

HIV-Specific Cellular Immune Responses Are Stimulated by Structured Treatment Interruption in Chronically HIV-1 Infected Koreans

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We evaluated the enhancing effect of structured treatment interruptions (STIs) on HIV-specific immunity in chronically HIV-1 infected Korean patients. A prospective case-control study was done with a total of 10 subjects for a period of 26 weeks. Six subjects were on STIs and four subjects were on continuous HAART for comparison. The STI subjects underwent four periods of STIs. For those on STIs, HAART was stopped at week 0 for two weeks, and resumed thereafter for six weeks. Viral load and CD4+/CD8+ T cells were measured by HIV RNA RT-PCR and flow cytometry, and HIV-specific immunity was measured by an ELISPOT assay. HIV-specific cytotoxic T cell immunity was more pronounced in the STI subjects than in the continuous HAART subjects after 26 weeks ($p=0.011$). The difference in cytotoxic T cell response in the STI group was more prominent than in the continuous HAART group ($p=0.011$). Viral load after 26 weeks was higher in the STI subjects than in the continuous HAART subjects ($p=0.008$). An HIV-specific cellular immune response can be stimulated by STIs in chronically HIV-infected Koreans. A larger study is warranted in order to further characterize viral and immunological parameters of treatment with STIs in cases of chronic HIV infection.

Key Words: HIV, HIV infection, immunotherapy, cellular immunity

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Highly active antiretroviral therapy (HAART) is associated with both virological and immunological benefits in human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). HAART has also been known to reduce AIDS-related mortality.¹ Despite these benefits, when used alone HAART has proven insufficient to completely eliminate viral reservoirs, even after decades of treatment.² Structured treatment interruptions (STIs) have been investigated as a means of improving viral control via the augmentation of HIV-1-specific cellular immune responses, with the additional potential benefits of reduced drug exposure and lower costs.

Results have thus far been encouraging for patients who undergo STIs following HAART when initiated during the period of acute infection.^{3,4} However, the benefits of STIs in those chronically infected is less clear. Studies indicate that only a minority of chronically infected individuals are able to maintain viral suppression after STIs without HAART, although most patients exhibit increased antiviral cellular immune responses.⁵ Furthermore, rapid declines in peripheral CD4+ T-cell counts have been observed during STIs.^{6,7} These CD4+ T-cell declines are inversely proportional to increases in plasma HIV-1 RNA levels during STIs, and are generally followed by a return to pre-interruption CD4+ T-cell levels after the re-initiation of HAART.⁷

To date, there has been no study conducted regarding the effects of STIs in HIV infected

Koreans. Therefore, in this report we have evaluated the effect of STIs with respect to the augmentation of HIV-specific immunity and controlling viral replication in chronically HIV-1 infected Korean patients.

A prospective case-control study was performed with a total of 10 patients. Six were on STIs and four were on continuous HAART for comparison purposes. Study subjects were enrolled in the study if they met the following criteria: chronically HIV-1 infected Koreans between the ages of 18 and 50 years old; having a viral load (RT-PCR) in plasma < 25 copies/mL for 12 months after HAART; and CD4+ cell counts ≥ 300 cells/ μ L. Informed consent was obtained from all subjects.

The STI subjects underwent four periods of STI. For those subjects on STIs, HAART was stopped at week 0 of the study for two weeks, and was resumed thereafter for six weeks (week 2 to week 8). This cycle was repeated four times. Antiretroviral therapy for all study subjects consisted of a protease inhibitor and two nucleoside

reverse-transcriptase inhibitors (standard doses of lamivudine and zidovudine). The protease inhibitors were indinavir or nelfinavir. After each interruption, medical visits were scheduled at day 0 and were continued weekly thereafter. Viral load and CD4+/CD8+ T cell levels were assessed via a HIV RNA RT-PCR and flow cytometry. HIV-specific cellular immunity was measured by an interferon- γ ELISPOT assay. The interferon- γ ELISPOT assay, as described previously, was performed on frozen PBMCs (peripheral blood mononuclear cells) from all 10 subjects.^{8,9} Cells in triplicate were stimulated by pools of 9-11 synthetic 15-mer peptides overlapping by 11 amino acids, which spanned the entire HIV-1 gag sequence (each peptide, 2 μ g/mL). Negative and positive controls were administered medium alone and phytohemagglutinin (1 μ g/mL), respectively. Results are expressed as the number of spot-forming cells (SFC) per 10^6 PBMCs. The number of HIV-1 specific interferon- γ producing CD8+ T-cells was calculated by subtracting the number of spot-forming cells in the

Table 1. Baseline Characteristics and Immunological and Virological Parameters in the Structured Treatment Interruption (STI) and Continuous Highly Active Antiretroviral Therapy (HAART) Subjects

Demographic/Immunological/Virological parameters	STI (n = 6)	HAART (n = 4)	p value
Age (yrs)	45 (35 - 47)	43 (41 - 45)	NS
Sex (male:female)	5:1	3:1	
Protease inhibitors received, no. of patients			NS
Indinavir	4	2	
Nelfinavir	2	2	
Initial CD4+ T cells (/ μ L)	698.0 (446.7 - 893.5)	728.0 (713.5 - 786.7)	NS
Final CD4+ T cells (/ μ L)	652.5 (456.5 - 830.0)	788.5 (741.5 - 825.7)	NS
Initial CD8+ T cells (/ μ L)	590.5 (441.2 - 985.7)	789.5 (646.5 - 967.0)	NS
Final CD8+ T cells (/ μ L)	1139.0 (672.5 - 1355.7)	780.0 (641.2 - 972.0)	NS
Initial plasma HIV RNA (copies/mL)	< 25	< 25	NS
Final plasma HIV RNA (copies/mL)	6,386.0 (118.0 - 20,325.0)	< 25	0.008
Initial CTL responses (SFCs/ 10^6 PBMCs)	108.0 (34.7 - 147.7)	107.5 (45.0 - 140.0)	NS
Final CTL responses (SFCs/ 10^6 PBMCs)	515.5 (243.0 - 741.5)	112.5 (66.7 - 141.0)	0.011
Changes of CTL response (SFCs/ 10^6 PBMCs)	406.0 (214.7 - 596.0)	11.0 (4.5 - 27.2)	0.011

NS, not significant.

Data are shown as 'median value (interquartile range)'.

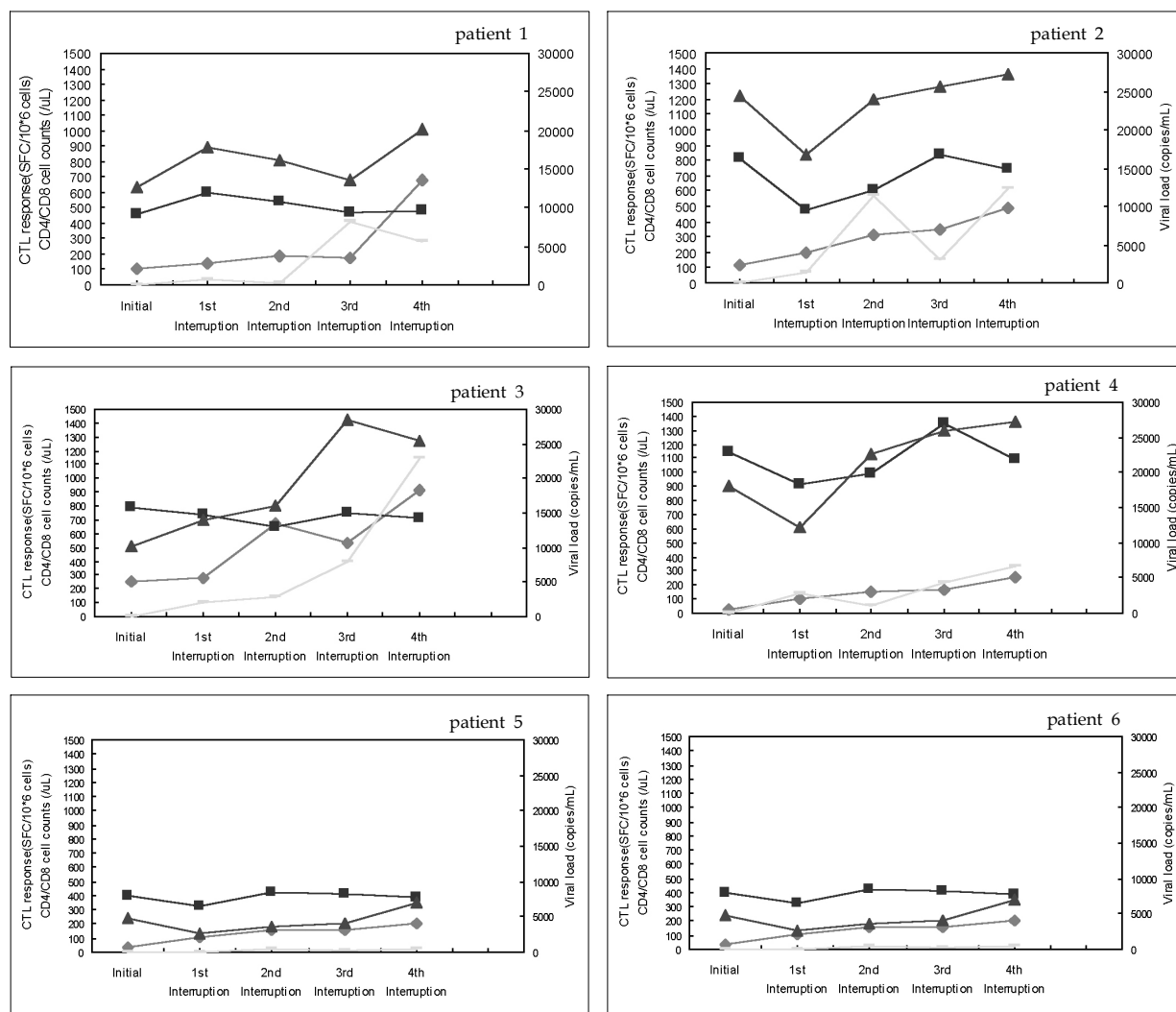


Fig. 1. Changes of immunological and virological parameters after treatment interruption schedules in the STI group. (■, CD4 cell counts (μL); ▲, CD8 cell counts (μL); ◆, Cytotoxic T lymphocyte responses (SFC/ 10^6 PBMC); —, Viral load (copies/mL))

negative control from the peptide-stimulated cells.

We then compared the HIV-specific immunity response, immunological parameters, and virological parameters between the STI and the continuous HAART subjects. Continuous variables were compared using the Mann-Whitney U test. A p -value < 0.05 was considered to be statistically significant. The SPSS (version 11.0) software package was used for all analyses. Data were expressed as the 'median value (interquartile range)'.

There were no statistically significant differences between the STI subjects and the continuous HAART subjects with respect to age, sex, or dura-

tion of known HIV-1 infection. No patient had conditions defining AIDS during the study.

Table 1 displays the immunological and virological parameters in both the STI subjects and the continuous HAART subjects. Final CD4⁺ T cell counts were lower in STI subjects than in the continuous HAART subjects, but this difference was not significantly different (652.5 (456.5-830.0) μL vs. 788.5 (741.5-825.7) μL). Plasma HIV RNA levels after 26 weeks were higher in the STI subjects than in the continuous HAART subjects (6,386.0 (118.0-20,325.0) copies/mL vs. < 25 copies/mL, $p = 0.008$). HIV-specific cytotoxic T cell immunity after 26 weeks, as measured by the

ELISPOT assay, was more pronounced in the STI subjects than in the continuous HAART subjects (515.5 (243.0-741.5) SFCs/ 10^6 PBMC vs. 112.5 (66.7-141.0) SFCs/ 10^6 PBMC, $p = 0.011$). The changes of CTL (cytotoxic T lymphocyte) response in the STI group were more prominent than in the continuous HAART group (406.0 (214.7-596.0) SFCs/ 10^6 PBMC vs. 11.0 (4.5-27.2) SFCs/ 10^6 PBMC, $p = 0.011$).

Fig. 1 shows the changes of immunological and virological parameters after each scheduled STI. After STIs, cytotoxic T lymphocyte (CTL) responses were stimulated, CD8 + T cell counts increased, and the viral load gradually increased in most patients.

It has been hypothesized that cyclical interruptions of antiretroviral therapy may boost HIV-1-specific immune responses, reset viral loads, and might constitute a novel modality of immune-based therapy in cases of HIV-1 infection, but only when initiated at the period of acute infection.³ However, these immune-specific responses seem to be weaker when STIs were performed during a chronic HIV infection. Moreover, there is contradictory evidence regarding the possibility of an association between these immune responses and the reset of the viral load set-point, which occurs in a low proportion of patients (approximately 20% of patients with chronic HIV infection).⁵

This study demonstrates that HIV-1 specific cellular immune responses were augmented following cycles of STIs in chronically HIV-1 infected Koreans. Chronically HIV-infected Korean individuals exhibited similar immunological responses to STIs as reported in other studies, which suggests that race may not be involved in the factors influencing an STI response.^{3-5,7} Recently, a prospective, randomized trial of STIs for chronically HIV-1 infected Thai patients was reported.¹⁰ This STI trial demonstrated that adequate immunological function could be preserved by the use of a CD4 cell count-guided method or a week-on/week-off approach to therapy withdrawal and reintroduction, compared with the continuous antiretroviral therapy in Thai patients. The study found that the week-on/week-off therapy was associated with a higher virological failure rate.

There are several important safety issues in-

herent in the STI protocols.¹¹ One concern is the potential development of drug resistance. We did not assess drug resistance by genotyping, and so are unaware of any mutations known to be associated with resistance to reverse transcriptase or protease inhibitors in the study population. Another concern was the possibility that viremia would not be re-suppressed upon the re-initiation of therapy. Viral loads dropped below 25 copies/mL in all patients within one month of re-introduction of the same antiretroviral regimen. These findings are consistent with the observations in other previous studies on antiretroviral therapy interruption.¹² Another safety concern associated with the STI protocols tested in this study was the potential drop in total CD4 T-cell counts after the viral load rebound. In another study, the CD4+ T-cell count decline was inversely proportional to increases in plasma HIV-1 RNA levels during STIs, and were generally followed by a return to pre-interruption CD4+ T-cell levels after the reinstatement of HAART.⁷ In our study, final CD4+ T cell counts were lower in STI subjects than in continuous HAART subjects, but this difference was not significant.

Chronically HIV-1 infected subjects are characterized by a significantly higher degree of immunosuppression than primary HIV-1 infected subjects, and they also tend to harbor more diverse viral sequences, requiring broader immune responses for viral control.^{13,14} Therefore, strategies designed to stimulate broader immunity and potentiate sufficient helper T cell functions are necessary for immune-based therapies, such as STIs, in terms of controlling viral replication in these chronically HIV-infected patients.

Our study has some limitations, the most important of which is the small number of patients enrolled. In addition, we interrupted HAART according to the duration of HAART, but took neither CD4+ T cell counts nor the viral load into consideration.

In conclusion, HIV-specific cellular immune response can be stimulated by STIs in chronically HIV-infected Koreans. A larger study is warranted in order to further characterize viral and immunological parameters after STIs in cases of chronic HIV infection.

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