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IMMUNE RECONSTITUTION IN HIV-1 INFECTED PATIENTS TREATED FOR TWO YEARS WITH HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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ABSTRACT: The aim of this paper was to evaluate the immune reconstitution of HIV-1 patients subjected to highly active antiretroviral therapy (HAART) for two years or more according to $CD_{45}RA$ and $CD_{45}RO$ cell count; determination of IL-2, IFN-γ, IL-4, IL-10 and TNF-α serum levels; CD_4^+ T and CD_8^+ T lymphocyte count; and plasma viral load (VL) determination. For this purpose, a cross sectional study was carried out in the Tropical Diseases Area, Botucatu School of Medicine, São Paulo State University, UNESP, Botucatu, São Paulo, Brazil. Between June 2001 and April 2002, 37 HIV-1 infected patients were evaluated, 13 with treatment indication but untreated (G1), 9 subjected to HAART for 5-7 months (G2), and 15 treated for two years or more (G3); both treated groups used medication regularly and without failure. Forty-nine normal individuals were studied as controls (GC-1 and GC-2). There was a tendency ($p<0.10$) for the predominance of two nucleoside reverse transcriptase inhibitors (NRTI) associated with one non-nucleoside reverse transcriptase inhibitor (NNRTI) regimen in G2; and two NRTI associated with a protease inhibitor (PI) in G3. Statistical differences between groups were seen for $CD_{45}RA$ (G1<[G3=GC-2]; $p<0.05$) and $CD_{45}RO$ (G1<GC-

2<G3; p<0.01) cells, and CD_4^+ T lymphocyte count (G1<G3; G2-intermediate; p<0.05), VL determination (G1>[G2=G3]; p<0.001), TNF-α serum determination ([G1>G3; G2=intermediate]>GC-1; p<0.001), IL-2 (G1<[G2=G3=GC-1]; p<0.01), IFN-γ ([G1=GC-1]<[GC-2=G3]; p<0.001), IL-4 and IL-10 ([G1=G2=G3]>GC-1; p<0.001), serum cytokine profiles, with a higher proportion of subtype 2 in G1 and mature subtype 0 in G2 and G3 (p<0.005). There was no statistical difference for CD_8^+ T lymphocyte counts (G1=G2=G3; p<0.50). Consistency was seen between positive correlations of profile 1 definer cytokines (IL-2 and IFN-γ), $CD_{45}RA$ and $CD_{45}RO$ cells, and CD_4^+ T lymphocyte counts and between positive correlations of profile 2 definer cytokines (IL-4 and IL-10) with TNF-α, and VL. The negative correlations were also consistent as they expressed the inverse of the positives. The variables with the highest number of correlations were IL-2, IFN-γ, and VL, followed by $CD_{45}RA$ and $CD_{45}RO$ cells, and IL-10. The variables with the lowest number of correlations were CD_4^+ T and CD_8^+ T lymphocytes. The results express the partial but important immune reconstitution in HIV-1 infected individuals with the interference of HAART and the importance of cytokines especially IL-2 and IFN-γ, and $CD_{45}RA$ and $CD_{45}RO$ cells as surrogate markers of this reconstitution.

KEY WORDS: HIV, immune reconstitution, antiretroviral, HAART, cytokines, $CD_{45}RA$, $CD_{45}RO$.

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INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) was a major milestone in the attempt to recuperate HIV-1 infected individuals' immune systems. Despite this recuperation being slow, partial, and variable, the suppression of HIV-1 replication has permitted a higher level of control over associated diseases with a consequent decrease in mortality and improvement in quality of life. (10)

The main surrogate markers of natural history and therapeutic efficacy currently used in HIV infected patient follow-up are clinical condition, CD_4^+ T lymphocyte count, and plasma viral load (VL) determination (21, 23). However, after VL drops below detectable levels, it does not allow us to consistently express the behavior of immune response reconstitution. Also, CD_4^+ T lymphocyte count can show irregular behavior without significant elevation even in individuals with major clinical improvement (4) and not demonstrate whether this elevation expresses the real immune system reconstitution through the production of naive cells, which have $CD_{45}RA$ surface molecules, or just the recirculation of memory cells, also known as $CD_{45}RO$ (10, 13, 20). In addition, $CD4^+$ T lymphocyte count does not characterize the quality of the immunomodulatory substances they produce.

Other indices might be useful for a better evaluation of these patients' immune reconstitution, considering both qualitative and quantitative aspects. From these we can highlight serum cytokine profile (1, 5, 6, 7, 9, 17, 22, 26, 28) and $CD_{45}RA$ and $CD_{45}RO$ cell count. (10, 13, 20)

PARTICIPANTS

Between June 2001 and April 2002, 37 HIV-1 patients on or not on HAART were studied. They were treated at the Special Outpatient Clinic and Infirmary of Tropical Diseases, Botucatu School of Medicine, UNESP. Twenty-five were male and twelve female, aged between 18 and 62 years (X=37.6 years).

There were also 49 normal individuals, controls, blood donors from the Botucatu Hemocenter. Out of these, 36 were male and 13 female, aged between 18 and 57 years (X=33.2 years).

METHODS

Groups were formed as follows: Group G1, with 13 HIV-1 infected individuals, untreated but indicated for HAART; Group G2, with nine HIV-1 infected individuals on HAART for 5 to 7 months; Group G3, with 15 HIV-1 infected individuals on HAART for more than 24 months; and GC-1 with 30 normal individuals and GC-2 with 19 normal individuals, both control groups without clinical complaints and negative for anti-HIV-1/2, HTLV-1/2, hepatitis B and C, syphilis and Chagas' disease antibodies.

Exclusion criteria were: other causes of immunosuppression such as neoplasias, transplants, treatment with immunosuppressive substances, or autoimmune diseases, those using immunostimulants, those in any stage of pregnancy, those irregularly using antiretrovirals and/or with therapeutic failure, and those who did not give informed consent.

The antiretroviral (ARV) regimens used in each group were: without treatment; two nucleoside reverse transcriptase inhibitors (NRTI) associated with one non-nucleoside reverse transcriptase inhibitor (NNRTI); and two NRTI associated with a protease inhibitor (PI).

All HIV-1 infected individuals were subjected to VL, and serum TNF-α, IFN-γ, IL-2, IL-4 and IL-10 determination; and CD_4^+ T and CD_8^+ T lymphocyte count. CD_{45}RA and CD_{45}RO cell counts were performed in six G1 and eight G3 individuals. Other routine laboratory examinations such as hemogram and blood biochemistry were also carried out.

All 30 GC-1 individuals were subjected to serum TNF-α, IFN-γ, IL-2, IL-4, and IL-10 determination; normal values were obtained by mean + 2SD (Table 1). Serum cytokine profiles were determined as per subtypes described by Spellberg & Edwards-Jr. (27) (Table 2). All 19 GC-2 individuals were subjected to CD_{45}RA and CD_{45}RO cell count.

Exams were performed by the Clinical Analysis Laboratory of Botucatu University Hospital and the Applied Immunology Laboratory of Tropical Diseases Area, both of Botucatu School of Medicine, UNESP, and the Botucatu Hemocenter.

Quantitative determination of T lymphocytes with CD_4^+ and CD_8^+ markers was performed by flow cytometry using FACSCount reagent kits (Becton Dickinson). CD_{45}RA and CD_{45}RO cell counts were performed by flow cytometry using specific CD_{45}RA

monoclonal antibody kits, clone ALB11, mouse isotype IgG1 conjugated with fluorescein isothiocyanate (FITC), and $CD_{45}RO$, clone UCHL1, mouse isotype IgG2a conjugated with phycoerythrin (PE). To obtain the absolute number of counted cells, their relative number was multiplied by the total number of lymphocytes from the hemogram. The following controls were used: mouse IgG, subclasses IgG1-FITC and IgG2a-PE, all manufactured by Beckman Coulter.

Sera aliquots were stored at -70°C for determination of TNF-α, IFN-γ, IL-2, IL-4, and IL-10 cytokines; levels were determined by ELISA using commercial kits (R&D Systems). The assay detection limit varied from 3 to 5 pg/ml, depending on the cytokine.

HIV-1 VL was determined using NucliSens HIV-1 QT (Organon Teknika). The assay detection limit was 80 copies of plasma RNA/ml.

Statistical Analysis

Group comparisons were made with the non-parametric Kruskal-Wallis test and analysis of variance for entirely randomized experiments (ANOVA-ERE). The χ^2 test was used for serum cytokine profile proportion comparisons in the three HIV-1 groups, and for PI proportion comparisons in the two HIV-1 groups under treatment. When the proportion was zero, Yates correction was applied. For the χ^2 test, statistical significance was when $p<0.05$ and the value equal or higher than 3.84 for one degree of freedom, and equal or higher than 5.99 for two degrees of freedom. In all other analyses, statistical significance was $p<0.05$. For the correlations between pairs of variables, Spearman coefficient of rank correlation was calculated with significance $\alpha=0.05$ and $\alpha=0.01$, (30).

This study was approved by the Research Ethics Committee of Botucatu School of Medicine - UNESP.

RESULTS

Table 3 shows groups distribution according to sex and age. Males predominated in all groups, except G1. Mean age between groups was from 31 to 41 years. The highest mean age in HIV-1 infected groups was in G3. The mean age of HIV-1 individuals was

slightly higher than that of controls. The prevailing mode of transmission in all infected groups was heterosexual.

Groups were not homogeneous to ARV treatment regimens as they were grouped using this parameter. G1 patients had not yet been treated; six G2 patients received two NRTI associated with one NNRTI, and three G2 patients, two NRTI associated with one PI; 11 G3 patients received two NRTI associated with one PI, and four G3 received two NRTI and one NNRTI. Although there was no significant difference between groups under treatment, PI tended to predominate in G3 (χ^2_1=3.70; p<0.10).

At exams collection, G1 had the highest number of individuals (six) with AIDS defining diseases; there were also 2 oligosymptomatic and 5 asymptomatic patients. In G2, asymptomatic was predominant; there were no oligosymptomatic, and only one individual with AIDS, who showed paradoxical reaction to tuberculosis. All G3 were asymptomatic.

Analyzing data from Table 4, statistical difference was seen between G1, and G3 and GC-2 for $CD_{45}RA$ cell count, with patients in G1 showing lower counts than those in G3 and GC-2 which were similar, and between G1, G3, and GC-2 for $CD_{45}RO$ cell count, with G1 showing lower counts than GC-2, and GC-2 patients showing lower counts than G3.

Table 4 shows patient characterization in relation to serum cytokine level determination. There was a progressive TNF-α serum level decrease from G1 to G3, and all showed higher TNF-α than GC-1. Statistical difference was seen between G1 and G2, G3, and GC-1 for IL-2, with serum levels in G1 being less elevated than in groups G2, G3, and GC-1, which had no statistical difference between them. There was no difference in IFN-γ serum levels between G1 and GC-1, but they were less elevated than in G2 and G3, which were not statistically different either. There was no statistical difference between G1, G2 and G3 for IL-10 and IL-4 serum levels, however these were higher than in GC-1 individuals. Statistical difference was seen for serum cytokine profile with predominance of profile 2 in G1, and pronounced predominance of mature profile 0 in G2 and G3 (χ^2_2 =13.65; p<0.005). In G3, only mature profile 0 was seen. No patient in the HIV-1 groups showed profile 1.

According to data in Table 4, there was statistical difference between groups for CD_4^+ T lymphocyte count with progressive increase from G1 to G3; this did not occur with CD_8^+ T lymphocyte count.

In G1, all individuals had detectable VL and in G2 there were seven undetectable and two detectable VL patients; in G3 there were nine undetectable and six were greater than 80 copies/ml; statistical difference was seen between groups, with G1 being statistically greater than both G2 and G3 which had no difference between them (Table 4).

Table 5 shows a linear correlation between the pairs of variables studied. There was a significantly positive correlation (α=0.05) between TNF-α and IL-4; IL-2 and CD_4^+ T lymphocytes; IFN-γ and CD_{45}RA cells; IFN-γ and CD_{45}RO cells; IL-10 and VL; and CD_8^+ T lymphocytes and CD_{45}RA cells. There was a significantly strong positive correlation (α=0.01) between TNF-α and IL-10; TNF-α and VL; IL-2 and IFN-γ; IL-2 and CD_{45}RA cells; IL-10 and IL-4; CD_4^+ T lymphocytes and CD_{45}RA cells; CD_4^+ T lymphocytes and CD_{45}RO cells; and CD_{45}RA and CD_{45}RO cells. Analysis of the table also shows that there was a significantly negative correlation (α=0.05) between IL-10 and CD_{45}RA cells; IL-4 and CD_{45}RO cells; VL and CD_{45}RA cells; and VL and CD_{45}RO cells. A significantly strong negative correlation was seen (α=0.01) between TNF-α and IL-2; TNF-α and IFN-γ; IL-2 and IL-10; IL-2 and IL-4; IL-2 and VL; IFN-γ and IL-10; IFN-γ and IL-4; IFN-γ and CV; and CD_4^+ T lymphocytes and VL.

The highest number of significant correlations was for IL-2, IFN-γ, and VL, with seven correlations each; this was followed by IL-10, CD_{45}RA and CD_{45}RO cells with six, TNF-α and IL-4 with five, CD_4^+ T lymphocytes with four, and CD_8^+ T with only one significant correlation.

Table 1: Normal values of TNF-α, IFN-γ, IL-2, IL-4, and IL-10 serum cytokines from control group GC-1.

Cytokines	TNF-α (pg/ml)	IL-2 (pg/ml)	IFN-γ (pg/ml)	IL-10 (pg/ml)	IL-4 (pg/ml)
Mean	96	110	186	5	7
Median	98	107	206.5	0	7
Standard Deviation	28.111	33.194	56.654	7.146	3.856
Variation	49-143	63-183	97-310	0-27	0-15
Normal Values (Mean + 2 SD)	152	176	300	19	14

Table 2: Serum cytokine profiles according to subtypes by Spellberg & Edwards-Jr. (27).

Profile	IL-2 (pg/ml)	IFN-γ (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
1	> 176	> 300	< 14	<19
Mature 0	*	> 300	> 14	*
2	< 176	< 300	> 14	> 19

* Normal, low or high.

Table 3: Group distributions according to sex and age.

Characteristics	G1	G2	G3	GC-1	GC-2
Sex (M/F)*	6/7	7/2	12/3	21/9	15/4
Age (years)					
Mean	33.2	39	40.7	35.3	31.0
Variation	22-53	25-60	18-62	21-57	18-46

G1: HIV-1 infected individuals before the start of HAART.

G2: HIV-1 infected individuals under HAART for between five and seven months.

G3: HIV-1 infected individuals under HAART for more than 24 months.

GC-1 & GC-2: Normal blood donors from the Botucatu Hemocenter, without clinical problems, negative for anti-HIV-1/2, HTLV-1/ 2, hepatitis B & C, syphilis, and Chagas' disease antibodies.

* M - Male F - Female

Table 4: Distribution of the 37 HIV-1 infected individuals and 49 normal controls by group, according to TNF-α, IFN-γ, IL-2, IL-4, and IL-10 serum cytokines, cytokine profile, $CD_{45}RA$ and $CD_{45}RO$ cell count, CD_4^+ T and CD_8^+ T lymphocyte count, and plasma viral load (VL).

Group		TNF-α (pg/ml)	IL-2 (pg/ml)	IFN-γ (pg/ml)	IL-10 (pg/ml)	IL-4 (pg/ml)	Cytokine Profiles	CD_{45} RA (cel/mm^3)	CD_{45} RO (cel/mm^3)	CD_4^+ (cel/mm^3)	CD_8^+ (cel/mm^3)	VL Copies/ml
G1	X	616	69	288	61	51	2	375	401	152	809	200769
	Md	617	56	217	66	54		410	498	113	790	200000
	SD	143.598	23.558	185.614	12.809	12.303		202.002	209.216	121.691	443.787	177586.202
	Variation	417-826	46-127	94-714	38-76	29-71		50-549	110-576	4-382	273-1793	12000-580000
G2	X	452	99	522	41	44	Mature 0	———	———	275	803	1477
	Md	310	98	540	29	46				226	891	U*
	SD	338.9	25.849	212.683	21.638	14.330				191.870	334.132	3952.101
	Variation	127-1040	63-146	240-840	21-81	31-78				108-592	281-1299	U*-12000
G3	X	363	112	676	41	39	Mature 0	954	916	363	902	1612
	Md	327	98	626	32	41		733	767	314	843	U*
	SD	211.246	35.447	224.402	21.983	10.192		555.629	332.099	204.423	388.824	3358.901
	Variation	106-741	48-176	341-1061	18-96	26-56		472-2045	622-1449	59-780	263-1690	U*-13000
GC1/2	X	96	110	186	5	7	Normal	996	732	———	———	———
	Md	98	107	207	0	7		927	668			
	SD	28.111	33.194	56.654	7.146	3.856		338.946	213.128			
	Variation	49-143	63-183	97-310	0-27	0-15		587-1705	484-1412			
Statistics		H=47.785; p<0.001	H=16.091; p<0.01	H=41.532; p<0.001	H=51.634; p<0.001	H=51.232; p<0.001	$\chi^2_{2=}$13.65 p<0.005	F=6.19; p<0.05	F=7.64; p<0.01	H=8.786; p<0.05	H=0.521; p<0.50	H=26.030; p<0.001
Comments		(G1>G3; G2=I†) > GC1	G1< (G2=G3=GC1)	(G1=GC1) < (G2=G3)	(G1= 2=G3) > GC1	(G1=G2=G3) > GC1	(G2=G3) > G1	G1< (G3=GC2)	G1< GC2 < G3	G1< G3; G2=I†	G1=G2=G3	G1>(G2=G3)

G1: HIV-1 infected individuals before the start of HAART.

G2: HIV-1 infected individuals under HAART for between five and seven months.

G3: HIV-1 infected individuals under HAART for more than 24 months.

GC-1 & GC-2: Normal blood donors from the Botucatu Hemocenter, without clinical problems, negative for anti-HIV-1/2, HTLV-1/ 2, hepatitis B & C, syphilis, and Chagas' disease antibodies.

* U = Below detection limit (< 80 copies/ml). † I = Intermediate.

Table 5: Linear correlation between pairs of variables: TNF-α, IL-2, IFN-γ, IL-10, IL-4, CD_4^+ T and CD_8^+ T lymphocytes, plasma viral load (VL), and CD_{45}RA and CD_{45}RO cell count for six G1, three G2, and eight G3 individuals, all with HIV-1, sick or not.

Correlation of pairs of variables	IL-2	IFN-γ	IL-10	IL-4	CD_4^+	CD_8^+	VL	CD_{45}RA	CD_{45}RO
TNF-α	-0.76**	-0.79**	0.87**	0.67*	-0.33	-0.23	0.60**	-0.41	-0.36
IL-2	-	0.73**	-0.67**	-0.64**	0.55*	0.33	-0.58**	0.37	0.60**
IFN-γ	-	-	-0.74**	-0.62**	0.44	0.19	-0.62**	0.53*	0.56*
IL-10	-	-	-	0.72**	-0.31	-0.08	0.55*	-0.48*	-0.37
IL-4	-	-	-	-	-0.08	-0.09	0.33	-0.40	-0.46*
CD_4^+	-	-	-	-	-	0.43	-0.65**	0.63**	0.59**
CD_8^+	-	-	-	-	-	-	-0.41	0.52*	0.40
VL	-	-	-	-	-	-	-	-0.49*	-0.50*
CD_{45} RA	-	-	-	-	-	-	-	-	0.67**

Critical r values:

α=0.05 => r=0.46 $^{(*)}$

α=0.01 => r=0.57 $^{(**)}$

DISCUSSION

For a better evaluation of the qualitative aspects of the immune system behavior in patients on HAART, this paper used three other markers including serum cytokine determination and $CD_{45}RA$ and $CD_{45}RO$ cell count.

In relation to patient homogeneity, the sex behavior, age and transmission mechanism are in agreement with the most recent epidemiological condition of HIV-1 infected individuals in Brazil (18).

Predominance of infected individuals with AIDS defining diseases among untreated patients is strong evidence for the benefit of the treatment (11).

$CD_{45}RA$ cell count increased significantly with the treatment in patients treated for at least 2 years, with predominance of PI; levels reached the same values as in control group individuals, which did not occur with CD_4^+ cells. According to literature, as treatment goes on, there is a progressive increase in the number of naive CD_4^+ T lymphocytes (10). These findings suggest that $CD_{45}RA$ was more significant than CD_4^+ and VL in the evaluation of immune reconstitution. They perhaps suggest the partial preserved capacity of thymus regeneration (4,10). There was a significant increase in $CD_{45}RO$ cells in this study for patients treated for over two years. It should be mentioned that the mean of values from this group was higher than that from controls. This is in agreement with Mezzaroma *et al.* (20), Haase (10), and Powderly *et al*. (24). This count, similar to that of $CD_{45}RA$, was more sensitive than CD_4^+ count and VL values as indicators of immune reconstitution of patients on HAART. Another result of this study, which in a way reinforces these statements, was the strongly positive correlation between CD_4^+ T lymphocytes and $CD_{45}RA$ and $CD_{45}RO$ cells, at the same time that there was a weak negative correlation between both $CD_{45}RA$ and $CD_{45}RO$ and VL.

There were few references in literature where serum cytokines (2) have been determined as surrogate markers for HAART. Meira *et al.* (19) showed the importance of serum cytokine determination in HIV-1 infected patients classified into groups according to CD_4^+ T lymphocyte count. These authors (19) emphasized the use of such markers, together with $CD_{45}RA$ and $CD_{45}RO$ cell counts, as qualitative parameters in the evaluation of immune reconstitution of patients on HAART.

Serum TNF-α values decreased as treatment went on with lower levels in the over-two-years-treatment group, with PI predominance. This decrease, even though at levels about four times higher than in controls, seems to suggest a compatibility with clinical improvement since all patients in this group were asymptomatic. Ledru *et al.* (15) have reported that the decrease in TNF-α levels is compatible with the decrease of viral replication and apoptosis once this cytokine induces these phenomena (1, 15). Lew *et al.* (16) have reported a decrease in the number of T cells producers of TNF-α in the early weeks of HAART. Kaufmann *et al.* (13) showed a decrease in TNF-α secretion with the same treatment. Aukrust *et al.* (2) have reported data agreeing with this study, showing that plasma and *in vitro* TNF-α levels fall with treatment but not to normal levels. These authors (2) associate the persistent activation of TNF-α system components to therapeutic, virological, and immunological failure.

Serum IL-2 increased in the two under-treatment groups as treatment went on, reaching levels statistically equal to those of normal individuals. In absolute values, the G3 mean was higher than that of control group. This increase indicates a recuperation of the infected individual's immune system. Kaufmann *et al.* (13) report an increase in IL-2 secretion and Imami *et al.* (12), an elevation in specific RNA expression by RT-PCR in the first weeks of treatment. Weiss *et al.* (29) found increased levels of CD_4^+ T cells, producers of IL-2; in most studied patients, IL-2 production capacity under stimulation was similar to that of seronegative control individuals.

At pretreatment phase, IFN-γ levels were statistically equal to those of normal individuals, increasing significantly with treatment, which is in agreement with Imami *et al*. (12). This increase may be explained by the maintenance of large quantities of CD_8^+ T lymphocytes, which are the main IFN-γ producers. (8)

IL-4 and IL-10 were not statistically different between infected groups as treatment went on, but were always higher than in controls. However, in absolute values, in the pretreatment group, they were higher than in the other infected groups; this is in agreement with Imami *et al*. (12).

Characterization of serum cytokine profiles in patients from the three study groups showed none with profile 1 (Th-1). Most studied patients were considered as having mature 0 profile (Th-0), or 2 (Th-2). Meira *et al.* (19) called attention to the

predominance of Th-2 (53.1%) and Th-0 (38.7%) profiles in their patients. Although the study group formation was different, results agreed. Other authors (14, 25) have also reported a very small number of profile 1 patients, agreeing with this study. These authors (14, 25), however, did not mention profile 0 in their findings.

When only patients under treatment are considered, absolute serum cytokine median values show patients treated for over 2 years with higher IFN-γ and lower IL-4. These variations, although small, are important because they may be interpreted as an evolution of the mature 0 related to the duration of treatment and predominance of PI.

There was a progressive increase in CD_4^+ T lymphocyte counts in patients on HAART for over 2 years, with PI predominance; the median passed 300 cells/mm^3 by a small margin. Therefore, according to Haase (10), the increase in CD_4^+ T lymphocyte count with treatment was progressive but partial.

There was no difference in CD_8^+ T lymphocyte count behavior with treatment. This therefore did not offer any support for the evaluation of immune recuperation. Autran *et al.* (3) and Kaufmann *et al.* (13) found similar results.

HIV-1 VL showed a marked decrease with treatment duration, with some individuals remaining with VL at detectable levels (>80 copies/ml). These results agree with literature (3, 20).

The relationships of pairs of variables showed coherence of positive correlations between profile 1 defining cytokines (IL-2 and IFN-γ), $CD_{45}RA$ and $CD_{45}RO$ cells, and CD_4^+ T lymphocyte count, as well as for the association between them. These correlations also show coherence of correlations between profile 2 defining cytokines (IL-4 and IL-10) and TNF-α, and VL, as well as for the association between them. The negative correlations are also coherent as they express the reverse of positive associations. The variables with the highest number of positive or negative correlations were IL-2, IFN-γ, and VL, followed by $CD_{45}RA$ and $CD_{45}RO$ cells, and IL-10. The variables with the lowest number of correlations were CD_4^+ T and CD_8^+ T lymphocytes.

These results show the importance of cytokines especially IL-2 and IFN-γ, VL, and $CD_{45}RA$ and $CD_{45}RO$ cells as surrogate markers of HIV-1 infection with HAART interference. In literature (21), however, most authors consider CD_4^+ T lymphocyte count

and VL are the markers of natural history, biological activity, and therapeutic efficacy in HIV-1 individuals.

The best performance was in the group of patients treated for over 2 years, and the analysis of all parameters used perhaps suggests that their immune reconstitution may be attributed to longer treatment time and participation of PIs. Hence there is still the need to perform further studies with these variables with more patients and longer treatment duration.

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