Journal of Clinical Immunology, Vol. 25, No. 2, March 2005 (© 2005)
DOI: 10.1007/s10875-005-2817-z

Flow Cytometry Evaluation of the T-Cell Receptor Vβ Repertoire Among HIV-1 Infected Individuals Before and After Antiretroviral Therapy

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Accepted: November 19, 2004

HIV-1 infection leads to serious impairment of the immune system and perturbations in the T cell receptor Vβ repertoire are also described. Immune reconstitution can be potentially achieved in response to HAART. In the present study 10 patients were investigated for the Vβ pattern expression before and after six months of HAART. TCR were analyzed for T CD4+ and CD8+ subsets, separately, by flow cytometry, using a monoclonal antibody set of 24 different Vβ chains. Compared to eight Brazilian healthy controls, no differences in Vβ pattern of expression was observed for patients before or on antiretroviral therapy. Some chains such as Vβ 3, 14, 16, 20 and 21.3 were over utilized by both T subsets, independently of HIV infection and/or antiretroviral treatment, differing from the ones described for individuals of other nationalities. However, when each patient was taken individually, particular alterations were detected for the Vβ gene usage, compared to controls, for all individuals. After treatment, significant Vβ usage changes were observed for seven patients. One or more chains on both T subsets were engaged in this process, defining a preferential oligoclonal profile for TCR repertoire distribution, after HAART. Although no pattern of specific Vβ changes was detected in the circulating T cells, we cannot exclude that differential immune responses to HIV or other important antigens are being focused by these cells.

KEY WORDS: HIV-1; TCR Vβ repertoire; HAART; clonality.

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INTRODUCTION

More than two decades after the onset of the acquired immunodeficiency syndrome (AIDS), major progress has been made in the understanding of the pathogenesis of human immunodeficiency virus infection (1). It is now widely accepted that a delicate balance among multiple virologic and host factors are responsible for the possibility of preventing HIV infection, initial control of virus spreading and/or the establishment of chronic infection, and the severity of the clinical course of HIV disease (2).

Both cellular and humoral immune response have an important participation on this scenario, since the temporal relationship between the appearance of a vigorous HIV-specific immune response, with consequent down regulation of viremia and resolution of the acute viral syndrome, has been clearly established (3, 4). Preservation of immune function and low levels of virologic parameters are common findings in HIV-infected subjects with nonprogressive disease, whereas loss of immune response and high levels of virus are associated with rapid progression (5–7).

Nevertheless, which immunologic factors are really determinant for the extensive control of HIV infection is not clear, since patients may experience different rates of disease progression, despite the presence of vigorous HIV-specific immune response (8). Analysis of HIV-specific cellular responses in long-term non-progressors (LTNP) individuals demonstrated a relatively stable HIV-specific effective repertoire, which was correlated with maintenance of the non-progressor status (9). Indeed, alterations in certain T cell receptor (TCR) Vβ members are detected in long-term non-progressors (10). Moreover, prospective studies demonstrated that HIV infected subjects with mobilization of a restricted

mono-oligoclonal TCR repertoire during acute infection generally experience a rapid progression to AIDS, while a slower disease progression was associated with a broader TCR repertoire (11, 12). This association has also been suggested in simian immunodeficiency virus model (13). Thus, qualitative differences in the primary T cell immune response may be related with different clinical outcomes.

The introduction of highly active antiretroviral therapy (HAART) for HIV infection in the mid 1990s has resulted in dramatic declines in HIV-1 associated morbidity and mortality (14, 15). Improvement of the immune system is observed, as depicted by the increase in total CD4+ cell count, involving both memory and naive fractions (16–19). Reconstitution of pathogen-specific immunity with marked reduction of major opportunistic infections (17, 20–22), and reversion on chronic levels of immune activation and T-cell maturation abnormalities are also observed (17, 23–26), in parallel with a decline in HIV viral load (18). Indeed, a previous study demonstrated that perturbations in the T cell receptor Vβ during primary HIV infection are stabilized with HAART introduction (27), suggesting a positive influence of the therapy on the dynamics of T-cell mediated immune responses. However, this reconstitution is not complete, since studies of chronically infected individuals under HAART have shown that HIV-specific CD4+ lymphocyte proliferative responses are rarely recovered (17, 26, 28, 29). Reconstitution of these responses has been reported in some individuals in early stages of disease (30) and also in individuals with transient interruptions of HAART (31, 32). These results suggest that re-exposure to HIV-1 antigens may reconstitute these responses and that this could be dependent on the pre-existing repertoire. So, this environment of restoration and virus control are giving us important insights for better understanding the natural immune response against the virus.

In the present work, we evaluated HIV infected patients on chronic clinical phase of disease, for the TCR Vβ repertoire by flow cytometry. Since this analysis is an appropriate approach for determining the diversity and dynamics of the cell mediated immune response among different individuals, healthy control individuals and HIV-infected adults ongoing disease progression, previous and posterior to submission to HAART, were evaluated in order to identify differences and/or changes in TCR profiles among them and thus evaluate the T repertoire reconstitution on late stages of disease.

MATERIALS AND METHODS

Study Population

Ten patients naive of treatment and with indication to HAART were recruited by the Evandro Chagas Clinical Research Institute, FIOCRUZ, Rio de Janeiro-RJ, Brazil. Therapy submission criteria were in accordance with recommendations from the Brazilian Ministry of Health. All patients presented absolute CD4 T cells counts below 500 cells/mm^3 (range 15–379), viral load above 10,000 copies/ml (range 10^4 to 1.8×10^6) and clinical manifestations at entry (Table I). According to the CDC illness definition, all patients, except VB019, were reported aids cases. Patients of both sexes were enrolled, with age ranging from 26 to 49 years old. Triple antiretroviral regimen was adopted and combined two nucleoside analogues with one non-nucleoside reverse transcriptase inhibitors. Analyses were performed on two time points, at baseline and after 24 weeks of therapy. Eight non-HIV infected healthy individuals, with age ranging from 20 to 50 years old, were randomly selected for the control group and evaluated at one moment only. Protocols were approved by the institutional ethic committee and informed consent was signed by each patient before sample collection.

Viral Load Evaluation

Quantification of plasma viral load was determined for all patients before and after 24 weeks of HAART by nucleic acid sequence-based amplification, NASBA (Organon Teknika, Boxtel, The Netherland), with a detection limit of 80 copies/ml.

Analysis of the Vβ Repertoire

Peripheral blood mononuclear cells (PBMC) were collected from patients and control individuals and criopreserved until use. PBMC were thawed and directly stained with different anti-TCRBV antibodies from a Vβ monoclonal antibodies kit (IOTest Beta Mark, Beckman Coulter-Immunotech, Marseille, France), which labels 24 members from different Vβ families, covering approximately 70% of the normal human TCR Vβ repertoire. Using a two colors staining method, which combines TCR antibodies labeled with fluorescein, phycoerytrin and fluorescein/phycoerytrin, this kit allowed us to determine the Vβ chain expression on each T cell subset, employing anti CD4 phycoerythrin-cyanine 5 (PC5) and anti CD8 phycoerythrin – Texas red (ECD) antibodies. Samples were prepared according to manufacturers instructions and 2×10^5 PBMC were analyzed for each antibody combination using a Coulter XL-MCL flow cytometer. Previous to Vβ analysis, all samples were evaluated with isotype control antibodies with PC5 and ECD labels. The Vβ repertoire analysis protocol was developed as previously described (33). Briefly, an initial gate was set around the lymphocytes based on forward and side light scatter properties. At least 2×10^4 events were counted in this lymphogate,

Table I. Epidemiological, Clinical and Laboratory Status of 10 Studied Patients at Baseline and 24 Weeks After HAART

Patient identification number	Age (years)	Sex	Disease stage	CD4 cell count/mm^3		CD8 cell count/mm^3		Plasma viral load (RNA copies/ml)		HAART drugs
				Baseline	HAART (24 weeks)	Baseline	HAART (24 weeks)	Baseline	HAART (24 weeks)	
VB010	26	M	C2	379	397	1094	934	52,000	<LDL	AZT, 3TC, EFZ
VB012	44	F	C3	42	89	251	542	10,000	820	AZT, 3TC, EFZ
VB013	42	F	C2	360	356	872	677	42,000	<LDL	AZT, 3TC, EFZ
VB014	28	M	B3	77	97	730	942	290,000	<LDL	AZT, 3TC, EFZ
VB015	42	M	C3	15	182	700	698	1,800,000	<LDL	AZT, 3TC, EFZ
VB017	38	M	B3	43	226	622	568	1,800,000	<LDL	AZT, 3TC, EFZ
VB018	33	F	B3	40	85	525	591	150,000	<LDL	AZT, 3TC, EFZ
VB019	49	F	B2	209	230	695	674	12,000	110	AZT, 3TC, EFZ
VB020	40	M	C2	292	425	355	638	190,000	<LDL	AZT, 3TC, EFZ
VB021	45	M	C3	20	534	976	1153	560,000	<LDL	d4T, 3TC, EFZ

Note. AZT, zidovudine; 3TC, lamivudine; d4T, stavudine; EFV, efavirenz; LDL, lower detection limit.

in order to guarantee an accurate determination of minor Vβ populations. Additional gates defined both CD4 and CD8 T subsets, and consisted of a small lymphocyte light scatter gate as well as a gate on T bright lymphocytes. Percentages of each Vβ member were then determined. Parallel samples were labeled with three colors stain anti-CD3, anti-CD4 and anti-CD8 monoclonal antibodies to confirm the percentage of such T cell populations (CD3/CD4 and CD3/CD8) in order to guarantee that all CD4+ and CD8+ bright gated cells were indeed T lymphocytes.

In order to determine the repertoire perturbations, normal ranges were established for each Vβ member based on confidence intervals of 95% (CI 95%) obtained from eight healthy control individuals. Perturbations (increments/declines) of Vβ members were considered to be significant in patients when they were beyond these normal intervals. However, for Vβ families expressed at very low levels (i.e., <2%), only four-fold changes in relation to median of controls were considered significant, in order to avoid false positive alterations. When post-therapy changes were analyzed a similar criteria was used, but Vβ frequencies from each patient before treatment were used as references and alterations of at least two-fold were considered significant.

RESULTS

TCR Vβ Expression on T-Cell Subsets of Control Individuals

For a better analysis of alterations in T-cell repertoires in CD4+ and CD8+ T cells, an understanding of the normal peripheral repertoire must be obtained. So, the T-cell Vβ chain profiles presented by eight normal subjects were evaluated first. In human peripheral blood there is a marked usage of individual Vβ families with several members making up the majority of the repertoire. In our control group, these were Vβ3, 14, 16, 20 and 21.3 for both T cell CD4+ and CD8+ subpopulations, as shown in Fig. 1. Moreover, Vβ12 was preferentially utilized by the T CD4+ subset, while Vβ8 was detected on more than 5% of the T CD8+ circulating cells. In addition, other Vβ members were particularly utilized by above 5% of T cells in some individuals, but these were isolated mobilization cases, since no significant differences were detected among healthy individuals.

On the other hand, the frequencies of other TCR Vβ members such as Vβ4, 11, 18 and 22 were below than 1%. Particularly low expressions were also detected for Vβ23 on T CD4+ lymphocytes and for Vβ2, 5.1 and 13.6 on the T CD8+ subpopulation. The median levels of other Vβ segments varied from 1 to 5%, in both T subsets. Similar patterns were observed for T repertoire distributions on both CD4 and CD8 cells, but three Vβ chains frequencies were significantly different between the two subsets. Both Vβ5.1 and 12 were more mobilized by CD4+ rather than CD8+ cells, while CD8+ cells utilized Vβ23 more frequently than did CD4 lymphocytes ($p > 0.05$).

All 24 TCR Vβ chains were represented in the T repertoires of the control group and very few were expressed in more than 15% of T cells. Only two cases of very high expression were observed among them, the Vβ16 chain in control 01, expressed on 25.9% of T CD8+ subset and the Vβ1, observed on 27% of CD4+ lymphocytes in control subject 07. In reviewing the clinical report of these subjects, they had no known concurrent illnesses at the time of sample collection. So, it becomes evident that T lymphocytes of healthy donors use TCR Vβ genes non-randomly, and that particular mobilizations may occur.

Comparison of Vβ Repertoires from Controls and HIV-Infected Patients

All 10 patients recruited for this study were immunocompromised at the moment of HAART submission, presenting depressed T CD4+ cell counts (<500 /mm^3) and

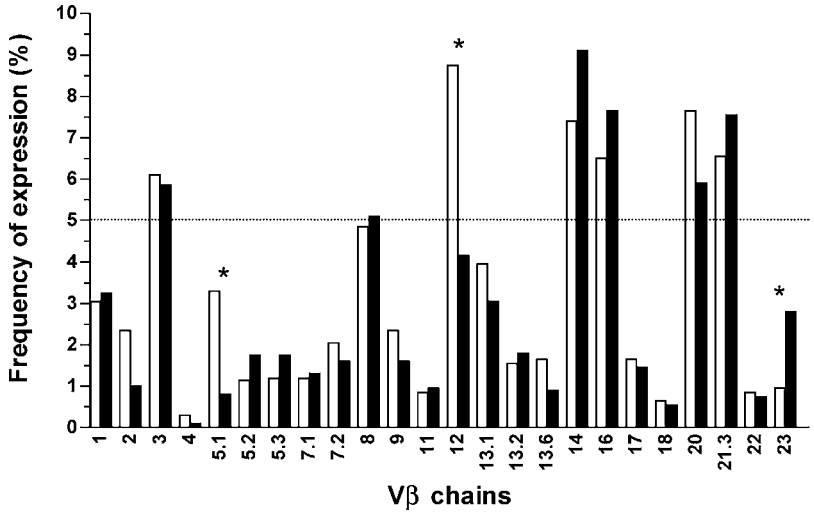

Fig. 1. Median relative values of TCR Vβ usage by CD4+ (open bars) and CD8+ (closed bars) peripheral T-cell subsets from eight healthy control subjects. $^{*}p < 0.05$.

enhanced T CD8+ circulating cells, as shown in Table I. The RNA viral load levels were also elevated ($>10^4$ copies/ml) and all patients presented clinical symptoms, being reported as aids cases, with only one exception. Under this scenario, it was expected that biased T cell repertoires would occur. However, when whole median values of Vβ gene usage from patients before HAART submission and normal controls were compared, no differences were obtained. Figures 2 and 3 show TCR Vβ repertoire distribution of 10 patients evaluated in relation to the confidence intervals of 95% (CI 95%) obtained from control individuals (boxes), for T CD4+ and CD8+ subpopulations, respectively. Similar patterns of TCR Vβ mobilization were detected in the patients. As observed for healthy individuals, Vβ3, 14, 20 and 21.3 were also the most used chains for both T cell CD4+ and CD8+ cells among the patients. Vβ12 was frequently more mobilized for CD4+ circulating cells while Vβ8

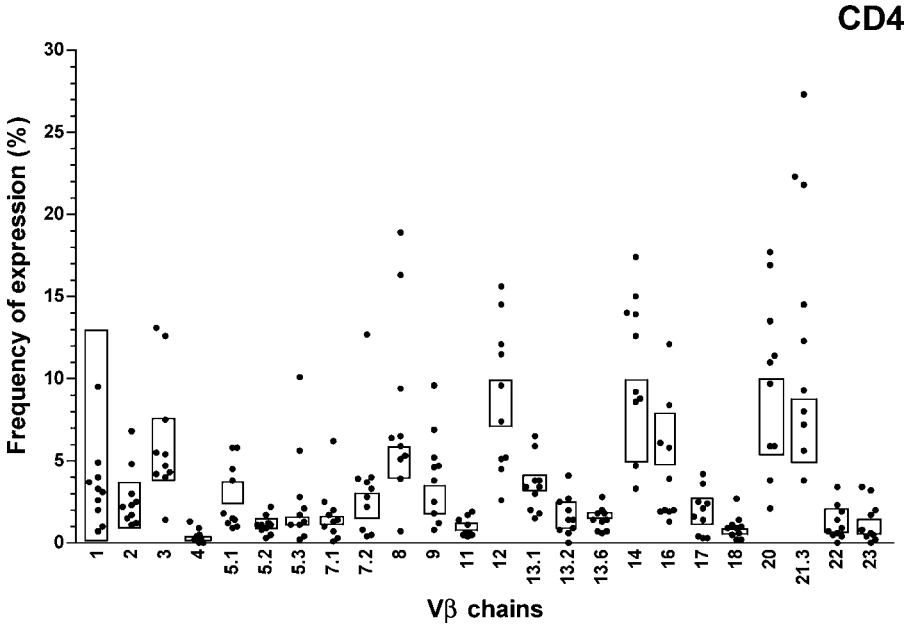

Fig. 2. TCR Vβ expression frequency distribution on T CD4+ cells from 10 HIV-infected patients, before starting antiretroviral therapy. Boxes represent normal confidence intervals (95%).

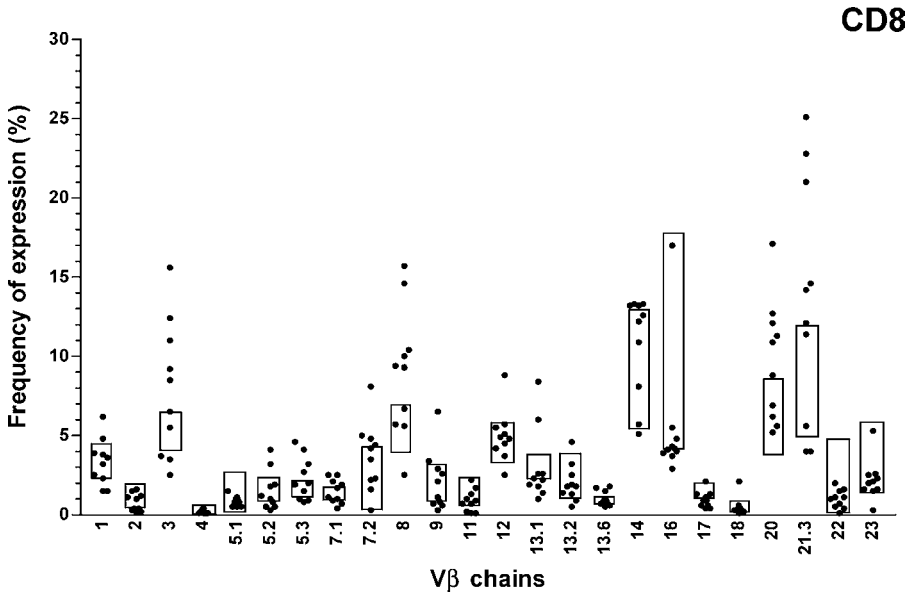

Fig. 3. TCR Vβ expression frequency distribution on T CD8+ cells from 10 HIV-infected patients, before starting antiretroviral therapy. Boxes represent normal confidence intervals (95%).

was utilized by both T subsets. Interestingly, Vβ16 expression was decreased in the CD8+ subset from 9 out of 10 patients, with expression values ranging from 2.9 to 5.5% in these individuals, while the Vβ16 median observed for controls was 7.6 (CI 95%, 4.2–17.8%). This reduction was considered significant for five patients, since the frequencies of expression were beyond defined ranges. Meanwhile, this chain remained frequently utilized by CD4+ cells. Less than 1% of circulating peripheral blood cells of HIV-1 patients expressed Vβ4, 11, 18 and 22. Similar mobilization patterns were observed between CD4 and CD8 cells for the TCR Vβ repertoire in patients, but significant differences on frequency of utilization ($p < 0.05$) remained for Vβ5.1 and 12, which were much more expressed by the T CD4+ subpopulation, while Vβ23 was more frequently mobilized on T 8+ cells.

On the other hand, skewed repertoires were identified when each patient was particularly evaluated separately. Significant expansions or reductions were observed for all 10 patients, when their repertoire profiles were compared to control medians, for both T subsets, and these were represented by the points outside normal CI 95% intervals in Figs. 2 and 3. Nevertheless, some of these points, corresponding to perturbations of Vβ segments expressed at low frequency (i.e. <2%), were not considered important, since these were not four-fold changes. On T CD4+ cells, for example, the median value observed for Vβ5.3 expression in control group was 1.2% (CI 95%, 0.6–2.4%), but only expansions above 4.8% were considered significant changes. The majority of abnormalities in the HIV population represented proportional expansions on gene expression levels and were more frequently observed on T CD4+ cells.

Figure 4 exemplifies the frequencies of Vβ mobilization observed for both T CD4+ and CD8+ cells of four HIV-infected individuals. Analysis of T CD4+ cells of patient VB013 detected the expansion (E) of Vβ2, 5.1, 9 and 22 and the reduction (R) of Vβ12, 14, 16 and 21.3 in relation to normal range values (CI 95%). Because all patients were analyzed only at the ultimate time point before starting antiretroviral therapy and not at any other earlier moment, we decided to employ the "mono," "oligo" or "polyclonal-like" terminology, in order to describe the repertoire profile pre-HAART submission, since the dynamic analysis of the Vβ expressions throughout the chronic phase of HIV-infection was not carried out.

As shown in Fig. 4, although some Vβ perturbations were detected for T CD4+ cells from the patient VB013, no apparent dominant Vβ expression was detected over the repertoire and therefore the Vβ expression was characterized as "polyclonal-like." The same was observed for the T CD8+ cells from another patient (not shown). On the other hand, the VB013 patient T CD8+ subset presented almost all Vβ frequencies within the normal range values, but a clonal dominance of Vβ14-bearing cells was observed. Although moderate (13.2%), this expression was responsible for shaping a "monoclonal-like" status for the T CD8+ repertoire, since

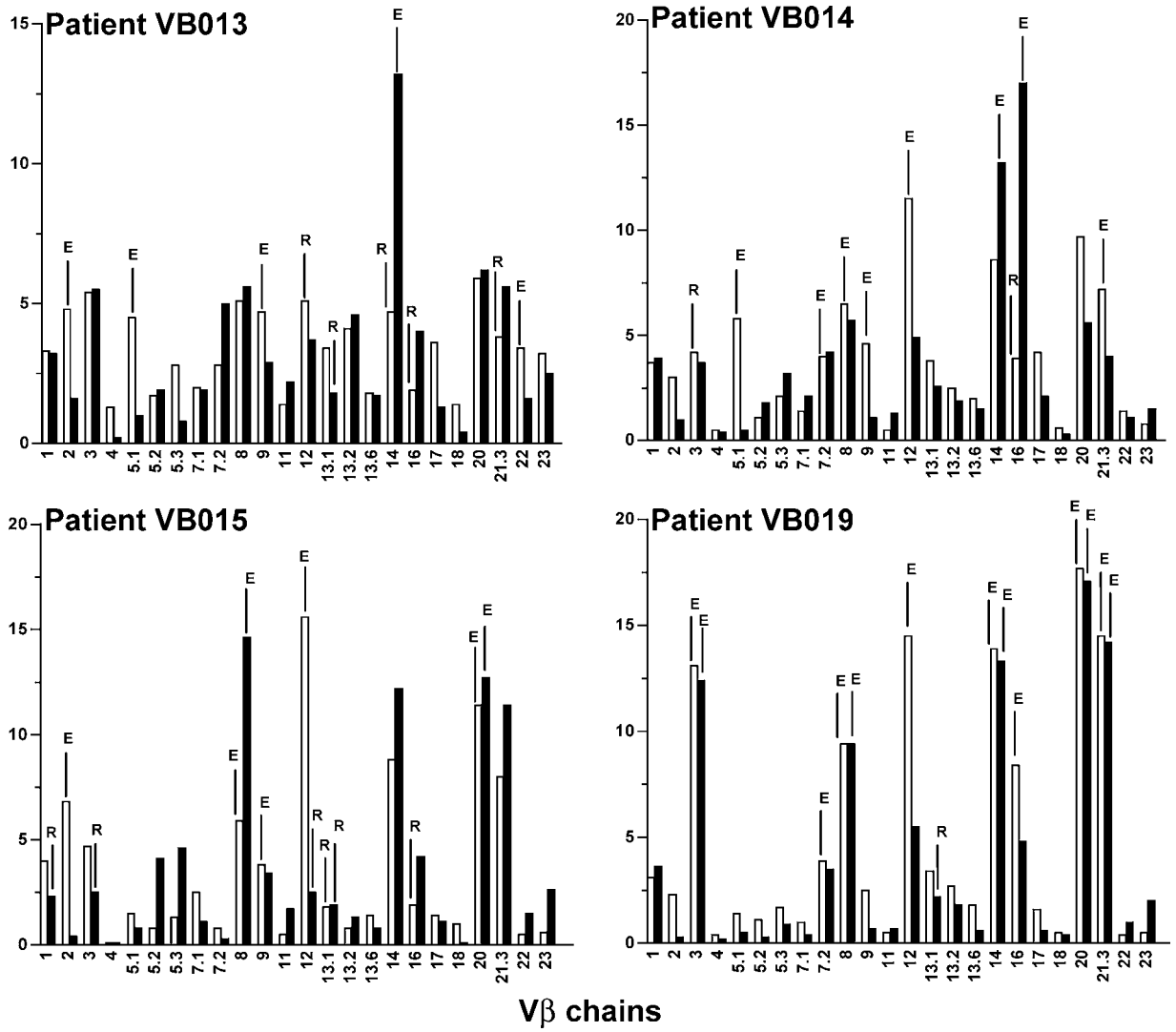

Fig. 4. TCR Vβ frequency distribution on T CD4+ cells (opened bars) and T CD8+ cells (closed bars) from four HIV-infected patients, before starting antiretroviral therapy. E, expansions and R, reductions of TCR Vβ chain usage in relation to median normal ranges.

only frequencies around 5% were detected for all the other Vβ members.

When the T cell repertoire was shaped by two or more Vβ chains expressed on approximately 10% or more than the circulating cells, an "oligoclonal-like" profile was identified. For patient VB014, eight significant alterations were observed among Vβ members of the T CD4+ subset, with both expansions (Vβ5.1, 7.2, 8, 9, 12 and 21.3) and reductions (Vβ3 and 16), and an "oligoclonal-like" distribution of TCR Vβ expression was identified on these cells. This profile was more clearly identified when the T CD8+ subset from this patient was evaluated. The "oligoclonal-like" T cell distributions shaped by expansions and reductions of Vβ chains were also observed for patients VB015 and VB019, in both T cell subpopulations. Thus, this skewed profile was the most frequent pattern of Vβ mobilization observed among the studied patients, being detected for at least one T cell subset, from nine chronically HIV-1 infected individuals.

Comparison of Vβ Repertoires from HIV-Infected Patients, Previous to and After HAART

The antiretroviral scheme used for the patients included in the present study was able to control the viral replicative process, since the viral load fell to undetectable levels 24 weeks after treatment for 80% of patients, while clinical manifestations disappeared and

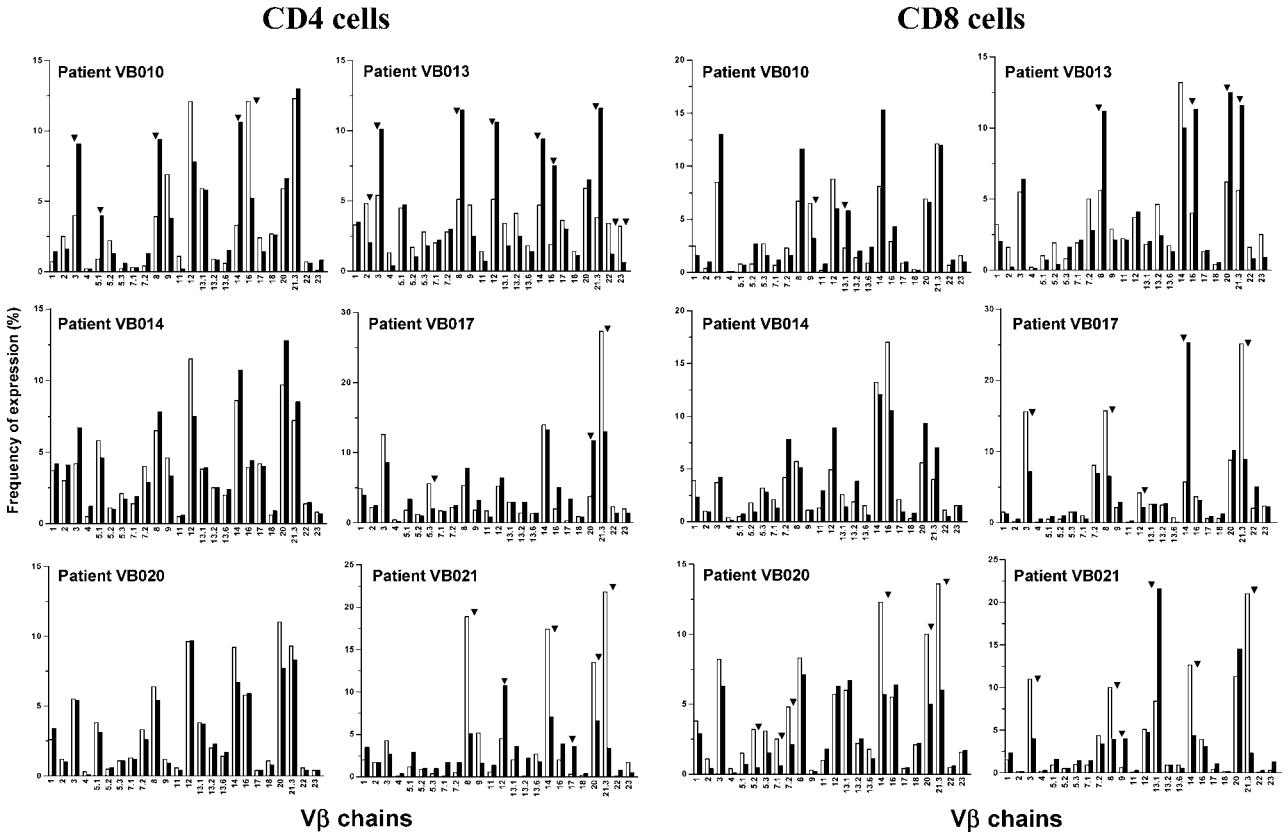

Fig. 5. Distribution of the TCR Vβ repertoire of six HIV-infected individuals at the HAART submission time point (open bars) and after 24 weeks of treatment (closed bars), observed on both T CD4+ (right panel) and T CD8+ (left panel) cells. Arrows indicate significant changes of Vβ utilizations after therapy.

T CD4+ counts were enhanced by up to 340%, from a median of 60 to 204 cells/mm^3, although only one patient presented values above 500 cells/mm^3 (Table I). In order to investigate the effect of the response to antiretroviral therapy on the TCR Vβ peripherally circulating repertoire, the patients were also evaluated after 24 weeks of treatment submission. Seven out of 10 patients presented some alterations on Vβ frequencies, when they were compared to their own initial values observed before HAART. Expression of some chains declined while others expanded, affecting previously observed repertoire distribution in most of the patients. Figure 5 shows the changes in TCR mobilization observed for six patients, in T CD4+ (left panel) and CD8+ (right panel) subpopulations. Here, we were able to identify the dynamic patterns of repertoire mobilization profiles presented for each patient, since two time points were investigated.

The patient VB010 had the Vβ3, 5.1, 8 and 14 expanded and the Vβ16 reduced on T CD4+ cells. However, the initial "oligoclonal-like" distribution profile was preserved, defining an oligoclonal pattern of TCR mobilization in this patient post-HAART, although shaped by other Vβ chains. The same was observed for the T CD8+ subset from patient VB021. For T CD8+ cells from VB010, the same Vβ chains shaped the repertoire and the oligoclonal distribution was preserved after 24 weeks of treatment. When the patient VB013 was analyzed, the previous "polyclonal-like" distribution profile gave rise to an oligoclonal mobilization in the T CD4+ subset, since significant expansions and reduction were detected. This also happened in T CD8+ cells from patient VB012. The T CD8+ cells repertoire from VB013 also experienced Vβ changes and the former of oligoclonality set up. On the other hand, the previous "oligoclonal-like" profile gave rise to a polyclonal distribution for patient VB020 (T CD8+ subset) and patient VB021 (T CD4+ subset), and to a monoclonal distribution for the T CD8+ cells from patient VB017. For three patients, no expansions or reductions were observed after 24 weeks of HAART, neither on T CD4+ nor on CD8+ cells, although some chains were slightly mobilized, indicating the dynamics of the T cell repertoire. Therefore, HAART submission induced an oligoclonal distribution profile in 9 out of 10

patients, on at least one T subset, inducing other Vβ genes of the TCR Vβ repertoire.

DISCUSSION

In this study we evaluated the T cell Vβ repertoire status of HIV-infected individuals, naive of anti-retroviral treatment, previous to and after HAART. All patients were immunodepressed at study enrollment, presenting low T CD4+ counts, high virus levels and clinical symptoms. After 24 weeks of antiretroviral therapy, a reduction of virus replication was observed, since the plasmatic viral load dropped, an elevation on T CD4+ numbers on peripheral blood occurred and the clinical manifestations disappeared. This reversion of viral and immunological parameters was in accordance with that observed in several studies (16–19, 26) for HIV-1 patients under HAART.

The T cell repertoire analysis was conducted by flow cytometry using a kit, which allows analysis of 24 TCR Vβ chains on previously fractionated T CD4+ and CD8+ subpopulations, in a very simple way, using a four-colour stain system. Primarily, we investigated eight control cell preparations, in order to define the normal frequency and distribution of TCR Vβ chains in the peripheral blood of adult humans in the studied population. It has became evident from many studies that T lymphocytes of healthy blood donors use TCR Vβ genes randomly. Although all known TCR Vβ gene members are expressed in peripheral blood T cells, some chains tend to be much more expressed at the cell population level, as Vβ2, Vβ6, Vβ8 and Vβ17, while some others such as Vβ12 and Vβ24 tend to be under-represented (34, 35). In addition to non-random expressions, some TCR Vβ chains are preferentially used in T CD4+ cells (i.e. Vβ5.1, 6.7, 8, 9 and 12), others in CD8+ cells, such as Vβ1, Vβ5.2, Vβ9, Vβ14 and Vβ23 (36–41). In our control group, Vβ3, 14, 16, 20 and 21.3 dominated the repertoire for both T subsets and together, as these chains answered for about 43 and 40% of the circulating T CD4+ and CD8+ cells, respectively. Meanwhile, the Vβ4, 11, 18 and 22 were under-utilized among T repertoires from our healthy individuals. Indeed, different frequencies of expression were also observed for Vβ5.1 and Vβ12, with higher usage on T CD4+ cells and Vβ23, with higher usage on T CD8+ subpopulation.

Our results differed from those described in the literature and did not correlate with data supplied by Beckman-Coulter in the kit, which related Vβ2, 5.1 and 17 as the more expressed chains and Vβ16 and 23, as those less used. Of note, Vβ16 was frequently observed in our control group, but it was under-represented in the reference data. Moreover, the frequency of T cell expansion in healthy individuals appears to be similar in genetically distinct populations such as Scandinavian and Japanese (42). In concordance with this observation is the TCR Vβ gene usage in peripheral blood T cells from healthy Turkish blood donors (43). These studies concluded that the TCR Vβ repertoires are shaped without genetic influence. However, our data are not in accordance with such observation as we identified differences in TCR Vβ distribution in our Brazilian study group. Akolkar *et al*. (44) documented the influence of HLA genes on TCR V segment frequencies and expression levels in peripheral blood T lymphocytes, but this same group also proposed non-HLA genes and environmental effects in determining the TCR repertoire in healthy individuals (45, 46). Our results suggest that genetic factors, maybe different from HLA genes or non-polymorphic HLA determinants and environment elements, like peripheral antigen-driven selection are responsible for TCR Vβ repertoire distribution.

When the whole Vβ repertoire distribution was compared between HIV-infected patients and control group individuals, no differences in the pattern of Vβ utilization was observe for any of the T lymphocyte subsets, as also reported by Mion *et al*. (47). This data confirmed the non-random pattern of TCR mobilization observed in our studied population. However, an exception was detected for Vβ16 on T CD8+ cells, for which an apparent reduction of expression were detected among the patients and may be the result of an in progress clonal exhaustion process in these immunodepressed individuals with ongoing aids disease. This phenomenon was already described as occurring during the acute phase of HIV-infection (48). Individual analysis reported skewed TCR Vβ utilizations, with a more frequent "oligoclonal-like" profile distribution. The "polyclonal-like" distribution was observed for two patients, on T CD4+ or CD8+ cells, while a "monoclonal-like" was detected on T CD8+ subset form only one patient. Skewed Vβ repertoires are well documented among HIV-infected individuals (47, 49) and the mono-oligoclonal mobilization during the acute phase of HIV infection has been associated with a bad prognosis to aids progression (11, 12). Therefore, the TCR Vβ repertoire aspects observed here are in accordance with the immunodeficiency status of the patients.

As already discussed, antiretroviral therapy is able to restore the number of peripheral blood T CD4+ and T CD8+ cells to at least near-normal levels, but it remains uncertain whether these therapies will result in restoration of the immune system once severely damaged. Our

results demonstrate that significant changes on TCR Vβ mobilization has occurred in 7 out of 10 studied patients after 24 weeks on HAART. Although no specific pattern of Vβ mobilization could be detected in the circulating CD4+ or CD8+ T cells, the oligoclonal TCR Vβ repertoire distribution was commonly identified. Soudeyns *et al*. (27) showed that HAART leads to a progressive stabilization of the Vβ profile during primary HIV-infection, reflecting the reduction of TCR Vβ repertoire oligoclonality. However, the analysis of TCR Vβ perturbations in chronically HIV-1-infected patients with moderate immunodeficiency along 36 months on HAART showed the maintenance of the oligoclonal profile (50). In fact, Cossarizza *et al*. (51) observed an improvement of the CD4+ and CD8+ T lymphocytes repertoire after antiretroviral therapy among individuals with acute HIV infection. Conversely, changes on the repertoire were only detected for T CD8+ cells in chronically severe immunosuppressed individuals, suggesting that HAART could restore the T cell repertoire in HIV positive individuals whose immune system were not severely compromised. Although oligoclonal profiles were also observed on our studied group, Vβ chains other than those initially mobilized were evolved, suggesting that other important antigens are being focused by these cells, after control of viral replication and immune activation has been achieved upon antiretroviral therapy. This could reflect the proliferation of preexisting cells in the T cell reconstitution processes of these individuals, depending on their immune status during chronic infection, previous to HAART. However, the clonal nature of T cell expansions or reductions was not investigated and no data about the CDR3 region was obtained, thus not permitting us to deduce the T cell source. Furthermore, Pantaleo *et al*. (12) analyzed the Vβ repertoire longitudinally, during primary HIV infection and during and after transition to the chronic phase, describing three major patterns of Vβ perturbations: alteration of a single Vβ member (type 1), alteration of two chains (type 2) or no detectable alterations (type 3). This study observed that type 1 individuals progressed to aids one year after seroconversion, while type 2 presented significantly higher T CD4+ counts than type 1, and type 3 individuals presented T CD4+ counts above 500 cells/mm^3, at the same time period. So, a close relationship was established between rates of disease progression and patters of Vβ perturbations. After HAART, almost all of our patients presented alterations involving multiple Vβ chains or no detectable alterations, for T CD4+ and CD8+ cells, what may also suggest better prognosis for T cell immune response reconstitution, although oligoclonal TCR Vβ repertoire distributions were dominant.

ACKNOWLEDGMENTS

We thank Vera Bongertz (Laboratory of AIDS and Molecular Immunology, FIOCRUZ, Rio de Janeiro, Brazil) for reviewing this paper. This project was supported by the FIOCRUZ Integrated AIDS Program/Brazilian Ministry of Health. Carmem Beatriz Wagner Giacoia-Gripp is personally supported by a grant from the Brazilian Research Council (CNPq).

REFERENCES

1. Soudeyns H, Pantaleo G: The moving target: mechanisms of HIV persistence during primary infection. Immunol Today 20:446–450, 1999
2. Graziosi C, Soudeyns H, Rizzardi GP, Bart PA, Chapuis A, Pantaleo G: Immunopathogenesis of HIV infection. AIDS Res Hum Retroviruses Suppl 2:S135–S142, 1998
3. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD: Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 68:4650–4655, 1994
4. Moore JP, Cao Y, Ho DD, Koup RA: Development of the anti-gp120 antibody response during seroconversion to human immunodeficiency virus type 1. J Virol 68:5142–5155, 1994
5. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC: Brief report: Absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. New Engl J Med 26(332):228–232, 1995
6. Pantaleo G, Menzo S, Vaccarezza M, Graziosi C, Cohen OJ, Demarest JF, Montefiori D, Orenstein JM, Fox C, Schrager LK, Margolick JB, Buchbinder S, Giorgi JV, Fauci AS: Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. New Engl J Med 26(332):209–216, 1995
7. Haynes BF, Pantaleo G, Fauci AS: Toward an understanding of the correlates of protective immunity to HIV infection. Science 271:324–328, 1996
8. Pantaleo G, Fauci AS: New concepts in the immunopathogenesis of HIV infection. Annu Rev Immunol 13:487–512, 1995
9. Propato A, Schiaffella E, Vicenzi E, Francavilla V, Baloni L, Paroli M, Finocchi L, Tanigaki N, Ghezzi S, Ferrara R, Chesnut R, Livingston B, Sette A, Paganelli R, Aiuti F, Poli G, Barnaba V: Spreading of HIV-specific CD8+ T-cell repertoire in long-term nonprogressors and its role in the control of viral load and disease activity. Hum Immunol 62:561–576, 2001
10. Bodman-Smith MD, Williams I, Johnstone R, Boylston A, Lydyard PM, Zumla A. T cell receptor usage in patients with non-progressing HIV infection. Clin Exp Immunol 130:115–120, 2002
11. Pantaleo G, Demarest JF, Soudeyns H, Graziosi C, Denis F, Adelsberger JW, Borrow P, Saag MS, Shaw GM, Sekaly RP, Fauci AS: Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV. Nature 370:463–467, 1994
12. Pantaleo G, Demarest JF, Schacker T, Vaccarezza M, Cohen OJ, Daucher M, Graziosi C, Schnittman SS, Quinn TC, Shaw GM, Perrin L, Tambussi G, Lazzarin A, Sekaly RP, Soudeyns H, Corey L, Fauci AS: The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression

independent of the initial level of plasma viremia. Proc Natl Acad Sci USA 94:254–258, 1997
13. Chen ZW, Shen L, Regan JD, Kou Z, Ghim SH, Letvin NL: The T cell receptor gene usage by simian immunodeficiency virus gag-specific cytotoxic T lymphocytes in rhesus monkeys. J Immunol 156:1469–1475, 1996
14. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD: Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. New Engl J Med 338:853–860, 1998
15. Valdez H, Chowdhry TK, Asaad R, Woolley IJ, Davis T, Davidson R, Beinker N, Gripshover BM, Salata RA, McComsey G, Weissman SB, Lederman MM: Changing spectrum of mortality due to human immunodeficiency virus: Analysis of 260 deaths during 1995–1999. Clin Infect Dis 32:1487–1493, 2001
16. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, Jones M, Facey K, Whitacre C, McAuliffe VJ, Friedman HM, Merigan TC, Reichman RC, Hooper C, Corey L: Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. New Engl J Med 334:1011–1017, 1996
17. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, Katlama C, Debre P, Leibowitch J: Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 277:112–116, 1997
18. Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, Richman DD, Valentine FT, Jonas L, Meibohm A, Emini EA, Chodakewitz JA: Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. New Engl J Med 337:734–739, 1997
19. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, Eron JJ Jr, Feinberg JE, Balfour HH Jr, Deyton LR, Chodakewitz JA, Fischl MA: A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. New Engl J Med 337:725–733, 1997
20. Brodt HR, Kamps BS, Gute P, Knupp B, Staszewski S, Helm EB: Changing incidence of AIDS-defining illnesses in the era of antiretroviral combination therapy. AIDS 11:1731–1738, 1997
21. Egger M, Hirschel B, Francioli P, Sudre P, Wirz M, Flepp M, Rickenbach M, Malinverni R, Vernazza P, Battegay M: Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: Prospective multicentre study. Swiss HIV Cohort Study. BMJ 315:1194–1199, 1997
22. Ledergerber B, Telenti A, Egger M: Risk of HIV related Kaposi's sarcoma and non-Hodgkin's lymphoma with potent antiretroviral therapy: Prospective cohort study. Swiss HIV Cohort Study. BMJ 319:23–24, 1999
23. Pakker NG, Notermans DW, de Boer RJ, Roos MT, de Wolf F, Hill A, Leonard JM, Danner SA, Miedema F, Schellekens PT: Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: A composite of redistribution and proliferation. Nat Med 4:208–214, 1998
24. Bisset LR, Cone RW, Huber W, Battegay M, Vernazza PL, Weber R, Grob PJ, Opravil M: Highly active antiretroviral therapy during early HIV infection reverses T-cell activation and maturation abnormalities. Swiss HIV Cohort Study. AIDS 12:2115–2123, 1998
25. Bouscarat F, Levacher M, Landman R, Muffat-Joly M, Girard PM, Saimot AG, Brun-Vezinet F, Sinet M: Changes in blood CD8+ lymphocyte activation status and plasma HIV RNA levels during antiretroviral therapy. AIDS 12:1267–1273, 1998
26. Connick E, Lederman MM, Kotzin BL, Spritzler J, Kuritzkes DR, St Clair M, Sevin AD, Fox L, Chiozzi MH, Leonard JM, Rousseau F, D'Arc Roe J, Martinez A, Kessler H, Landay A: Immune reconstitution in the first year of potent antiretroviral therapy and its relationship to virologic response. J Infect Dis 181:358–363, 2000
27. Soudeyns H, Campi G, Rizzardi GP, Lenge C, Demarest JF, Tambussi G, Lazzarin A, Kaufmann D, Casorati G, Corey L, Pantaleo G: Initiation of antiretroviral therapy during primary HIV-1 infection induces rapid stabilization of the T-cell receptor beta chain repertoire and reduces the level of T-cell oligoclonality. Blood 95:1743–1751, 2000
28. Plana M, Garcia F, Gallart T, Miro JM, Gatell JM: Lack of T-cell proliferative response to HIV-1 antigens after 1 year of highly active antiretroviral treatment in early HIV-1 disease. Immunology Study Group of Spanish EARTH-1 Study. Lancet 352:1194–1195, 1998
29. Pontesilli O, Kerkhof-Garde S, Notermans DW, Foudraine NA, Roos MT, Klein MR, Danner SA, Lange JM, Miedema F: Functional T cell reconstitution and human immunodeficiency virus-1-specific cell-mediated immunity during highly active antiretroviral therapy. J Infect Dis 180:76–86, 1999
30. Al-Harthi L, Siegel J, Spritzler J, Pottage J, Agnoli M, Landay A: Maximum suppression of HIV replication leads to the restoration of HIV-specific responses in early HIV disease. AIDS 14:761–770, 2000
31. Haslett PA, Nixon DF, Shen Z, Larsson M, Cox WI, Manandhar R, Donahoe SM, Kaplan G: Strong human immunodeficiency virus (HIV)-specific CD4+ T cell responses in a cohort of chronically infected patients are associated with interruptions in anti-HIV chemotherapy. J Infect Dis 181:1264–1272, 2000
32. Ruiz L, Martinez-Picado J, Romeu J, Paredes R, Zayat MK, Marfil S, Negredo E, Sirera G, Tural C, Clotet B: Structured treatment interruption in chronically HIV-1 infected patients after long-term viral suppression. AIDS 14:397–403, 2000
33. MacIsaac C, Curtis N, Cade J, Visvanathan K: Rapid analysis of the Vbeta repertoire of CD4 and CD8 T lymphocytes in whole blood. J Immunol Methods 283:9–15, 2003
34. Choi YW, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J: Interaction of Staphylococcus aureus toxin "superantigens" with human T cells. Proc Natl Acad Sci USA 86:8941–8945, 1989
35. Malhotra U, Spielman R, Concannon P. Variability in T cell receptor V beta gene usage in human peripheral blood lymphocytes. Studies of identical twins, siblings, and insulin-dependent diabetes mellitus patients. J Immunol 149:1802–1808, 1992
36. Cossarizza A, Kahan M, Ortolani C, Franceschi C, Londei M: Preferential expression of V beta 6.7 domain on human peripheral CD4+ T cells. Implication for positive selection of T cells in man. Eur J Immunol 21:1571–1574, 1991
37. Grunewald J, Janson CH, Wigzell H: Biased expression of individual T cell receptor V gene segments in CD4+ and CD8+ human peripheral blood T lymphocytes. Eur J Immunol 21:819–822, 1991
38. Clarke GR, Humphrey CA, Lancaster FC, Boylston AW: The human T cell antigen receptor repertoire: Skewed use of V beta gene families by CD8+ T cells. Clin Exp Immunol 96:364–369, 1994
39. Muraro PA, Jacobsen M, Necker A, Nagle JW, Gaber R, Sommer N, Oertel WH, Martin R, Hemmer B: Rapid identification of local T cell expansion in inflammatory organ diseases by flow cytometric T cell receptor Vbeta analysis. J Immunol Methods 246:131–143, 2000

40. van den Beemd R, Boor PP, van Lochem EG, Hop WC, Langerak AW, Wolvers-Tettero IL, Hooijkaas H, van Dongen JJ: Flow cytometric analysis of the Vbeta repertoire in healthy controls. Cytometry 40:336–345, 2000
41. Melenhorst JJ, Eniafe R, Follmann D, Nakamura R, Kirby M, Barrett AJ: Molecular and flow cytometric characterization of the CD4 and CD8 T-cell repertoire in patients with myelodysplastic syndrome. Br J Haematol 119:97–105, 2002
42. Shigematsu M, Nagai S, Mikuniya T, Izumi T, Wigzell H, Eklund AG, Grunewald J: T cell receptor (TCR) V gene segment use in HLA-typed Japanese healthy subjects. Clin Exp Immunol 103:149–154, 1996
43. Esin S, Gul A, Hodara V, Jeddi-Tehrani M, Dilsen N, Konice M, Andersson R, Wigzell H: Peripheral blood T cell expansions in patients with Behcet's disease. Clin Exp Immunol 107:520–527, 1997
44. Akolkar PN, Gulwani-Akolkar B, Pergolizzi R, Bigler RD, Silver J: Influence of HLA genes on T cell receptor V segment frequencies and expression levels in peripheral blood lymphocytes. J Immunol 150:2761–2773, 1993
45. Akolkar PN, Gulwani-Akolkar B, Robinson MA, Silver J: The influence of non-HLA genes on the human T-cell receptor repertoire. Scand J Immunol 42:248–256, 1995
46. Silver J, Gulwani-Akolkar B, Akolkar PN: The influence of genetics, environment, and disease state on the human T-cell receptor repertoire. Ann N Y Acad Sci 756:28–52, 1995
47. Mion M, Indraccolo S, Feroli F, Minuzzo S, Masiero S, Zamarchi R, Barelli A, Borri A, Chieco-Bianchi L, Amadori A: TCR expression and clonality analysis in peripheral blood and lymph nodes of HIV-infected patients. Hum Immunol 57:93–103, 1997
48. Pantaleo G, Soudeyns H, Demarest JF, Vaccarezza M, Graziosi C, Paolucci S, Daucher MB, Cohen OJ, Denis F, Biddison WE, Sekaly RP, Fauci AS: Evidence for rapid disappearance of initially expanded HIV-specific CD8+ T cell clones during primary HIV infection. Proc Natl Acad Sci USA 94:9848–9853, 1997
49. Soudeyns H, Rebai N, Pantaleo GP, Ciurli C, Boghossian T, Sekaly RP, Fauci AS: The T cell receptor V beta repertoire in HIV-1 infection and disease. Semin Immunol 5:175–185, 1993
50. Giovannetti A, Pierdominici M, Marziali M, Mazzetta F, Caprini E, Russo G, Bugarini R, Bernardi ML, Mezzaroma I, Aiuti F: Persistently biased T-cell receptor repertoires in HIV-1-infected combination antiretroviral therapy-treated patients despite sustained suppression of viral replication. J Acquir Immune Defic Syndr 34:140–154, 2003
51. Cossarizza A, Poccia F, Agrati C, D'Offizi G, Bugarini R, Pinti M, Borghi V, Mussini C, Esposito R, Ippolito G, Narciso P: Highly active antiretroviral therapy restores CD4+ Vbeta T-cell repertoire in patients with primary acute HIV infection but not in treatment-naive HIV+ patients with severe chronic infection. J Acquir Immune Defic Syndr 35:213–222, 2004