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Structured Treatment Interruptions Following Immediate Initiation of HAART in Eight Patients with Acute HIV-1 Seroconversion

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Abstract
Background: The immunological and clinical benefits of structured treatment interruptions (STIs) during primary HIV-1 infection remain largely unclear.
Patients and Methods: Eight patients identified during primary HIV-1 infection were immediately treated with HAART and underwent subsequent STIs after reaching complete viral suppression of HIV-RNA in peripheral plasma. HAART was re-initiated if either HIV-1 RNA >5000 copies/ml, CD4-cells <200 cells/μl or symptomatic HIV-1 disease was observed.
Results: After treatment discontinuation, four of eight patients were able to persistently control HIV-1 viremia below 5000 copies/ml until the last time point of follow-up (median 3 years). CD4-cell counts were within the interquartile range of untreated individuals compared to historical reference data from the MACS cohort. In the remaining study subjects persistent virological control was not reached despite repeated STIs. Moreover, compared to the MACS cohort repetitive virological failures during STIs appeared to induce an accelerated decline of CD4-cells.
Conclusion: Spontaneous HIV-1 control after treated primary HIV-1 infection was possible in four out of eight individuals, however, if STIs after treated primary infection ameliorate the overall HIV-1 disease progression remains unknown. In the absence of viral control, repetitive viral exposure during STIs might be associated with accelerated decline of CD4-cell counts.

Key words: HIV-1, primary HIV-1 infection, HAART, Investigational therapies, structured treatment interruption

Introduction:

Structured or supervised treatment interruptions during HIV-1 infection represent immunotherapeutic interventions that involve the repetitive exposure to the autologous virus after periods of treatment with highly active antiretroviral therapy (HAART). This approach has not been successful during chronic HIV-1 infection [1, 2], but was associated with at least transient containment of viremia when treatment was started during primary infection in humans or animals infected with AIDS-associated retroviruses [3-5]. The main theory underlying this immunotherapeutic intervention is that early initiation of HAART can preserve, or even strengthen, HIV-1-specific CD4+ T cell responses [6-8], while the repetitive exposure to the autologous virus during subsequent STIs increases the magnitude and breadth of HIV-1-specific CD8+ T cells [9-11].

In a previous observational pilot study [3], it was shown that eight out of 14 individuals treated during primary infection achieved spontanous viral control below 5000 copies/ml for more than 180 days during a subsequent treatment interruption, however, the ability to contain viremia below this levels over the long term (>720 days) was only maintained in three individuals [12]. Here, we report on the virological and immunological outcomes of eight study persons who had been treated according to a similar protocol.

Patients and Methods:

Study Population

Eight individuals with acute HIV-1 infection were enrolled in this study between January 2001 and September 2002. Acute HIV-1 infection was defined by the presence of HIV-1 RNA in the plasma, and the detec-

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tion of no more than 3 bands in an HIV-1 immunoblot in the presence of a positive or negative HIV-1 Elisa. All patients were from the University of Bonn Medical Center and were recruited after providing written informed consent. The study was conducted in agreement with good clinical practice and the declaration of Helsinki and its subsequent revisions.

Initiation of HAART and Structured Treatment Interruptions

All patients were immediately treated with HAART (lopinavir/ritonavir 400/100 mg b.i.d., stavudine 40 mg b.i.d. (n = 7) or zidovudine 300 mg b.i.d. (n = 1), and lamivudine 150 mg b.i.d.). Structured treatment interruption were allowed after suppression of HIV-RNA below the level of detection (< 50 copies/ml plasma) for at least 3 months. After treatment discontinuation, HAART was restarted if viral load exceeded 5,000 copies/ml on any single occasion or immunological failure (decline of CD4-cells < 200/μl) or symptomatic disease occurred, requiring treatment according to present guidelines.

HLA Typing

HLA genoype was determined via reverse hybridization using the Inno-LiPa HLA-A Multiplex kit, HLA-B Multiplex kit and HLA-DRB decoder kit by Innogenetics N.V., Ghent, Belgium.

Chemokine Receptor Mutations

Genomic DNA was extracted from 200 μL EDTA treated blood and the CCR5-delta 32 mutation samples using the QIAamp Blood Mini Kit (Qiagen) according to the manufacturer's protocol. Primers flanking the CCR5-_32 mutation were chosen to amplify 189-bp (wild-type) and 157-bp (delta 32-deletion) fragments of the CCR5 gene, respectively [13]. For the detection of the CCR2-V64I and the SDF 1-3_ A mutation, the polymorphic gene sequences were amplified in a real-time PCR on a LightCycler according to a previously published protocol [14, 15]. Alleles were identified with linear allele-specific hybridization probes.

Synthetic HIV-1-Peptides

Peptides corresponding to previously described optimal HIV-1-specific CD8+ T cell epitopes [16] were synthesized at the MGH Peptide Core Facility on an automated peptide synthesizer using Fmoc technology, as described. Peptides overlapped by 10 amino acids and spanned the entire HIV-1 clade B 2001 consensus sequence.

Elispot Assays

Elispot assays were carried out as described previously [9]. The numbers of spots per well were counted using an automated Elispot plate reader (AID EliSpot reader system, Autoimmune Diagnostika GmbH, Strassberg, Germany). Samples were analyzed at baseline, i.e. the last available specimen prior to the beginning of the first STI, at 8 (± 4 weeks), 24 (± 8 weeks) and 48 (± 12 weeks) weeks. In the case patients entered a second or third STI, times were added to the length of the first STI, to allow comparison with patients maintaining virological control during the first STI.

Statistical Analysis

Descriptive statistical analysis was based on non-parametric tests (Wilcoxon matched pairs signed rank test, Fishers Exact Test, Kruskal-Wallis signed rank Test). Statistical analysis was performed using SPSS (SPSS Inc., Chicago, Illinois, USA) or Prism (GraphPad Software Inc., San-Diego, California, USA) software packages.

Results

Baseline Characteristics of Study Subjects

Seven male and one female individual, median age 35 years, were diagnosed with acute HIV-seroconversion (Table 1). Seven patients had symptoms compatible with the acute retroviral syndrome, while patient #1 was symptomless and was diagnosed during routine follow-up of a needle-stick injury. At the time of diagnosis HIV 1/2 ELISA was negative in 4 patients, weakly positive in one patient and positive in another 3 patients. HIV-1 immunoblot was negative in 5 patients, 1 patient showed one, one patient two and another patient three bands, respectively. Median HIV-RNA at diagnosis was 962,700 copies/ml (range 31,400 – 21,895,600) and CD4 T-cell count was a median of 406 cells/μl (range 261 – 1076). HAART was started within a median of 8 days (range 0-40) after diagnosis of HIV-infection and continued for a median of 194 days.

Evolution of HIV-1 Viremia

After HAART discontinuation, 4 patients (Controller, #1-4) were able to persistently control HIV-viral load below 5000 copies/ml plasma during the entire time of follow-up, (median of 1096 days, range 889 - 1444). In contrast, in the remaining four patients, virological rebound > 5000 copies/ml was observed within a median of 63 days (range 35 – 190) after the first treatment cessation. No statistically significant difference between controllers and non-controllers was seen regarding the time of HIV-1 diagnosis and the time of HAART initiation during primary infection. (Table 1). After re-initiation of HAART, patient #5 of the group of non-controllers was lost to follow-up and consequently did not enter another STI. Neither a second (patients #6 – 8) nor a third STI (patient #8) allowed for persistent control of viremia below 5000 copies/ml in the group of non-controllers.

Effect of Treatment Interruptions on CD4+ T Cell Counts

To analyze the immunological response of the study subjects, CD4+ T cell counts observed during the first 9 months of treatment interruption were compared

Table 1. Baseline characteristics of patients.

Patient	Sex	Age [years]	Symptoms	ELISA	Immuno Blot [bands]	HIV-RNA [copies/ml]	CD4-cells [/μl]	Time to Tx [days]	HAART
Controllers									
1	female	26	none	negative	0	31.400	1076	11	LPV/r, 3TC, d4T
2	male	35	rash, LAS	negative	0	100.944	261	0	LPV/r, 3TC, AZT
3	male	42	fever, rash	positive	2	2.766.400	300	3	LPV/r, 3TC, d4T
4	male	38	fever, LAS	positive	3	127.636	585	40	LPV/r, 3TC, d4T
Non-Controllers									
5	male	24	flu-like symptoms, fever	negative	0	2.651.500	340	10	LPV/r, 3TC, d4T
6	male	35	flu-like symptoms, fever	positive	0	1.667.400	392	6	LPV/r, 3TC, d4T
7	male	30	flu-like symptoms	weakly pos.	1	258.000	420	22	LPV/r, 3TC, d4T
8	male	44	rash, fever	negative	0	21.895.600	828	4	LPV/r, 3TC, d4T

Baseline characteristics of patients: LPV/r Lopinavir/ritonavir 400/100 mg b.i.b.; 3TC lamivudine 150 mg b.i.d.; d4T stavudine 40 mg b.i.d.; AZT zidovudine 250 mg b.i.d.; LAS lymphadenopathy syndrome

with corresponding data from 6,973 HIV-1 infected individuals sequentially enrolled in the Multi-Center AIDS Cohort Study (MACS). Overall, data from all 7 evaluable study persons did not significantly differ from the CD4+ T counts observed in the MACS cohort (Fig. 1, p = 0.37). However, controllers showed relatively stable CD4+ T cell counts throughout the two years of treatment interruption, with all four patients matching the interquartile range of the MACS-cohort. In contrast, individuals with repetitive treatment interruptions in the absence of HIV-1 control had CD4+ T cell counts ranging in the lowest 25% of the MACS-cohort. However, none of these patients required HAART re-initiation due to clinical signs of immunodeficiency or progressive loss of CD4+ T cells.

Magnitude and Breadth of HIV-1-specific CD8+ T Cells

In 7 (all except patient#2) of the study individuals, sufficient cryopreserved PBMC specimens were available to analyze HIV-1-specific CD8+ T responses by interferon-γ elispots, using previously-described epi-

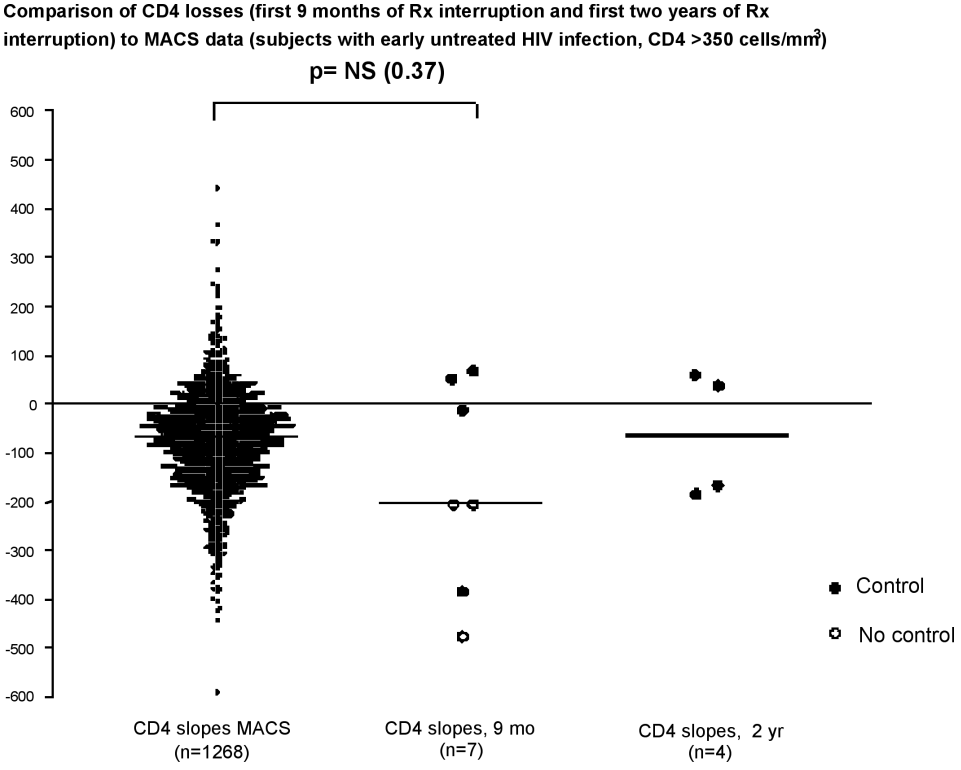

Fig.1. Comparison of CD4 losses with MACS cohort. Y-axis: loss of CD4-positive T-cells per year [/μl]; Historical control: 1268 subjects with early untreated HIV-infection, CD4 > 350 cells /μl from the MACS-cohort; Open circle: non-controller; Closed circle: controller.

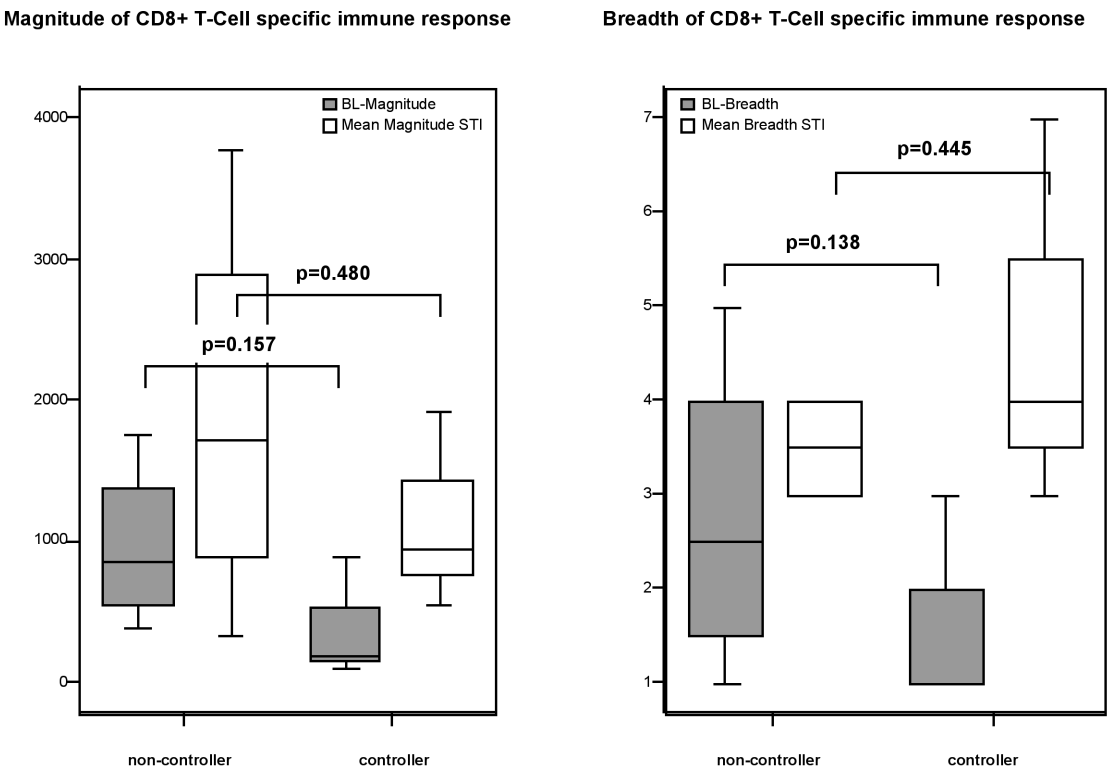

Fig. 2. Magnitude and breadth of CD8 T-cell mediated HIV-specific immune-response, as measured by interferon-γ ELISPOT; Magnitude: numbers of spot forming cells per 10^6 PBMC; Breadth: number of HIV-1 epitopes targeted.BL = baseline, Mean STI = mean breadth and magnitude of recognized epitopes / numbers of spot forming cells during structured treatment interruption.

Table 2. Host immunogenetics.

Patient	CCR5	CCR2	SDF 1-3´A	HGV	HLA
Controllers					
1	wt/wt	wt/wt	wt/wt	pos	A 02 / 11 B 27 / 44
2	wt/wt	wt/wt	wt/mut	neg	A 01 / 24 B 07 / 08
3	wt/wt	wt/wt	wt/wt	neg	A 24 B 35 / 44
4	wt/mut	wt/mut	wt/wt	neg	A 01 / 03 B 44 / 57
Non-Controllers					
5	wt/wt	wt/wt	wt/wt	pos	A 02 B 51
6	wt/wt	wt/wt	wt/mut	neg	A 11 / 68 B 14 / 35
7	wt/wt	wt/mut	wt/wt	pos	A 03 / 68 B 07 / 13
8	wt/mut	wt/wt	wt/wt	neg	A 24 B 44 / 51

Host immunogenetics: CCR5, CCR2 and SDF 1-3´A receptor polymorphisms, wt wildtype, mut mutant; HGV Hepatitis G Virus in peripheral serum (PCR-technique) at baseline, pos positive, neg negative; HLA human leukozyte antigen, typing for HLA A and B was performed.

topic HIV-1 peptides corresponding to the patients' HLA class I types. Compared to the baseline evaluation, patients showed a significant increase in the magnitude of HIV-1-specific CD8 T-cell responses during the treatement interruptions ($p < 0.023$; Fig. 2). Patients also showed an increase in the breadth of immune responses as defined by the number of epitopes recognized (statistical trend, $p < 0.063$). Comparing controllers versus non-controllers, there was no significant difference in the magnitude or the breadth of response, at all time points.

Host Genetics

Two of study subjects with spontaneous viral control during the first STI were HLA-B57 or –B27 positive, which has been associated with a beneficial HIV-1 disease outcome in a number of previous studies [17, 18], while none of these two protective HLA class I alleles were observed in patients with rapid virological rebound (Table 2; $p = 0.27$, Fisher's exact test). Moreover, patients controlling or non-controlling HIV viremia during STIs did not differ with regard to CCR5 Δ32 heterozygosity [19].

Discussion

In our study we present a detailed follow-up of eight individuals identified during primary HIV-1 infection, of whom four achieved spontaneous long-term virological control after antiretroviral treatment discontin-

uation. In contrast, progressive HIV-1 viremia was observed in four remaining study subjects who were unable to control HIV-1 RNA below 5000 copies/ml during the first, the second or the third treatment interruption.

With 50% of our study persons achieving long-term virological control, our data appear to suggest that STIs after treated primary infection are associated with a superior virological performance compared to natural HIV-1 infection without similar immunotherapeutic interventions. Despite the limitations due to the small sample size of our cohort and the fact that two study subjects carried protective HLA class I alleles, it is noteworthy that all of our study subjects who achieved spontaneous viral control shortly after treatment cessation maintained viral containment for at least two years (until the last time of follow-up), which is in clear contrast to the limited duration of viral control that was observed in the majority of individuals being treated with a similar protocol in a previous study [12].

The reasons accounting for viral control in two of our study subjects without protective HLA class I alleles are unclear at present, but do definitely not reflect a stronger magnitude of HIV-1-specific interferon-γ secreting CD8+ T cells, as both controlling and noncontrollling individuals have comparable total magnitudes of HIV-1-specific CD8+ T cells [20]. Instead, recent data appear to suggest that protective cellular immunity is rather associated with the proliferative capacity of HIV-1-specific CD4+ and CD8+ T cells [7, 21], the clonal composition of HIV-1-specific CD8+ T cells and their specific structural interaction with the peptide-MHC class I complex [22] as well as viral fitness costs induced by HIV-1-specific CD8+ T cell mediated viral escape [23].

If the present data are insufficient to determine the potential advantages of STIs after treated primary infection, are they at least adequate to rule out any major harmful effects potentially associated with this treatment strategy? Although no case of rapid or severe clinical deterioration has ever been described in HIV-1 patients undergoing STIs and emerging resistance against common HIV-1 drugs appears to be well controllable, our data suggest that an accelerated loss of CD4+ T cells mediated by repetitive exposure to high level viremia may occur as a potential side effect of this treatment approach. Indeed, our finding of a faster than usual CD4+ T cell decline during STIs in the presence of high level viremia, together with similar findings made in some individuals in a previous study [12], clearly indicate that STIs after treated primary infection should only be performed in the setting of clinical studies in which detailed and meticulous patient supervision is guaranteed.

Overall, these data show that selected HIV-1 patients are able to achieve HIV-1 control during structured treatment interruptions after early initiation of antiretroviral treatment, even in the absence of protective HLA class I alleles. However, repetitive exposure to high level viremia during STIs in individuals failing to achieve spontaneous viral control appears to be associated with accelerated loss of CD4+ T-cells. Prospective controlled clinical trials will be necessary to determine the ultimate risks and benefits associated with this treatment approach.

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