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T cell receptor excision circles (TRECs), CD4+, CD8+ and their CD45RO+ and CD45RA+ subpopulations in hepatitis C virus (HCV)-HIV-co-infected patients during treatment with interferon alpha plus ribavirin: analysis in a population on effective antiretroviral therapy

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Summary

Interferon (IFN)-α induced CD4+ T lymphopenia is a toxic effect of the treatment of chronic hepatitis C virus (HCV) in human immunodeficiency virus (HIV)-co-infected patients. To increase the knowledge about this secondary effect, we performed an analysis of the evolution of the T cell receptor excision circles (TRECs), CD4+ and CD8+ T cells and of their CD45RO+ and CD45RA+ subpopulations during the treatment of chronic hepatitis HCV with peginterferon alpha (pegIFN-α) + ribavirin. Twenty HCV/HIV-co-infected patients, with undetectable HIV load after highly active antiretroviral therapy (HAART), were treated with pegIFN-α + ribavirin. TRECs were determined using real-time polymerase chain reaction. CD4+ and CD8+ T cells and their CD45RO+ and CD45RA+ subpopulations were analysed by two-colour flow cytometry. Median baseline CD4+ and CD8+ T cells were 592 mm3 and 874 mm3, respectively. Median baseline CD45RO+ subpopulation was 48% for CD4+ T and 57% for CD8+ T lymphocytes. A progressive decrease in both T cell populations, as well as of their CD45RO+ and CD45RA+ subpopulations, was detected, with a difference between the baseline and nadir levels approaching 50%. The evolution of T cell populations and TRECs was independent of the response to the treatment. T lymphocytes and their subpopulations returned to baseline levels at 24 weeks after the end of treatment, with the exception of the T CD4+ CD45RA+ subpopulation. The ratio of CD4+ CD45RO+/CD4+ CD45RA+ increased from 0·89 (baseline) to 1·44 (24 weeks after the end of the therapy). TRECs/ml did not return to the basal values. In conclusion, a significant reduction of CD4+ and CD8+ T cells, and of their CD45RA+ and CD45RO+ subpopulations, in HIV/HCV co-infected patients treated with pegIFN-α was observed. Both subpopulations increased after the suppression of treatment, but the CD4+ CD45RA subpopulation did not reach the basal levels as a consequence, at least in part, of a decrease in thymic production.

Keywords: hepatitis C infection, HIV, interferon alpha, T lymphocytes, thymic production

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Introduction

The combination of interferon (IFN)-α and ribavirin is the accepted treatment for chronic hepatitis C. IFN-α-induced CD4+ T lymphopenia is a toxic effect which is presumed to be involved in the appearance of opportunistic infections in patients with immunodeficiency [1,2]. This secondary effect imposes a risk in the treatment of chronic hepatitis C virus (HCV) in human immunodeficiency virus (HIV)-co-infected patients [3–6]. Hypothetical mechanisms of action include an inhibiting effect on different haematopoietic growth factors [7–9] that could affect lymphoid differentiation at an early stage [10], as well as changes in the cellular distribution induced by IFN [5]. Ribavirin modulates the cytokine-mediated lymphocyte response [11,12] and produces mutations in RNA virus [13], but lymphopenia is not an adverse effect attributed to this drug. However, a profound analysis of the cellular modifications induced by this combination (IFN-α + ribavirin) has not been performed.

In 1998, Douek *et al*. provided evidence for the existence of a functional thymus in adults and suggested a reduced thymic output in HIV infection [14]. Later, Nobile *et al*. [15] and Franco *et al*. [16] demonstrate that *de novo* CD4 T cell generation is preserved for a long time during the course of HIV infection and it is even increased in young HIV-1-infected patients during early-stage disease. At more advanced stages of disease and in older patients, no such increased T cell production could be observed. The determination of thymic output by quantification of T cell receptor excision circles (TRECs) has been described recently. TRECs are by-products of T cell receptor (TCR) gene rearrangements, generated during lymphocyte maturation in the thymus [14,17]. They are stable, not duplicated during mitosis, and diluted rapidly in proliferating T cell subpopulations. It has been shown that TREC levels are increased after highly active antiretroviral therapy (HAART) in adult patients [14,17], suggesting a renewed thymic function.

The CD45RA and CD45RO isoforms have been considered to be markers of different stages of lymphocyte differentiation [18,19]. CD45RA is detected on the cell membrane after thymic differentiation and prior to confrontation with the antigen (naive cells). After antigenic contact, usually in the lymphatic nodes, $CD45RA^+$ expression is lost and CD45RO is detected on the membrane of T cells (memory cells) [20,21]. Thus, in HIV/HCV co-infected patients, the serial analysis of the TRECs and CD45 isoforms after treatment with IFN-α and ribavirin could differentiate the modifications of T cells as attributable to a decrease in the thymic production or an effect of peripheral redistribution.

To gain further insight into the pathogenesis of decrease and recuperation of $CD4^+$ cell counts and the effect of anti-HCV therapy in HIV-infected patients, with undetectable HIV load after HAART, we designed a prospective study in which we analysed: (1) the dynamic of naive and memory $CD4^+$ and $CD8^+$ T cells by flow cytometric analysis; and (2) the output of $CD4^+$ and $CD8^+$ from the thymus by TREC analysis.

Patients and methods

Patients

We carried out a prospective study of 20 HIV/HCV co-infected patients in the Hospital Universitario Puerta del Mar (Cadiz, Spain). Patients were selected from those individuals attending the infectious disease unit. The inclusion criteria were those used for standard treatment of chronic HCV infection [22,23]: (1) HCV co-infection, defined as a positive serology result by a second- or third-generation enzyme-linked immunosorbent assay and the detection of HCV-RNA; (2) a maintained increase of serum aminotransferase levels for at least 6 months was required; and (3) finally, all had undergone an interpretable liver biopsy in the last 6 months. A minimum fibrosis score ≥ 1, according to the histological index proposed by Knodell and modified by Scheuer and Desmet [24], was the indication for therapy.

Exclusion criteria were the following: (1) clinical or biochemical criteria of decompensated cirrhosis; (2) positivity of hepatitis B surface antigen; (3) other infectious, autoimmune, tumoral, biliary or vascular-associated liver disease; and (4) active alcohol or drug dependence – for the purpose of this work, alcoholism was defined as an enolic ingestion greater than 50 g alcohol/day for at least 5 years; (5) a Karnofsky index < 80; (6) absolute counts of neutrophils of < 1500 cells/μl, platelets of $< 90\,000$ cells/μl or haemoglobin concentration of $< 11{\cdot}0$ g/dl; (7) poorly controlled psychiatric disease; (8) substantial co-existing medical conditions; (9) inability to use contraceptive measures, for any reason; and (10) previous IFN or ribavirin therapy.

It was assumed that the date of HIV and HCV infection was the date of the first transfusion or of the first intravenous drug use [25]. In four patients the date of acquisition of the infection could not be determined reliably (sex transmission).

All patients had been receiving HAART for at least the last 4 years and all of them had attained an HIV viral load in the undetectable (< 50 copies/ml) range. $CD4^+$ T cell count was higher than 250 μl and no active opportunistic disease was present. Indications for HAART were based on individual clinical, immunological and virological status according to the periodic *Recommendations of the International AIDS Society* [26,27]. All patients reported therapy compliance of 95% or higher.

The study protocol was approved by the Institutional Ethical Committee and all patients and controls gave their informed consent.

Study design

Patients were selected to receive treatment with pegylated IFN (pegIFN)-2α (Pegasys, Hoffman-La Roche, Nutley, NJ, USA) at a dose of 180 μg, subcutaneously each week. Daily dose of ribavirin was 800 mg. Treatment was continued for 48 weeks.

All patients were followed-up in a similar way, with a visit for selection during the 35 days prior to the start of the treatment. Once the patient had been selected, clinical and analytical evaluations were performed at weeks 0, 4, 12, 24, 48 and 72. At each evaluation, anamnesis and physical examination, complete blood counts, routine liver and renal tests, determinations of HIV-RNA and HCV-RNA load and $CD3^+$, $CD4^+$ and $CD8^+$ T cells and $CD45RO^+$ and $CD45RA^+$ subpopulations were performed. At those evolution points, peripheral blood mononuclear cells (PBMC) were isolated and kept frozen in liquid nitrogen until further analysis of TRECs.

Side effects and toxicity of therapy were classified according to usual standards [23,28]. Colony-stimulating factors

were not administered. Cotrimoxazole prophylaxis was applied in those patients with less than 200 $CD4^+$ T cell/μl during the follow-up.

The criteria for interruption of the study were: (1) appearance of opportunistic infections; (2) concurrent disease that required the use of immunosuppressive therapy; (3) virological failure in the control of HIV infection; and (4) decision of the patient to not continue the treatment.

Routine laboratory determinations

Haemogram, routine biochemistry, coagulation tests and elemental study of urine were performed by automated tests. Sera reactivity for anti-HIV was performed by enzyme immunoassay (EIA) (Abbott Laboratories, North Chicago, IL, USA) and confirmed by Western blot (Pasteur Institute, Paris, France). Plasma HIV viral load was quantified by reverse transcription–polymerase chain reaction (RT–PCR) (Monitor HIV, Roche Diagnostics, Basel, Switzerland). Anti-HCV antibodies, as detected by both a second-generation EIA (EIA-2) (Ortho Diagnostic System, Raritan, NJ, USA) and a second-generation recombinant immunoblot assay (RIBA-2) (Ortho Diagnostic System, Raritan, NJ, USA), were present in every patient. Plasma samples were tested for HCV RNA by RT–PCR (Monitor HCV, Roche Diagnostics, Basel, Switzerland).

Flow cytometry

Lymphocyte populations and subpopulations were analysed in complete blood samples containing ethylenediamine tetraacetic acid (EDTA). Previous studies have shown clearly that CD45RA defines at least 95% of naive CD4 T cells. Therefore, CD45RA is considered a valid marker of naive T cells [29,30]. The analysis was performed by two-colour flow cytofluorometry (FACScan, Becton Dickinson Co, San Jose, CA, USA) using specific monoclonal antibodies against the following antigens: $CD3^+$, $CD4^+$, $CD8^+$, $CD45RA^+$ and $CD45RO^+$ (Becton Dickinson Co, San Jose, CA, USA).

Quantification of TREC levels

TRECs were determined using RT–PCR using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) for quantification of both the characteristic signal-joint sequences harboured by TRECS and genomic porphobilinogen gene sequences (to normalize by DNA content) [15,31]. DNA was extracted from PBMC with the high pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany). RT–PCR was performed according to the manufacturer's instructions. Extracted DNA (50 ng) was amplified in a total volume of 20 μl, by using the Fast Start DNA Master Plus HybProbe (Roche Diagnostics, Mannheim, Germany) [16]. After an initial denaturation step at 95°C for 10 min, amplification was performed by 45 cycles of denaturation (95°C for 0 s), annealing (63°C for 40 s) and extension (72°C for 40 s). A standard curve was made with serial dilutions of a plasmid that included a 376-base pairs (bp) fragment of the TREC sequence, provided by Dr Lissen (University of Seville, Spain).

All follow-up samples from each individual were measured simultaneously in duplicate PCR reactions. All samples available were PCR positive for TRECs, with a distribution of between 100 and 12 000 TRECs/10^6 PBMCs.

In addition to TRECss/10^6 PBMCs, the absolute counts of TRECs/μl were derived from each sample. Because all the TRECs in PBMCs are included inside the $CD3^+$ subpopulation [17], the TRECs/10^6 PBMC proportion was corrected by the percentage of $CD3^+$ cells found by flow cytometry in the PBMCs, yielding the number of TRECs/10^6 $CD3^+$ cells. This proportion was then multiplied by absolute $CD3^+$ cell counts obtained by flow cytometry.

Statistical analysis

Quantitative variables are presented as mean ± standard deviation (s.d.) if they follow a normal distribution, according to the Shapiro–Wilk test or, in the remaining cases, as median and interquartile range (Q1–Q3). Qualitative variables are presented as absolute number and percentage. The data from two independent groups were compared with the Mann–Whitney *U*-test. The significance of the parameters within each group was tested by the Wilcoxon matched-pairs signed rank test. For qualitative variables, ×2 with Yates's correction or Fisher's exact test was used. Correlations were assessed by Spearman's rank method. A *P*-value lower than 0·05 was considered significant. Statistical analysis was performed using the SPSS version 11·5 program (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the patients

Twenty patients [median (Q1–Q3) age, 34 (33–39) years, male sex: 15 individuals (75%), drug use as the risk factor for HIV-1 infection: 15 patients (75%)] were included. Patients had a median duration of HIV-1 infection of 16 years (Q1–Q3 [14–19]) and had been receiving effective (as evidenced by the negativization of HIV-1 load) HAART at least during the last 4 years (median (Q1–Q3) of HAART treatment, 6 years, 5–8 years). Twelve (60%) patients were receiving zidovudine, lamivudine and lopinavir and eight (40%) zidovudine, lamivudine and nelfinavir.

Before beginning HAART, the median HIV-1 load was 5·07 $\log_{10}$ copies/ml (4·32–5·41) and the median $CD4^+$ T cell count was 203 (60–388), with 50% of the individuals (10 subjects) presenting with a count lower than 200 $CD4^+$ T cells/μl. At the beginning of the study, all patients presented undetectable HIV load (lower than 50 copies/ml).

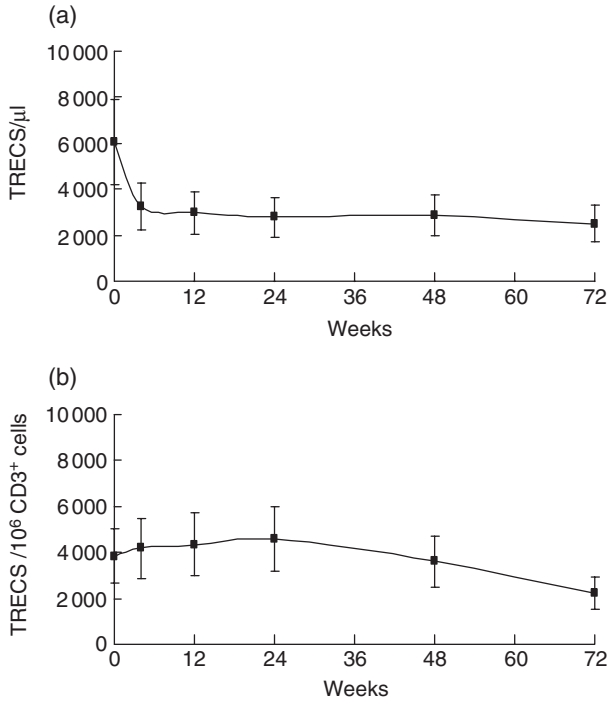

Fig. 1. Evolution of the T cell receptor excision circles (TRECs)/μl (a) and TRECs/10^6 $CD3^+$ lymphocytes (b) during treatment with interferon (IFN)-α and ribavirin of HIV/HVC co-infected patients. Data are presented as median (interquartile range) values.

At entry into the study, median $CD3^+$, $CD4^+$ and $CD8^+$ T cells were 1666 μl (Q1–Q3, 1182–2101), 592 μl (394–839) and 874 μl (638–1522), respectively. Median baseline memory subpopulations ($CD45RO^+$) were 48% for $CD4^+$ T and 57% for $CD8^+$ T lymphocytes. TRECs/10^6 $CD3^+$ lymphocytes and TRECs/μl were 3841 (2057–6171) and 6050 (2909–13834), respectively.

At baseline, the thymic function-related markers were not interrelated. Thus, the correlation between TRECs/10^6 $CD3^+$ lymphocytes and both naive $CD4^+$ ($r = -0{\cdot}126$, $P = 0{\cdot}631$) and naive $CD8^+$ ($r = 0{\cdot}190$, $P = 0{\cdot}465$) T cell counts was not statistically significant.

With respect to chronic HCV infection, median HCV RNA levels were $2{\cdot}06$ $(0{\cdot}43–10{\cdot}0) \times 10^6$ copies/ml; 55% of the patients (11 cases) presented infection by HCV genotype 1. Median fibrosis detected at the liver biopsy was 2 (1–3).

Patients' response to pegIFN and ribavirin

A maintained viral response (negativization of HCV-RNA 6 months after finishing the therapy) was detected in nine patients (45%).

No significant change was detected in the values of TRECs/10^6 $CD3^+$ cells during treatment. By contrast, when the absolute number of TRECs was considered (TRECs/μl) there was a significant decrease in TRECs/μl for the overall population after starting pegIFN + ribavirin. This thymic function-related marker was already significantly decreased *versus* baseline within 4 weeks (Wilcoxon's signed rank test, $P = 0{\cdot}031$) and was maintained during the treatment (Fig. 1).

A progressive decrease in both T cell populations, as well as of $CD45RO^+$ and $CD45RA^+$ subpopulations, was detected, with a difference between the baseline and nadir levels approaching 50% in all populations and subpopulations analysed (Table 1, Fig. 2). However, $CD4^+$/$CD8^+$, $CD4^+$ $CD45RA^+$/$CD8^+$ $CD45RA^+$ and $CD4^+$ $CD45RO^+$/$CD8^+$ $CD45RO^+$ ratios did not show significant changes during the entire follow-up. The lowest $CD4^+$ lymphocyte count was attained at the 12th week, with a decrease of 41% with respect to baseline count.

Analysis of these simultaneous decreases showed a direct relationship between early changes in naive T cells and TRECs/μl count. The change at week 12 (*versus* baseline) in TRECs/μl counts was correlated significantly with changes in naive $CD4^+$ T cell ($r = 0{\cdot}565$, $P = 0012$), naive $CD8^+$ T cell ($r = 0{\cdot}454$, $P = 0049$) and the combined $CD4^+ + CD8^+$ naive T cell counts ($r = 0{\cdot}508$, $P = 0026$) counts. No more significant correlation was present at 24, 36 or 48 weeks between changes in TRECs/μl and naive T cells.

The evolution of TRECs and of T cell populations and subpopulations was independent of the response to the treatment. Every patient showed a decrease of all lymphocyte populations and subpopulations. The decline was observed in all populations and subpopulations of T cells. Nine patients (45%) attained a $CD4^+$ T cell count lower than 200 μl during the treatment period, and in these, prophylaxis with cotrimoxazole was initiated.

Patients were classified into two groups with according to whether or not their $CD4^+$ T cells decreased under 200 $CD4^+$ T cells/μl. Both groups presented several differential characteristics: (1) patients who attained less than 200 $CD4^+$ T cells/μl presented a nadir value (previous to HAART) of $CD4^+$ T cells lower than 200 $CD4^+$ T cells/μl with a higher frequency than those who maintained their $CD4^+$ T cell count above that value; (2) patients who reached less than 200 $CD4^+$ T cells/μl presented T cell counts lower than 500/μl at baseline; and (3) these patients also showed a lower

Table 1. Decrease of lymphocyte populations and subpopulations in HIV/HVC treated with peginterferon + ribavirin.

	Nadir/basal ratio (%) (median, Q1–Q3)
TRECs/10^6 CD3 + cells	90 (59–179)
TRECS/μl	68 (32–86)
$CD4^+$ T lymphocytes (cells/μl)	41 (39–49)
$CD4^+$ CD45RO T lymphocytes (cells/μl)	44 (33–48)
$CD4^+$ CD45RA T lymphocytes (cells/μl)	46 (30–52)
$CD8^+$ T lymphocytes (cells/μl)	42 (35–55)
$CD8^+$ CD45RO T lymphocytes (cells/μl)	48 (30–73)
$CD8^+$ CD45RA T lymphocytes (cells/μl)	42 (34–58)

TRECs: T cell receptor excision circles.

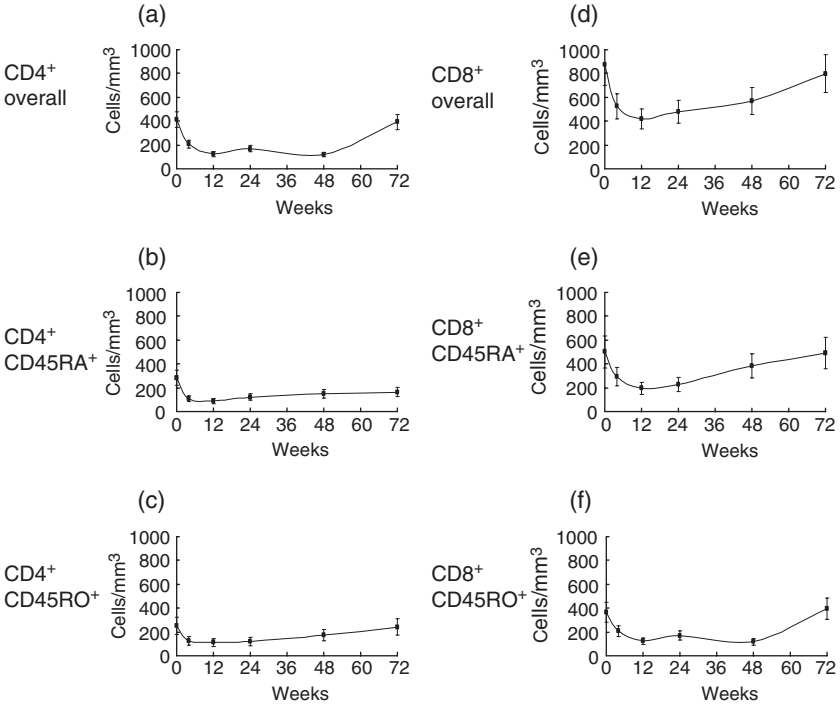

Fig. 2. Evolution of $CD4^+$ and $CD8^+$ T cell count during treatment with interferon (IFN)-α and ribavirin of HIV/HVC co-infected patients: (a) $CD4^+$, overall; (b) $CD4^+$ $CD45RA^+$ subpopulation; (c) $CD4^+$ $CD45RO^+$ subpopulation; (d) $CD8^+$, overall; (e) $CD8^+$ $CD45RA^+$ subpopulation; (f) $CD8^+$ $CD45RO^+$ subpopulation. Data are presented as median (interquartile range) values.

baseline (at entry into the study, prior to the beginning of pegIFN + ribavirin) percentage of $CD4^+$ $CD45RA^+$ (Table 2). The difference between groups of $CD4^+$ T cell response did not seem to be linked to various potential factors, such as Centers for Disease Control (CDC) stage of the disease, HAART regimen or HAART duration (data not shown).

Modifications in thymic function-related markers and in lymphocyte subpopulations after finishing the treatment with IFN + ribavirin

Twenty-four weeks after finishing the treatment, patients were reassessed. At this visit, TRECs/10^6 $CD3^+$ cells were reduced significantly with reference to values at the end of therapy ($P = 0{\cdot}036$). Absolute TRECs/μl had not reached the basal values (TRECs/μl at baseline *versus* TRECs/μl measured 24 weeks after finishing the treatment, $P = 0045$) (Fig. 1). However, lymphocyte populations and subpopulations attained the baseline levels, with the exception of the $CD4^+$ and $CD4^+$ $CD45RA^+$ T lymphocyte subpopulation (Fig. 2). Two patients persisted with a $CD4^+$ T cell count of less than 200 μl.

Median baseline memory $CD4^+$ $CD45RO^+$ had been 48%. Twenty-four weeks after finishing the treatment, this percentage had increased to 59%. Thus, the ratio of $CD4^+$ $CD45RO^+$/$CD4^+$ $CD45RA^+$ increased from 0·89 to 1·44.

Table 2. Differential characteristics of HIV-HCV co-infected patients in function of the lymphocyte response to peginterferon (pegIFN) + ribavirin.

	Patients whose $CD4^+$ T lymphocyte count decreased to less than 200 cells/μl ($n = 9$)	Patients whose $CD4^+$ T lymphocyte count did not decrease to less than 200 cells/μl ($n = 11$)	P
Age (years)	34 (30–40)	33 (33–39)	0·891
Number and percentage of men	7 (78)	8 (73)	0·624
Estimated duration of HIV infection (years)*	15 (13–17)	17 (14–23)	0·219
Previous intravenous drug use (n, %)	8 (89)	7 (64)	0·086
Nadir $CD4^+$ T cells/μl	118 (54–198)	296 (186–394)	< 0·001
Maximal HIV RNA load (log copies/ml)	5·1 (4·6–5·7)	5 (4·0–5·6)	0·920
Duration of HAART therapy (years)	5 (3–6)	5 (3–6)	1·000
Baseline TRECs/10^6 $CD3^+$ cells	4300 (1756–10507)	3840 (2336–5813)	0·815
Baseline TRECs/μl	8733 (2735–11789)	6060 (3029–8247)	0·219
Baseline $CD4^+$ T cells/μl	351 (286–445)	754 (667–1058)	< 0·001
Baseline $CD4^+$ $CD45RA^+$ T cells (% with respect to total $CD4^+$ T cells)	12 (8–14)	18 (12–20)	0·045
Baseline $CD8^+$ T cells/μl	874 (605–1622)	775 (628–1155)	0·550
Sustained HCV response to pegIFN + ribavirin (n, %)	4 (44)	5 (45)	

*Estimated in 16 patients. HAART: highly active antiretroviral therapy; TRECs: T cell receptor excision circles.

Interestingly, in the group of patients which did not reach their baseline values after the end of the follow-up, a higher proportion of patients showed basal counts of less than 500 $CD4^+$ T cells/μl and $CD4^+$ T cells lower than 200 μl during the treatment with pegIFN + ribavirin.

The viral HIV load was undetectable during the study and none of the patients developed an opportunistic infection during the follow-up.

Discussion

Infection by HCV significantly increases overall mortality as well as mortality from liver-related and AIDS-related causes in HIV-co-infected patients [32]. Thus, a beneficial effect of pegIFN + ribavirin, the accepted treatment for chronic hepatitis C, could be expected. However, a secondary effect is the appearance of $CD4^+$ T lymphopenia during therapy [33–35]. The IFN-induced decrease in lymphocyte count could be of greater relevance in HIV-infected patients [36–38]. This study has analysed the dynamic of changes in the populations and subpopulations of T cells in HIV-infected patients receiving treatment for chronic HCV infection.

In our series, all patients showed a reduction in the count of lymphocyte populations and subpopulations of about 50%. Forty-five per cent of patients attained a $CD4^+$ T cell count of less than 200 ml. Although no opportunistic event was detected during follow-up, the low incidence of opportunistic infections if an undetectable HIV viral load is maintained and cotrimoxazole prophylaxis is applied does not exclude risk [39].

It is remarkable that those patients who presented with less than 200 $CD4^+$ T cells/μl during anti-HCV treatment are those with a nadir lower than 200 $CD4^+$ T cells/μl prior to HAART treatment. Moreover, although at least a partial immune reconstitution was present at entry into the study (all patients had more than 350 $CD4^+$ T cells/μl), the baseline $CD4^+$ T cell count in this group was lower than 500 μl. Additionally, in these patients, a lower percentage of naive $CD4^+$ T cells was detected when compared with those who maintained their $CD4^+$ T cell count of over 200 μl during anti-HCV therapy. The difference between groups of $CD4^+$ T cell response did not seem to be linked to various potential factors, such as CDC stage of the disease, treatment regimen (e.g. zidovudine is known to have haematological toxicity and/or to induce lymphocyte apoptosis [40], but it was being used in all patients) or treatment duration. The observed difference also did not seem to be associated with distinct characteristics of HIV response to treatment, because plasma viral loads were undetectable.

The effect of IFN on $CD4^+$ T cell count has not been explained clearly, although the reduction in numbers of this lymphocyte subset has been linked to a redistribution [41]. The dynamic of the described changes could imply phenomena of ganglionar sequestration and/or thymic suppression from the beginning of the treatment with maintenance of these effects during the full period of administration. In our study, whereas median baseline memory $CD4^+$ $CD45RO^+$ was 48%, similar to the percentage reported in HIV-infected patients without immunological impairment [42], 24 weeks after finishing the treatment this percentage had increased. After finishing the treatment, the appearance of $CD4^+$ $CD45RO^+$ in a higher proportion than that observed at baseline, with an increased CD45RO/CD45RA ratio, supports the return of previously sequestered $CD4^+$ to the peripheral blood. However, the absence of recuperation of $CD4^+$ $CD45RA^+$ additionally implies a diminution in the thymic turnover, probably mediated by the effects of IFN on haematopoietic growth factors [7–9,43]. Thus, *in vitro* IFN-α inhibits the proliferation of peripheral T cells along the interleukin (IL)-7–p27 axis. These molecules are also involved in thymocyte proliferation [44].

To study the role of the thymus in the decrease of naive lymphocyte T cells during therapy of chronic HCV infection in HIV-infected adult patients, we have analysed the changes in TREC levels in this group of individuals. Patients in treatment with IFN + ribavirin presented a significant decrease in absolute TREC levels after the beginning of the therapy. Moreover, the diminution of absolute TREC levels correlated significantly with that of naive T cells.

Several processes affect TRECs in a cell population. TRECs measured in blood can increase due to the input of $TREC^+$ naive cells from the thymus or input of $TREC^+$ cells from an extrathymic source. Two processes lead to a decrease in TRECs: dilution of TRECs due to proliferation (without TREC replication) and loss of TRECs due to the death of $TREC^+$ cells [45]. Each of these mechanisms has a different effect on absolute TREC values (TRECs/μl) and TRECs per million cells [18,19,46]. The increase in absolute numbers of TRECs needs an active output of TRECs to peripheral blood, rather than a simple reduction in T cell division rates or the elimination of TREC-negative, activated T cells (which can alter TREC proportions). Consequently, although margination of naive lymphocytes is probable, our data suggest that the decrease in $CD45RA^+$ lymphocytes during therapy are, in part, related to the inhibition of thymic function by pegIFN + ribavirin.

Similarly, evaluation of T cell subpopulations and TREC values were performed after finishing the therapy. In all patients, absolute TREC values remained under the values detected at baseline, suggesting a prolonged effect of pegIFN + ribavirin on thymic function. In these patients, no correlation was established between TREC levels and the increase of $CD4^+$ naive T cells. A homeostatic peripheral expansion of naive T cells seems to be possible in HIV infection, being an alternative source of naive T cells [17]. Peripheral expansion of naive T cells will be in agreement with the significantly lower TREC proportion per naive T cells detected in this work. That this ratio actually did not increase also reinforces the hypothesis that thymic regeneration is impaired in treated patients.

Previous studies [47,48] have demonstrated that thymic output is more likely to affect TREC concentration within $CD4^+$ T cells than within $CD8^+$ T cells. In fact, it was notable that the naive $CD8^+$ cell count, but not that of $CD4^+$ T cells, increases after finishing therapy, suggesting a more intense and maintained effect on naive $CD4^+$ T cells than on $CD8^+$ T cells. It must be stated that TREC levels were measured in total PBMC, which makes it difficult to establish the relationship between changes in TRECs and in these cell populations.

It is, however, important to underscore that the increase in the *de novo* T cell generation could not be observed during later stages of the HIV infection (e.g. CD4 T cell counts less than 200) [15,49]. Based on these and our results, and although our patients were followed-up for only 24 weeks after finishing the treatment, T cell reconstitution in patients with nadir CD4 T cell counts less than 200 μl who begin IFN therapy may be hampered.

In conclusion, a significant reduction of $CD4^+$ and $CD8^+$ T cells, and of their naive and memory subpopulations in HIV-HCV co-infected patients treated with pegIFN and ribavirin, was observed. Both naive and memory subpopulations increase after treatment suppression but the $CD4^+$ T lymphocytes and $CD4^+$ $CD45RA^+$ subpopulation did not recover the basal levels 24 weeks after finishing treatment. This adverse outcome must be weighed against the salutary effects derived from the control of chronic hepatitis.

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