and hovered below this level for all visits to week 132 with one exception.

As can be seen in figure 3, a decrease in median plasma viremia post STI is temporally correlated with increasing levels of immunity. To determine whether an individual had better control of viremia after a series of STI, we compared viral loads determined after the same number of days off therapy during early and later cycles. RNA was determined in plasma obtained at the end of an early, short interruption and compared to RNA in plasma obtained after the same number of days off therapy at the mid-point of a later cycle. Five subjects completed cycles during which mid-point plasma was obtained. Plasma RNA levels mid-cycle (after 17 days off treatment) during Cycle 18 were lower (median 1.3 log decrease, range 0.32 to 2.10) than plasma RNA levels after a comparable number of days off treatment (17–18 days) at the end of Cycle 8 (Figure 3b). Plasma RNA at the end of the interruption for Cycle 18 was also lower than Cycle 8 (data not shown). We then examined the HIV-specific immune responses measured prior to the Cycle 8 and 18 treatment interruptions. As seen in figure 3b, HIV-specific CD8-mediated IFN-gamma frequencies measured in the preceding month were 4 to 30-fold higher prior to Cycle 18 than Cycle 8 in 4 of the 5 individuals. The one individual (PID 1) lacking an increase in HIV-specific immunity was an individual whose viremia remained relatively low (maximum of 11,071 cp/ml) throughout the study. Similar findings for viral load and HIV/vaccinia-specific IFN-gamma frequencies were observed when mid-point viral loads in other late cycles were compared to viral loads determined in the corresponding early cycles.

Absolute CD4 counts remained stable throughout the study (1222 at base line and 1064 at the last completed cycle. CD4 percentages declined slightly (median CD4% at baseline and last completed A cycle on study : 40.5% (n=8) vs 36.5% (n=8)), the change was not statistically different from zero, Wilcoxon sign rank test P=0.38). CD8 percentages increased [median CD8% at baseline and last completed A cycle on study: 24% (n=8) vs 29.5% (n=8), Wilcoxon sign rank test P=0.047]. The CD4 decline and increased CD8 percents stabilized around Cycle 10 and were similar to those of the continuous therapy cohort at baseline and throughout the study

Four subjects discontinued STI due to a study endpoint. One failed to suppress viremia to <50 cp/ml at Cycle 9 with a final plasma RNA=70 cp/ml (same sample retested at 20 cp/ml). Three individuals developed HIV with new primary drug resistance mutations for the antiretrovirals they were receiving (M184V; M184V, V82A; M184V, V82A and I84I/V). In spite of these mutations, viral replication was suppressed after resumption of their same antiretroviral therapy.

DISCUSSION

STI strategies have been used to reduce the discomfort, complications or cost of HAART. and have often concentrated on prolonged intervals off drugs, monitored by CD4 decline (20). These often demonstrated significant declines in CD4 T cells and one recent study (SMART) has also demonstrated that this approach may jeopardize the future health of individuals participating in this STI strategy. When the intent of the STI is to improve HIV-specific immunity, other protocols have been designed to limit exposure to virus by alternating drug holidays with treatment intervals. Previous strategies employed 1)

alternating weeks on and off therapy; 2) two weeks off and 8 weeks on therapy; or 3) even longer drug holidays. These approaches have all suggested that exposure to virus results in increased levels of CD8 T cell responses to HIV antigens. Douek showed that allowing HIV-specific CD4 T cells to be exposed to HIV will result in the death of these cells (21) reducing the HIV-specific CD4 T-cell help to maintain CD8 T cell responses. Short treatment interruptions during the early cycles permit only brief, low-level viral replication at a time when HIV-specific immunity is low, and avoid the high level viremia that would be expected during a prolonged treatment interruption. The increase in CD4 lymphoproliferative responses peaked several cycles prior to the peak CD8 responses and this decline may have demonstrated the death predicted by Douek. However, the fact that the HIV-specific responses seen in the STI cohort increased with successive cycles and persisted throughout the study also suggests that, as seen in other immunization processes, the cells produced the necessary help to maintain CD8 responses and their decrease in later cycles may represent circulation to other lymphoid compartments rather than true death. Lymphoproliferative responses by the control group never increased in magnitude.

The CD8 response was substantially higher than has been reported for children and adolescents with chronic HIV viremia (22). We noted a degree of inter-individual variability in the number of days off treatment prior to detecting a detectable increase in circulating virus, ranging from. Although most individuals became viremic after a 9-day treatment interruption, three developed viremia 5 days off therapy and one individual maintained HIV RNA <50cpm/ml for 29 days off therapy. Clearly a fixed protocol of days on and days off therapy with such inter-individual variability will not result in the same level of exposure to replicating virus across individuals. By slowly increasing the time off therapy we avoided high level viremia in the majority of individuals, with only one whose HIV RNA exceeded 100,000 cp/ml. The median time to peak viremia was Cycle 7 (15 days off therapy) but this varied between individuals. In general, the time required to suppress viremia, once it developed, was one or two 28-day intervals of treatment but was occasionally exceeded. Given the known variability in current viral measurement techniques, this requirement may have been too stringent as a value just slightly over 50 may be 2–3 fold higher or lower on a subsequent measurement. With this gradually advancing regimen of STI, viremia declined after cycle 10 and this was observed in all but one of the 8 subjects on the study until Cycle 13. The one exception failed to develop viremia >11,000 cp/ml throughout the study. Some STI studies have used a change in the individual's viral setpoint as a gauge of whether improved virologic control has been achieved. Such an outcome is difficult to monitor in a pediatric population with varying ages and durations of prior. Nevertheless, it should be noted that the "setpoint" of our patients just prior to achieving virologic control with HAART was considerably higher than the levels measured after their treatment interruptions.

In addition to establishing a correlation between the increase of HIV-specific immune response and the decrease of HIV RNA over time across the population studied, we also were able to look at the change in the pattern of viral rebound during early and later cycles of STI in 5 individuals and to correlate the changes with differences in their HIV-specific immune responses. In four of the five, the peak viral load during the STI declined markedly during the later cycles, indicating that the trajectory of the viral load rebound had been altered over time. All four individuals had entered the late STI with HIV-specific IFN-gamma responses of markedly higher frequency than were present at the start of the earlier STI, suggesting that the presence of increased HIV-specific immune responses preceding STI is associated with the reduced peak of viremia. One individual who had relatively low levels of viremia at both early and late STI had minimal change in immune response as well. Something other than IFN-gamma responses may be responsible for this individual's control of viremia.

New primary resistance mutations were documented in 3 of the 14 cycling individuals enrolled. These mutations were directed at lamivudine and protease inhibitors such as indinavir and ritonavir. Others have noted the development of both mutations involving resistance to lamivudine (i.e. M184V) and to non-nucleoside reverse transcriptase inhibitors (NNRTI) during STI studies, perhaps as a function of their relative long half-life upon interrupting therapy. We specifically excluded patients treated with NNRTIs to avoid this possibility. Study participants were required to have available treatment options should antiretroviral therapy occur. Two of the individuals who developed resistance mutations did so very early in their protocol (i.e. prior to Cycle 8). The other individual developed mutations prior to Cycle 13. All of these individuals were able to fully suppress their viremia without changing their therapy. Montaner has reported that M184V mutations appear and disappear in the course of an STI study, without clinical consequence (23). More recently, Kuritzkes' group noted that in select cases of multidrug-resistant HIV-1 infection, lamivudine contributed to suppression of HIV-1 replication, despite the presence of M184V mutations and lamivudine resistance (24). As this mutation has been associated with a loss of viral replication capacity, the loss of viral fitness may dwarf the potential harm of having the mutation develop. The development of new primary mutations is potentially problematic for patients with limited treatment options and this sort of STI should not be entertained in such a population but it is a viable option for individuals who have limited antiretroviral experience and can completely suppress viremia.

This study was the first pediatric study of STI as immune therapy. It also was unique in that it correlated the temporal increase in HIV-specific responses with the temporal decline of viremia, despite allowing more time for viremia to develop. Several important observations emerge from this limited number of study participants. Eight or more exposures to virus were needed to see increased levels of HIV-specific immune responses. The increases observed with STI markedly exceeded increases in the continuous treatment group despite the viral "blips" and outright treatment failures in a minority of the control individuals. Moreover, higher level of immune response correlated with declines in viremia across the STI group and within individuals as well. HIV-specific immune responses increased further with additional exposures although the magnitude of viremia that presumably stimulated the responses continued to decline. STI studies that have failed to find improved virologic control after the interruptions may have failed due to early exposure of the participants to high levels of virus. Our study suggests that high level viremia is not needed to induce immune responses. In many instances viremia continues to decline with increasing number of interruption cycles. One individual (PID 6) has continued to participate in cycling protocol beyond the length of the study and now is able to maintain undetectable RNA levels for more than 21 days (data not shown). Other studies may have failed due to an insufficient number of exposures to HIV; most of the studies had fewer than six STI. The results of our study might predict that other immunotherapeutic strategies involving HIV vaccines may require considerably larger number of immunizations to achieve virologic control than the typical 2–4 doses. While HIV vaccine immunotherapy may be a safe option for attempting to control viral replication, a recent study which therapy with a canarypox-based HIV vaccine with STI alone also noted that STI effects delayed viral rebound more effectively than vaccination (25). This may be due to the fact that exposure to autologous virus, as occurs in STI, is more likely to control viremia than exposure to a heterologous vector.

In summary, a subset of pediatric patients undergoing progressively lengthening STIs demonstrated improved virologic control despite experiencing longer periods off therapy. This effect is seen in those who have had 13 or greater interruptions, resulting in STI intervals that eventually exceed 27 days in length. Enhanced HIV-specific immune responses may likely have played a role in this phenomenon. While pediatric patients may

have different outcomes than adults due to the presence of more effective thymic output, a strategy such as ours may work as well with older individuals.

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Figure 1.
Graph of the plasma HIV RNA measurements at the end of the treatment interruption cycles (N) and at the end of retreatment cycles (A, B, C, and D).

Normalized to the number of cycles with virus >50cp/ml

Figure 2.

The median increase in number of PBMC interferon-gamma spot forming cells (SFC/ 10^6 PBMC) measured in response to whole-inactivated HIV (WHIV) and the sum of the responses to recombinant vaccinia vectors expressing HIV-1 gag, pol, env, and nef (vv-sum). The STI cohort is on the left and the continuously treated cohort on the right. X-axis shows number of cycles with detectable viremia in the left panel and weeks on study in the right panel, respectively.

FIGURE 3a

FIGURE 3b

Figure 3.

Figure 3a. The median plasma HIV RNA levels at the end of interruption and the median change from baseline in HIV-specific IFN-gamma SFC to whole inactivated HIV and vaccinia-expressing HIV gag, pol, env, and nef (vv-sum) determined at the end of 28 days of treatment following an interruption with detectable virus.

Figure 3b. Plasma HIV RNA (copies/ml) at the end of the interruption for Cycle 8 (gray bar) and at a comparable number of days off treatment at the mid-point of Cycle 18 (black bar) and the corresponding vv-sum IFN-gamma SFC/10⁶ PBMC determined at the end of the treatment interval antecedent to Cycle 8 (7A) and 18 (17A) as gray and black bars, respectively.