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# Recent thymic emigrants in lymphoma patients with and without human immunodeficiency virus infection candidates for autologous peripheral stem cell transplantation

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## Summary

**Signal joint T cell receptor excision circles (sjTRECs) have been reported as a clinical marker to measure the potential for recovery of the immune system after immunosuppressive treatments. The aim of this study was to investigate the thymic regenerative potential in 55 human immunodeficiency virus (HIV)-1 infected ($HIV^+$) and non-infected ($HIV^-$) lymphoma patients, candidates for autologous stem cell transplantation (ASCT). Moreover, the possible associations between sjTRECs and other immunological and clinical parameters were examined. SjTRECs levels in peripheral blood mononuclear cells (PBMCs) were quantified by real-time polymerase chain reaction and T lymphocyte subsets were analysed by flow cytometry. Our data showed that sjTRECs were reduced in lymphoma patients compared to healthy controls, although a weak significant association between low sjTRECs levels and increasing age was maintained [odds ratio (OR) = 4·00; 95% confidence interval (CI) 1·09–17·17]. We found that different chemotherapeutic treatments seem to induce similar effects on the thymic reservoir, independently from their intensity (type and number of cycles of previous chemotherapy). Results from multivariate models including adjustment for patients' sex, type of lymphoma and type of chemotherapy showed that thymic output was independent from HIV infection (OR, 0·95; 95% CI 0·20–4·48). SjTRECs levels correlated with naive T cell subsets in overall lymphoma patients and after stratification by HIV infection ($r > 0·37$). HIV replication should be maximally suppressed to properly evaluate thymic output by sjTREC markers. Our results suggested that *de novo* T cell generation is maintained partially in pretreated recurrent lymphoma patients, candidates for ASCT, and could contribute to restore the immune function after transplantation.**

**Keywords:** autologous stem cell transplantation, HIV infection, recurrent lymphoma, TCR excision circles

## Introduction

During thymocyte development, rearrangement of the T cell receptor gene leads to the excision of circular DNA fragments from genomic DNA, among which are signal joint T cell receptor excision circles (sjTRECs). These products are stable, unique for the T cells and are not duplicated during mitosis, which means that they are diluted out with each cellular division. SjTRECs recovery has been studied in depth in the peripheral blood of human immunodeficiency virus (HIV) negative ($HIV^-$) patients with different haemato-oncological malignancies receiving high-dose chemotherapy and allogenic [1–4] or autologous stem cell transplantation [5,6]. From these studies it appears that the sjTRECs value may be a clinical indicator of the potential for recovery of the immune system after infusion of stem cells.

After transplantation, functional recovery of lymphoid and immune effector cells occurs gradually, and reconstitution of normal humoral and cellular immunity may take 1 year or even longer [7]. In particular, T cell reconstitution occurs by either peripheral expansion of the already existing T cell pool or by renewal of thymopoiesis. In principle, a functional thymus, which is able to generate new naive T cells, would result in a higher degree of normalization of the skewed repertoire than the restoration of the T cell pool by expansion of existing T cells [8].

 

HIV disease is characterized by a progressive and profound immunodeficiency. CD4 T cell levels are the most accurate marker of the immune function decline and they provide important prognostic information. Moreover, different studies in HIV positive ($HIV^+$) patients during highly active anti-retroviral therapy (HAART) have suggested sjTRECs as a useful additional marker of HIV disease progression [9–12]. HIV-associated decreasing immunity yields increased morbidity and mortality from infectious diseases, but also higher incidence of lymphomas, compared to the non-infected population; furthermore, prior to the advent of HAART, the treatment of $HIV^+$ lymphoma subjects has been far less successful compared to $HIV^-$ patients [13,14]. Later, however, HAART keeping the virus replication under control changed the natural history of HIV infection by improving immunological and haematological functions. Higher response rates in patients with non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD) treated with chemotherapy (CT) and HAART [15] allowed $HIV^+$ patients with relapsed or refractory lymphomas to be candidates for high-dose CT and ASCT, as already occurring in $HIV^-$ subjects [16–19].

Conditions such as haematological malignancy, CT, altered immune status and viral infections seem to influence sjTRECs content, but their effective role on thymic function in patients with refractory/relapsed lymphoma candidates for ASCT has not yet been studied in depth. In particular, to our knowledge, only one study has reported about sjTRECs levels in NHL or HD $HIV^+$ patients who were candidates for high-dose therapy and ASCT. However, due to the small number of subjects, no definitive conclusion could be drawn on the role of the thymus [20].

Here, we studied the sjTRECs levels in a mono-institutional series of unselected patients with $HIV^-$ and $HIV^+$ recurrent lymphomas, candidates for ASCT, considering important demographic, clinical, virological and immunological characteristics including sex, age, kind of lymphoma, stage, type and cycles number of previous CT, time from the end of previous CT to the enrolment (TECT), HIV infection and T lymphocyte subpopulations.

SjTRECs, evaluated carefully, may contribute to the management of patients who may benefit from ASCT and from new strategies targeted to enhance thymic function which might significantly improve a compromised immune system.

## Materials and methods

### Patients and treatments

Between May 2001 and December 2005 a total of 55 consecutive patients (38 males, 17 females), 29 with high-grade NHL (23 relapsed and six refractory to the previous CT regimen) and 26 with HD (23 relapsed and three refractory), candidates for ASCT, were enrolled before the first cycle of debulking therapy. All patients were treated previously with conventional CT [23, 15, 14, respectively, cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)-like, etoposide, epirubicin, bleomycin, cyclophosphamide and prednisolone (VEBEP), other regimens] chosen according to the type of lymphoma. All $HIV^+$ subjects were receiving HAART at the time of enrolment.

Sixty-two healthy volunteers of comparable sex and age (median 44 years, range 20–84 years) were selected randomly from the general population and included for reference sjTRECs values. All patients and controls agreed to participate in the study by signing an informed consent approved by the Ethics Committee of our Institute, following the principles of the Declaration of Helsinki.

### Blood samples

Peripheral blood samples from patients and from healthy donors were collected in ethylenediamine tetraacetic acid (EDTA). Whole blood was processed immediately for cytofluorimetric analysis while peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll/Hypaque density gradient centrifugation and cryopreserved as dry pellets at −80°C before further analyses.

### T cell receptor (TCR) rearrangement excision circles assay

Genomic DNA was extracted from PBMCs by standard techniques [21]. To detect sjTRECs, a real-time polymerase chain reaction (PCR) method was used. PCR reactions were carried out in 25 μl using 200 ng of genomic DNA, 1× *Taq*Man Universal PCR Master Mix (PE Applied Biosystems, Foster City, CA, USA), 300 nM of primer forward 5′-CACATCCCTTTCAACCATGCT-3′, 300 nM of primer reverse 5′-GCCAGCTGCAGGGTTTCGG-3′ and 100 nM of probe Fam-5′-ACACCTCTGGTTTTTGTAAAGGTGCC CACT-3′-TAMRA [5]. Thermal cycling proceeded under the following conditions: 2 min at 50°C, 10 min at 95°C and 50 cycles each of 15 s at 95°C and 1 min at 60°C. The reactions were performed in a spectrofluorimetric thermal cycler (ABI PRISM 7900 Sequence detector; PE Applied Biosystems) and data collected were analysed using the Sequence Detection System Software (SDS version 2·1; PE Applied Biosystems). An amplicon for sjTRECs, defined by the 5′/3′ outer primers, was obtained from human genomic DNA derived from PBMCs of healthy donors and was cloned into the pCR2·1 vector (Invitrogen, Life Technologies, Carlsbad, CA, USA). In each experiment, 10-fold serial dilutions of this plasmid (from $3{\cdot}5 \times 10^7$ to $3{\cdot}5 \times 10^1$) were used to generate an external standard curve for the PCR-based assay.

In order to normalize the value of sjTRECs for cell equivalents, the β-globin gene was quantified under real-time PCR conditions similar to those used for sjTRECs quantification, as reported previously [22]. The β-globin standard curve was obtained from fourfold serial dilutions of human genomic


 

DNA quantified by spectrophotometry. The sjTRECs values were then expressed as the numbers of sjTRECs/$10^6$ PBMCs by using mean values from triplicate *Taq*Man assays for both sjTRECs and β-globin. Because only T cells contribute to the peripheral blood sjTRECs content, in addition to sjTRECs/$10^6$ PBMCs, the absolute counts of sjTRECs/$10^6$ $CD3^+$ cells were derived for each sample by using the percentage of $CD3^+$ cells found by flow cytometry in the gate of lymphocytes. The analyses generated OR and *P*-values similar to those of TRECs per $10^6$ PBMCs; therefore, only the discrepant findings have been illustrated separately.

Intra- and interassay variation was always lower than 20% and 10%, respectively, for sjTRECs and β-globin evaluation.

### Determination of HIV RNA levels in plasma samples

HIV RNA analysis was performed by Quantiplex HIV-RNA b-DNA assay (version 3·0; Bayer Diagnostics, Berkeley, CA, USA). This assay has a detection limit of 50 RNA copies/ml.

### Lymphocyte subpopulations in peripheral blood

The percentage values and absolute counts of T lymphocyte subsets were evaluated by a single platform whole blood lysing technique and an EPICS XL flow cytometer (Beckman-Coulter, Hialeah, FL, USA). Briefly, 100 µl of blood was added to fluorescent microbeads and to the appropriate monoclonal antibodies (mAbs) and incubated for 15 min; thereafter, the samples were lysed and fixed by using a commercial preparation (Immunoprep, Beckman-Coulter, Galway, Ireland). The CD3–PC5/CD4–RD1/CD8–ECD/CD45–fluorescein isothiocyanate (FITC) mAb combination (Beckman-Coulter, Fullerton, CA, USA) was used to stain peripheral blood T lymphocyte subpopulations. The proportion of $CD4^+$ or $CD8^+$ T cells expressing the memory or naive phenotype was defined by two- or three-colour flow cytometry using specific mAbs against CD45R0 or CD62L and CD45RA antigens, respectively, as described in Sempowski *et al.* [23] (Dako, Glostrup, Denmark; Immunotech, Marseille, France; Beckman-Coulter). Four-colour flow cytometry was used to analyse the expression of human leucocyte antigen D-related (HLA-DR) or CD38 activation markers on $CD4^+$ and naive $CD4^+$ or $CD8^+$ and naive $CD8^+$ T lymphocytes, respectively (Becton Dickinson, San Jose, CA, USA).

### Statistical analysis

Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated for some parameters after they were dichotomized or categorized into approximate tertiles on the basis of the distribution of the values in the group with lower median [24]. The dependent variables were: sjTRECs, naive $CD4^+$ and $CD8^+$ T cells levels (low *versus* high) and HIV infection ($HIV^+$*versus* $HIV^-$). The Spearman's rank correlation coefficient was used to analyse the correlation between sjTRECs values and naive T cell subsets. Given the low number of cases, the non-parametric Mann–Whitney *U*-test was used to compare immunological and virological parameters between $HIV^+$ subgroups. Analyses were performed with SAS System software, release 8·2 (SAS Institute Inc., Cary, NC, USA, 1999–2001).

A standard significance level of *P*-value $\leq 0{\cdot}05$ (two-sided) was chosen.

## Results

### Thymic output in overall lymphoma patients

When we examined the distribution of 55 lymphoma cases and 62 healthy controls according to sjTRECs levels, we found that normal individuals showed a significantly increased risk for high sjTRECs values compared to lymphoma patients: OR = 14·0 (95% CI 4·14–47·38) and 59·5 (95% CI 14·72–240·54), respectively, for 675–5632 sjTRECs/$10^6$ PBMCs and $\geq 5633$ *versus* < 675 (*P* for trend < 0·001).

Table 1 showed OR and 95% CI for some baseline demographic (sex and age) and clinical characteristics (lymphoma histology, type of previous CT, number of previous CT cycles, TECT) of the patients stratified by sjTRECs levels ($\leq$ 500 TRECs/$10^6$ PBMCs *versus* > 500). We found that low sjTRECs levels were associated significantly with increasing age: OR = 2·50 (95% CI 0·65–9·65) and 4·00 (95% CI 1·09–17·17; *P* for trend = 0·04), respectively, for 35–43 and $\geq$ 44 *versus* < 35 years. We could not find any significant association between the other parameters and sjTRECs. However, when sjTRECs were expressed per $10^6$ $CD3^+$ T cells, high sjTRECs values correlated with increased TECT values (data not shown). No association was observed between high or low naive $CD4^+$ and $CD8^+$ T cell levels and all the analysed variables (data not shown).

### Thymic output in $HIV^+$ and $HIV^-$ lymphoma patients: clinical data

Odds ratios and 95% CI for the main baseline demographic and clinical characteristics of the patients stratified by HIV infection were summarized in Table 2. A significantly lower risk for HIV infection was observed for females (OR = 0·14; 95% CI 0·03–0·57), while there was no statistically significant difference in age distribution between $HIV^+$ and $HIV^-$ lymphoma patients. In the $HIV^+$ group the proportion of NHL patients was significantly greater than in the $HIV^-$ patients (76·9% *versus* 31·0%, respectively; OR = 0·14; 95% CI 0·04–0·45), while the disease distribution by stage was comparable in the two groups. Among 26 $HIV^+$ patients, 14 had relapsed and six refractory high-grade NHL, five relapsed and one refractory HD; among 29 $HIV^-$ patients, nine had relapsed high-grade NHL, 18 relapsed and two refractory HD. Overall, the proportion of refractory lymphomas was 26·9%

 

**Table 1.** Odds ratios (OR) and corresponding 95% confidence intervals (CI) of some baseline characteristics of 55 lymphoma patients by high and low signal joint T cell receptor excision circles (sjTRECs) levels.

| | SjTRECs/$10^6$ PBMCs | | |
|---|---|---|---|
| | ≤ 500 *n* (%) | > 500 *n* (%) | OR (95% CI) |
| Sex | | | |
| Male | 22 (75·9) | 16 (61·5) | 1* |
| Female | 7 (24·1) | 10 (38·5) | 0·51 (0·16–1·63) |
| $\chi_1^2$; *P* | | | 1·30; *P* = 0·25 |
| Age (years) | | | |
| < 35 | 7 (24·1) | 12 (46·2) | 1* |
| 35–43 | 10 (34·5) | 8 (30·8) | 2·50 (0·65–9·65) |
| ≥ 44 | 12 (41·4) | 6 (23·0) | 4·00 (1·09–17·17) |
| $\chi^2$ trend; *P* | | | 4·34; *P* < 0·05 |
| Lymphoma histology | | | |
| high grade NHL | 16 (55·2) | 13 (50·0) | 1* |
| HD | 13 (44·8) | 13 (50·0) | 0·81 (0·28–2·35) |
| $\chi_1^2$; *P* | | | 0·15; *P* = 0·70 |
| Type previous CT† | | | |
| CHOP-like | 14 (50·0) | 9 (37·5) | 1* |
| VEBEP | 7 (25·0) | 8 (33·3) | 0·64 |
| Others | 7 (25·0) | 7 (29·2) | 0·73 |
| $\chi_2^2$; *P* | | | 0·52; *P* = 0·47 |
| *N* previous CT cycles† | | | |
| < 6 | 17 (73·9) | 19 (90·5) | 1* |
| ≥ 6 | 6 (26·1) | 2 (9·5) | 0·63 (0·13–2·93) |
| $\chi_1^2$; *P* | | | 0·35; *P* = 0·55 |
| TECT (months)† | | | |
| < 3·9 | 13 (50·0) | 7 (30·4) | 1* |
| ≥ 3·9 | 13 (50·0) | 16 (69·6) | 0·81 (0·28–2·35) |
| $\chi_1^2$; *P* | | | 0·15; *P* = 0·70 |

*Reference category. †The sum does not add up to the total because of missing values. NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; CT, chemotherapy; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; VEBEP, etoposide, epirubicin, bleomycin, cyclophosphamide and prednisolone; TECT, time elasped from previous chemotherapy; PBMCs, peripheral blood mononuclear cells.

in the HIV$^+$ patients and only 6·9% in the HIV$^-$ patients, although this difference was not statistically significant (OR = 4·97; 95% CI 0·93–26·62). All patients were treated previously with conventional CT chosen according to the type of lymphoma. A significant association between the HIV$^+$ patients and CHOP-like treatments was retrieved (OR = 0·25; 95% CI 0·06–0·98).

### Thymic output in HIV$^+$ and HIV$^-$ lymphoma patients: sjTRECs and naive T cell subsets

Table 3 shows the multivariate OR and 95% CI of the 55 lymphoma patients by HIV infection for tertiles of the immunological parameter levels, after adjustment for statistically significant variables at analysis, described in Table 2 (sex, lymphoma histology, type of previous CT). No association between the sjTRECs/$10^6$ PBMCs values and HIV infection was found. The same result was also obtained after univariate analysis (not shown). When the sjTRECs values were expressed per $10^6$ CD3$^+$ lymphocytes, a weak statistically significant positive association emerged between decreased sjTRECs/$10^6$ CD3$^+$ levels and HIV$^+$ patients at univariate analysis (OR = 0·26; 95% CI 0·07–1·00), which was lost after adjustment for sex, lymphoma histology and type of previous CT (OR = 0·46; 95% CI 0·09–2·29).

All patients at enrolment showed a moderate level of immunodeficiency (< 300 CD4$^+$ T cells/μl). However, HIV$^+$ subjects presented a significantly lower risk to have high CD4$^+$ T cell numbers and percentage values in comparison to the HIV$^-$ group (for CD4$^+$ cells/μl: OR = 0·03; 95% CI 0·01–0·48; for CD4$^+$%: OR = 0·07; 95% CI 0·01–0·46). The percentage values and the absolute counts of the naive CD4$^+$ T cell subset were not distributed differently between HIV$^+$ and HIV$^-$. With regard to CD8$^+$ T cells, only percentage values were associated significantly with HIV infection (OR = 12·39; 95% CI 2·15–71·47). The absolute counts and percentage values of the naive CD8$^+$ T cell subset were not distributed differently in HIV$^+$ compared to HIV$^-$, even if a weakly significant positive trend in risk was observed as naive CD8$^+$ absolute counts increased in HIV infection (OR = 4·24; 95% CI 0·87–20·77; *P* for trend = 0·05).

Spearman's correlation coefficients between sjTRECs levels and T cell subsets were reported in Table 4. Naive CD4$^+$ and CD8$^+$ T cell counts and percentage values were correlated positively with sjTRECs levels in the overall data set. Similar results were observed in the patients when stratified by HIV infection, with the exception of naive CD4$^+$ T cell counts in HIV$^+$ subjects ($r$ = 0·25; *P* = 0·25).

### CD4$^+$ and CD8$^+$ activation markers

No significant differences were found between HIV$^+$ and HIV$^-$ patients in the distribution of the proportion of CD4$^+$ and naive CD4$^+$ or CD8$^+$ and naive CD8$^+$ T cell subsets expressing HLA-DR or CD38 activation antigens, both after univariate (not shown) and multivariate analyses (Table 5). Moreover, the distribution of the ratios of CD4$^+$ or CD8$^+$ expressing CD45RA to CD4$^+$ or CD8$^+$ expressing CD45R0 antigens did not show differences between the groups of patients.

### Role of HIV replication on immunological parameters

In order to evaluate the impact of HIV replication on thymopoiesis, we analysed sjTRECs levels of viraemic patients in comparison with those of age-matched patients with undetectable viral load for at least 3 months before enrolment (Table 6). Although the median HIV RNA load was relatively low (median value, 1200 copies/ml; range, 204–305 426), we observed lower sjTRECs content in viraemic compared to non-viraemic patients (median 143 *versus* 508, respectively; *P* = 0·04). Moreover, the latter group of patients


 

**Table 2.** Odds ratios (OR) and corresponding 95% confidence intervals (CI) of the main baseline characteristics of 55 lymphoma patients by human immunodeficiency virus (HIV) infection.

| | HIV$^+$ ($n = 26$) $n$ (%) | HIV$^-$ ($n = 29$) $n$ (%) | OR (95% CI) |
|---|---|---|---|
| Sex | | | |
| Male | 23 (88·5) | 15 (51·7) | 1* |
| Female | 3 (11·5) | 14 (48·3) | 0·14 (0·03–0·57) |
| $\chi_1^2$; $P$ | | | 7·50; $P < 0·01$ |
| Age (years) | | | |
| < 40 | 10 (38·5) | 17 (58·6) | 1* |
| ≥ 40 | 16 (61·5) | 12 (41·4) | 2·27 (0·77–6·69) |
| $\chi_1^2$; $P$ | | | 2·20; $P = 0·14$ |
| Lymphoma histology | | | |
| high grade NHL | 20 (76·9) | 9 (31·0) | 1* |
| HD | 6 (23·1) | 20 (69·0) | 0·14 (0·04–0·45) |
| $\chi_1^2$; $P$ | | | 10·61; $P = 0·001$ |
| Stage (Ann Arbor)† | | | |
| 1–2 | 2 (8·0) | 4 (16·7) | 1* |
| 3–4 | 23 (92·0) | 20 (83·3) | 2·77 (0·91–8·39) |
| $\chi_1^2$; $P$ | | | 3·24; $P = 0·07$ |
| Lymphoma status after previous CT | | | |
| Relapsed lymphoma | 19 (73·1) | 27 (93·1) | 1* |
| Refractory lymphoma | 7 (26·9) | 2 (6·9) | 4·97 (0·93–26·62) |
| $\chi_1^2$; $P$ | | | 3·51; $P = 0·06$ |
| Type previous CT† | | | |
| CHOP-like | 18 (69·2) | 5 (19·2) | 1* |
| VEBEP | 3 (11·6) | 12 (46·2) | 0·11 (0·02–0·51) |
| Others | 5 (19·2) | 9 (34·6) | 0·25 (0·06–0·98) |
| $\chi_2^2$; $P$ | | | 7·41; $P < 0·01$ |
| $N$ previous CT cycles† | | | |
| < 6 | 20 (87·0) | 16 (76·2) | 1* |
| ≥ 7 | 3 (13·0) | 5 (23·8) | 0·63 (0·13–2·93) |
| $\chi_1^2$; $P$ | | | 0·35; $P = 0·55$ |
| TECT (months)† | | | |
| < 3·9 | 13 (50·0) | 7 (30·4) | 1* |
| ≥ 3·9 | 13 (50·0) | 16 (69·6) | 0·81 (0·28–2·35) |
| $\chi_1^2$; $P$ | | | 0·15; $P = 0·70$ |

*Reference category. †The sum does not add up to the total because of missing values. NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease. CT, chemotherapy; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; VEBEP, etoposide, epirubicin, bleomycin, cyclophosphamide and prednisolone; TECT, time elasped from previous chemotherapy.

showed a better immunological status and a lower proportion of activated T cell markers, even if not always statistically significant (median CD8$^+$ RA : RO ratio: non-viraemic 0·7 *versus* viraemic 0·2; $P = 0·02$).

## Discussion

High-dose CT followed by autologous haematopoietic stem-cell transplantation is the first-choice salvage treatment for patients with refractory/relapsed chemotherapy-sensitive NHL or HD. Initially, ASCT was not feasible in HIV$^+$ patients, but current anti-retroviral therapies suppress viral replication and improve immune deficiency, also allowing collection and grafting of peripheral blood stem cells in these patients [16,18,19,25]. However, to date, the role of thymus and other mechanisms of immune restoration after ASCT are not understood completely [20,26]. In order to gain insight into this issue, we evaluated the thymic activity by measuring the peripheral blood sjTREC content, a parameter reflecting the potential of immunological recovery from immune depression [27], in a mono-institutional cohort of HIV$^+$ and HIV$^-$ lymphoma patients, candidates for ASCT, after failure of previous CT. Moreover, we analysed phenotypically defined naive T cell subsets and some relevant potential influencing factors on these markers such as sex, age, type of haematological malignancy, type and number of cycles of previous CT, TECT, immune activation status and HIV replication.

 

**Table 3.** Odds ratios (OR) and corresponding 95% confidence intervals (CI) of signal joint T cell receptor excision circles (sjTRECs) and some lymphocyte subpopulations in 55 lymphoma patients by human immunodeficiency virus (HIV) infection.

| Parameters | HIV$^+$ ($n = 26$) $n$ (%) | HIV$^-$ ($n = 29$) $n$ (%) | Multivariate* OR (95% CI) |
|---|---|---|---|
| SjTRECs/$10^6$ PBMCs | | | |
| < 212 | 9 (34·6) | 9 (31·0) | 1† |
| 212–511 | 9 (34·6) | 5 (17·3) | 2·34 (0·37–15·01) |
| ≥ 512 | 8 (30·8) | 15 (51·7) | 0·95 (0·20–4·48) |
| $\chi^2$ trend; $P$ | | | 0·02; $P = 0·89$ |
| SjTRECs/$10^6$ CD3 | | | |
| < 356·9 | 12 (46·2) | 8 (27·6) | 1† |
| 356·9–1327·5 | 9 (34·6) | 8 (27·6) | 0·77 (0·16–3·68) |
| ≥ 1327·6 | 5 (19·2) | 13 (44·8) | 0·46 (0·09–2·29) |
| $\chi^2$ trend; $P$ | | | 0·88; $P = 0·35$ |
| CD4$^+$ cells/μl | | | |
| < 100 | 9 (34·6) | 4 (13·8) | 1† |
| 100–199 | 9 (34·6) | 9 (31·0) | 0·06 (0·01–0·93) |
| ≥ 200 | 8 (30·8) | 16 (55·2) | 0·03 (0·01–0·48) |
| $\chi^2$ trend; $P$ | | | 6·08; $P = 0·01$ |
| CD4$^+$ % | | | |
| < 15 | 13 (50·0) | 3 (10·3) | 1† |
| 15–24 | 6 (23·1) | 3 (10·3) | 0·40 (0·04–3·85) |
| ≥ 25 | 7 (26·9) | 23 (79·4) | 0·07 (0·01–0·46) |
| $\chi^2$ trend; $P$ | | | 7·94; $P < 0·01$ |
| CD4$^+$CD45RA$^+$CD62L$^+$ cells/μl‡ | | | |
| < 9 | 9 (37·5) | 4 (14·8) | 1† |
| 9–56 | 8 (33·3) | 13 (48·2) | 0·44 (0·09–2·30) |
| ≥ 57 | 7 (29·2) | 10 (37·0) | 0·54 (0·10–2·85) |
| $\chi^2$ trend; $P$ | | | 0·84; $P = 0·36$ |
| CD4$^+$CD45RA$^+$CD62L$^+$ %‡ | | | |
| < 6·3 | 7 (29·2) | 9 (33·3) | 1† |
| 6·3–32·0 | 9 (37·5) | 9 (33·3) | 1·91 (0·36–10·17) |
| ≥ 32·1 | 8 (33·3) | 9 (33·3) | 2·13 (0·41–11·14) |
| $\chi^2$ trend; $P$ | | | 0·95; $P = 0·33$ |
| CD8$^+$ cells/μl | | | |
| < 200 | 4 (15·4) | 7 (24·1) | 1† |
| 200–299 | 6 (23·1) | 12 (41·4) | 0·73 (0·10–5·33) |
| ≥ 300 | 16 (61·5) | 10 (34·6) | 1·80 (0·29–11·12) |
| $\chi^2$ trend; $P$ | | | 1·21; $P = 0·27$ |
| CD8$^+$ % | | | |
| < 29 | 3 (11·5) | 15 (51·7) | 1† |
| 29–38 | 2 (7·7) | 5 (17·2) | 5·21 (0·40–67·78) |
| ≥ 39 | 21 (80·8) | 9 (31·1) | 12·39 (2·15–71·47) |
| $\chi^2$ trend; $P$ | | | 8·10; $P < 0·01$ |
| CD8$^+$CD45RA$^+$CD62L$^+$ cells/μl‡ | | | |
| < 24 | 4 (18·2) | 7 (31·8) | 1† |
| 24–52 | 5 (22·7) | 8 (36·4) | 2·15 (0·33–14·26) |
| ≥ 53 | 13 (59·1) | 7 (31·8) | 4·24 (0·87–20·77) |
| $\chi^2$ trend; $P$ | | | 3·86; $P = 0·05$ |
| CD8$^+$CD45RA$^+$CD62L$^+$ %‡ | | | |
| < 7·4 | 7 (31·8) | 5 (22·7) | 1† |
| 7·4–23·6 | 8 (36·4) | 10 (45·5) | 1·52 (0·32–7·13) |
| ≥ 23·7 | 7 (31·8) | 7 (31·8) | 1·41 (0·24–8·16) |
| $\chi^2$ trend; $P$ | | | 0·02; $P = 0·88$ |

*Adjusted for statistically significant parameters at univariate analysis described in Table 1. †Reference category. ‡The sum does not add up to the total because of missing values.

**Table 4.** Spearman's rank correlation coefficients between signal joint T cell receptor excision circles (sjTRECs) levels and T cell subsets in overall, human immunodeficiency virus (HIV)$^+$ and HIV$^-$ patients.

| | SjTRECs/$10^6$PBMCs | |
|---|---|---|
| | $r$ | $P$-value |
| All patients* | | |
| CD4$^+$CD45RA$^+$CD62L$^+$ cells/μl | 0·43 | < 0·01 |
| CD4$^+$CD45RA$^+$CD62L$^+$ % | 0·37 | 0·01 |
| CD8$^+$CD45RA$^+$CD62L$^+$ cells/μl | 0·37 | 0·01 |
| CD8$^+$CD45RA$^+$CD62L$^+$ % | 0·59 | < 0·001 |
| HIV$^+$* | | |
| CD4$^+$CD45RA$^+$CD62L$^+$ cells/μl | 0·25 | 0·25 |
| CD4$^+$CD45RA$^+$CD62L$^+$ % | 0·40 | 0·05 |
| CD8$^+$CD45RA$^+$CD62L$^+$ cells/μl | 0·44 | < 0·05 |
| CD8$^+$CD45RA$^+$CD62L$^+$ % | 0·54 | < 0·01 |
| HIV$^-$ | | |
| CD4$^+$CD45RA$^+$CD62L$^+$ cells/μl | 0·58 | < 0·01 |
| CD4$^+$CD45RA$^+$CD62L$^+$ % | 0·39 | 0·05 |
| CD8$^+$CD45RA$^+$CD62L$^+$ cells/μl | 0·61 | < 0·01 |
| CD8$^+$CD45RA$^+$CD62L$^+$ % | 0·71 | < 0·001 |

*Few data are missing. PBMCs, peripheral blood mononuclear cells.

In lymphoma patients overall we did not find an association between sjTRECs levels and sex, mirroring results obtained by some authors in healthy donors [1,28,29]. Furthermore, in agreement with other reports [28,30,31] a significant, although weak, association of sjTRECs content with age was found; this correlation confirms that post-CT naive T cell production is a direct consequence of thymic output, suggesting that other phenomena, such as the release of previously sequestered TREC$^+$ naive T cells from the lymphoid microenvironment, could contribute only partially to mask the true thymic activity. We found that lower sjTRECs levels were associated significantly with lymphoma patients compared to normal individuals, confirming that thymic output is generally depressed in patients with haematological malignancy and/or after CT [1]. However, we observed that different histological types of lymphoma seemed to influence the thymic reservoir in the same way; it is worth noting that the design of our study does not allow to rule out entirely that the type of lymphoma could affect differently thymic reservoir. Indeed, some reports showed that distinct haematological malignancies had a different impact on thymic function at onset of disease [1,32,33]. In addition, we did not find any association between thymic output markers and different previous CT types or number of cycles, suggesting that multiple and various CT regimens could induce a similar reduction of thymic reservoir regardless of their intensity.

When we analysed demographic and clinical characteristics of lymphoma patients stratified by HIV infection, our data showed a statistically significant different distribution of gender between HIV$^+$ and HIV$^-$ lymphoma subjects, with a lower HIV$^+$ risk for females. Furthermore, the histological type of the haematological malignancy and type of previous

**Table 5.** Odds ratios (OR) and corresponding 95% confidence intervals (CI) of some activation markers in 55 lymphoma patients by human immunodeficiency virus (HIV) infection.

| | HIV$^+$ ($n = 26$) $n$ (%) | HIV$^-$ ($n = 29$) $n$ (%) | Multivariate* OR (95% CI) |
|---|---|---|---|
| CD4$^+$ HLADR$^+$ %† | | | |
| < 16·3 | 7 (31·8) | 7 (31·8) | 1‡ |
| 16·3–29·8 | 7 (31·8) | 8 (36·4) | 0·66 (0·13–3·39) |
| ≥ 29·9 | 8 (36·4) | 7 (31·8) | 0·77 (0·14–4·08) |
| $\chi^2$ trend; $P$ | | | 1·20; $P = 0·27$ |
| CD8$^+$CD38$^+$ %† | | | |
| < 6·6 | 4 (18·2) | 7 (30·4) | 1‡ |
| 6·6–19·0 | 7 (31·8) | 8 (34·8) | 1·17 (0·21–6·45) |
| ≥ 19·1 | 11 (50·0) | 8 (34·8) | 1·68 (0·37–7·62) |
| $\chi^2$ trend; $P$ | | | 0·67; $P = 0·41$ |
| CD4$^+$ naive HLADR$^+$ %† | | | |
| < 2·6 | 7 (31·8) | 8 (34·8) | 1‡ |
| 2·6–22·0 | 7 (31·8) | 8 (34·8) | 1·03 (0·21–4·95) |
| ≥ 22·1 | 8 (36·4) | 7 (30·4) | 1·28 (0·24–6·92) |
| $\chi^2$ trend; $P$ | | | 0·09; $P = 0·76$ |
| CD8$^+$ naive CD38$^+$ %† | | | |
| < 2·5 | 7 (31·8) | 2 (8·7) | 1‡ |
| 2·5–25·1 | 8 (36·4) | 15 (65·2) | 0·35 (0·08–1·59) |
| ≥ 25·2 | 7 (31·8) | 6 (26·1) | 0·63 (0·11–3·72) |
| $\chi^2$ trend; $P$ | | | 1·02; $P = 0·31$ |
| CD4$^+$ RA : RO ratio† | | | |
| < 0·2 | 11 (45·8) | 12 (44·4) | 1‡ |
| 0·2–0·5 | 8 (33·3) | 9 (33·3) | 2·66 (0·47–15·09) |
| ≥ 0·6 | 5 (20·8) | 6 (22·2) | 1·76 (0·31–9·97) |
| $\chi^2$ trend; $P$ | | | 0·80; $P = 0·37$ |
| CD8$^+$ RA : RO ratio† | | | |
| < 0·2 | 9 (40·9) | 8 (34·8) | 1‡ |
| 0·2–0·4 | 6 (27·3) | 9 (39·1) | 1·18 (0·24–5·80) |
| ≥ 0·5 | 7 (31·8) | 6 (26·1) | 2·56 (0·43–15·29) |
| $\chi^2$ trend; $P$ | | | 0·62; $P = 0·43$ |

*Adjusted for statistically significant parameters at univariate analysis described in Table 1. †The sum does not add up to the total because of missing values. ‡Reference category.

CT regimens administered were distributed differently between the two groups. Our univariate and multivariate analyses including adjustment for these potential confounding factors showed that sjTRECs/$10^6$ PBMCs levels were distributed similarly between HIV$^+$ and HIV$^-$ subjects, suggesting that HIV infection has a limited effect on thymic output. In addition, sjTRECs/$10^6$ CD3$^+$ seems not to be affected by HIV infection. Nevertheless, it is worth noting that to a certain extent this parameter could be influenced by sex, lymphoma histology and/or type of previous CT, as we found a loss of significance in the association between low sjTRECs/$10^6$ CD3$^+$ values and HIV infection after multivariate calculation. This observation, in conjunction with an association found between high sjTRECs/$10^6$ CD3$^+$ levels and increased TECT in overall patients (data not shown), indicates that thymic activity evaluation by sjTRECs/CD3$^+$ could be more sensitive and informative than sjTRECs/PBMCs. With regard to naive CD4$^+$ and CD8$^+$ T cell levels, our analyses also confirmed that HIV infection does not influence these parameters substantially.

Our data on overall lymphoma patients showed a positive correlation between sjTRECs values and the naive CD4$^+$ and CD8$^+$ T cell subsets, suggesting that repopulating naive T cells are indeed of thymic origin and confirming that the thymus of patients who have received CT might be able to generate T cells *de novo* [34]. When we stratified our data by HIV infection, this association with sjTRECs levels was maintained, except for absolute naive CD4$^+$ T cells numbers in HIV$^+$, where this subset could be an overestimate of T cell neogenesis. In fact, T cells expressing the RA isoform may expand in the absence of specific antigenic stimulation, maintaining CD45RA expression, or may also derive from expansions of a limited number of mature, post-thymic cell revertants from memory to naive phenotype [8,35]. Our observation, together with the absence of an association between naive T cells and age in the overall lymphoma cases (data not shown), suggests that the sjTRECs represents a more sensitive parameter to define the thymic output in this cohort of patients.

The relation between the TREC content and thymic output may be affected by several other parameters which can be altered in HIV infection, such as the dilution of TREC-bearing cells in the periphery by cell division, apoptosis rate and the longevity of naive T cells [36,37]. Therefore, we could not rule out that the absence of difference in sjTRECs values in our groups could be due to the selection of our HIV$^+$ population. In fact, HIV$^+$ patients overall in this study were receiving HAART regimens for a long time and most of them had undetectable viral load, leading possibly to reduction of cell activation, proliferation and apoptosis [38–40]. In fact, we observed no differences in the expression of lymphocyte activation parameters (HLA-DR and CD38 expression, RA : RO ratios) between HIV$^+$ and HIV$^-$ groups, while data on cell proliferation or apoptosis were not available. However, the influence of HIV replication on sjTREC levels may be supported by our results because, after subgrouping HIV$^+$ subjects, a certain level of T cell activation and lower sjTRECs values were found among HIV$^+$ patients with detectable HIV load within 3 months from baseline [36,41–43]. Regarding naive T cell longevity, it is well known that CT induces a loss of more than 90% of peripheral CD45RA$^+$ T cells; therefore, it can be speculated that the impact of previous treatments on the naive long-lived T cell subpopulation would be similar in both groups [44].

In conclusion, this is the first study that describes the sjTRECs levels in a relatively wide cohort of HIV$^-$ and HIV$^+$ patients (candidates for ASCT) with relapsed or refractory lymphoma. Although our data should be interpreted with caution, given the heterogeneity of the patient population, our analyses suggest that *de novo* T cell generation is kept partially in these patients and could contribute to restoring the immune function after transplantation. Different chemotherapeutic treatments induce similar effects on thymic

 

**Table 6.** Evaluation of signal joint T cell receptor excision circles (sjTRECs) levels and lymphocyte subpopulations between non-viraemic and viraemic human immunodeficiency (HIV)$^+$ patients.

| Parameter | HIV RNA ≤ 50 cp/ml Median (range) $n = 16$ | HIV RNA > 50 cp/ml Median (range) $n = 8$ | $P$-value* |
|---|---|---|---|
| Age | 41 (29–67) | 41 (29–58) | 0·71 |
| HIV viraemia cp/ml | – | 1272 (204–30 5426) | n.a. |
| SjTRECs/$10^6$PBMCs | 508 (5–8150) | 143 (32–511) | < 0·05 |
| CD4$^+$cells/μl | 181 (13–399) | 155 (35–293) | 0·60 |
| CD4$^+$ % | 19·5 (4·0–43·0) | 9·5 (6·0–19·0) | < 0·01 |
| CD4$^+$CD45RA$^+$CD62L$^+$ cells/μl | 45 (0–141)$^\dagger$ | 8·5 (1–198) | 0·31 |
| CD4$^+$CD45RA$^+$CD62L$^+$ % | 29·0 (0·0–79·0)$^\dagger$ | 7·5 (2·0–33·0) | 0·12 |
| CD8$^+$cells/μl | 315 (44–912) | 808 (96–3607) | < 0·05 |
| CD8$^+$ % | 60·1 (28·0–77·0) | 69·5 (40·0–89·0) | 0·11 |
| CD8$^+$CD45RA$^+$CD62L$^+$ cells/μl | 80 (6–327)$^\dagger$ | 34 (13–464) | 0·35 |
| CD8$^+$CD45RA$^+$CD62L$^+$ % | 24·3 (7·3–65·0)$^\dagger$ | 5·8 (1·5–46·4) | < 0·01 |
| CD4$^+$HLADR$^+$ % | 18·0 (4·8–39·4)$^\dagger$ | 41·0 (12·4–72·6) | 0·13 |
| CD8$^+$CD38$^+$ % | 14·9 (3·2–74·1)$^\dagger$ | 25·8 (8·0–84·3) | 0·42 |
| CD4$^+$ naive HLADR$^+$ % | 10·9 (2·0–97·1)$^\dagger$ | 10·1 (1·6–91·8) | 0·97 |
| CD8$^+$ naive CD38$^+$ % | 5·6 (0·3–100·0)$^\dagger$ | 16·2 (0·6–56·2) | 0·91 |
| CD4$^+$ RA : RO ratio | 0·4 (0·0–1·1)$^\dagger$ | 0·1 (0·0–0·5) | 0·12 |
| CD8$^+$ RA : RO ratio | 0·7 (0·1–4·3)$^\dagger$ | 0·2 (0·0–1·1) | < 0·05 |

*Patients' groups were compared by Mann–Whitney $U$-test. $^\dagger$Few data are missing; n.a., not applicable; PBMCs, peripheral blood mononuclear cells.

reservoir, independently from their intensity (type and number of cycles of previous CT). Moreover, HIV infection is not a detrimental factor on thymic output at the time of lymphoma relapse. Continuously suppressed HIV RNA levels, under effective HAART, should be a prerequisite in order to consider sjTRECs as a specific marker for the evaluation of thymic function. Our study suggests that sjTRECs, in conjunction with immunological parameters analyses, could be a useful clinical marker to identify lymphoma patients who require close immunological monitoring and who might benefit from new strategies to improve the immune system. Further studies, possibly with additional parameters as suggested by Dion *et al.* [45,46], should be undertaken to assess the residual thymic function in larger and more homogeneous cohorts of recurrent lymphoma patients and to understand the clinical relevance of sjTREC evaluation during post-ASCT follow-up.

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## References

1 Chen X, Barfield R, Benaim E *et al.* Prediction of T-cell reconstitution by assessment of T-cell receptor excision circle before allogeneic hematopoietic stem cell transplantation in pediatric patients. Blood 2005; **105**:886–93.

2 Hazenberg MD, Otto SA, de Pauw ES *et al.* T-cell receptor excision circle and T-cell dynamics after allogeneic stem cell transplantation are related to clinical events. Blood 2002; **99**:3449–53.

3 Talvensaari K, Clave E, Douay C *et al.* A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. Blood 2002; **99**:1458–64.

4 Weinberg K, Blazar BR, Wagner JE *et al.* Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. Blood 2001; **97**:1458–66.

5 Douek DC, Vescio RA, Betts MR *et al.* Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. Lancet 2000; **355**:1875–81.

6 Malphettes M, Carcelain G, Saint-Mezard P *et al.* Evidence for naive T-cell repopulation despite thymus irradiation after autologous transplantation in adults with multiple myeloma: role of *ex vivo* CD34+ selection and age. Blood 2003; **101**:1891–7.

7 Guillaume T, Rubinstein DB, Symann M. Immune reconstitution and immunotherapy after autologous hematopoietic stem cell transplantation. Blood 1998; **92**:1471–90.

8 Kolte L, Dreves AM, Ersboll AK *et al.* Association between larger thymic size and higher thymic output in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy. J Infect Dis 2002; **185**:1578–85.

9 Franco JM, Rubio A, Martinez-Moya M *et al.* T-cell repopulation and thymic volume in HIV-1-infected adult patients after highly active antiretroviral therapy. Blood 2002; **99**:3702–6.

10 Markert ML, Alvarez-McLeod AP, Sempowski GD *et al.* Thymopoiesis in HIV-infected adults after highly active antiretroviral therapy. AIDS Res Hum Retroviruses 2001; **17**:1635–43.

11 Pham T, Belzer M, Church JA *et al.* Assessment of thymic activity in human immunodeficiency virus-negative and -positive adolescents by real-time PCR quantitation of T-cell receptor rearrangement excision circles. Clin Diagn Lab Immunol 2003; **10**:323–8.

12 Touloumi G, Pantazis N, Karafoulidou A *et al.* Changes in T cell receptor excision DNA circle (TREC) levels in HIV type 1-infected


 

subjects pre- and post-highly active antiretroviral therapy. AIDS Res Hum Retroviruses 2004; **20**:47–54.
13 Spina M, Tirelli U, Zagonel V *et al.* Burkitt's lymphoma in adults with and without human immunodeficiency virus infection: a single-institution clinicopathologic study of 75 patients. Cancer 1998; **82**:766–74.
14 Vaccher E, Tirelli U, Spina M *et al.* Age and serum lactate dehydrogenase level are independent prognostic factors in human immunodeficiency virus-related non-Hodgkin's lymphomas: a single-institute study of 96 patients. J Clin Oncol 1996; **14**:2217–23.
15 Vaccher E, Spina M, Talamini R *et al.* Improvement of systemic human immunodeficiency virus-related non-Hodgkin lymphoma outcome in the era of highly active antiretroviral therapy. Clin Infect Dis 2003; **37**:1556–64.
16 Krishnan A, Molina A, Zaia J *et al.* Durable remissions with autologous stem cell transplantation for high-risk HIV-associated lymphomas. Blood 2005; **105**:874–8.
17 Gabarre J, Azar N, Autran B *et al.* High-dose therapy and autologous haematopoietic stem-cell transplantation for HIV-1-associated lymphoma. Lancet 2000; **355**:1071–2.
18 Re A, Cattaneo C, Michieli M *et al.* High-dose therapy and autologous peripheral-blood stem-cell transplantation as salvage treatment for HIV-associated lymphoma in patients receiving highly active antiretroviral therapy. J Clin Oncol 2003; **21**:4423–7.
19 Simonelli C, Michieli M. Autologous stem cell transplantation for HIV-infected individuals with relapsed lymphomas: no longer an experimental strategy. Blood 2005; **105**:439–40.
20 Benicchi T, Ghidini C, Re A *et al.* T-cell immune reconstitution after hematopoietic stem cell transplantation for HIV-associated lymphoma. Transplantation 2005; **80**:673–82.
21 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning, 2nd edn. Woodbury: Cold Spring Harbor Laboratory Press, 1989.
22 Bortolin MT, Pratesi C, Dolcetti R *et al.* Clinical value of Epstein–Barr virus DNA levels in peripheral blood samples of Italian patients with undifferentiated carcinoma of nasopharyngeal type. Cancer Lett 2006; **233**:247–54.
23 Sempowski G, Thomasch J, Gooding M *et al.* Effect of thymectomy on human peripheral blood T cell pools in myasthenia gravis. J Immunol 2001; **166**:2808–17.
24 Breslow NE, Day NE. Statistical methods in cancer research, vol. I: The analysis of case–control studies. IARC Scientific Publication. 32. Lyon: IARC Press, 1980.
25 Gabarre J, Marcelin AG, Azar N *et al.* High-dose therapy plus autologous hematopoietic stem cell transplantation for human immunodeficiency virus (HIV)-related lymphoma: results and impact on HIV disease. Haematologica 2004; **89**:1100–8.
26 Heitger A, Winklehner P, Obexer P *et al.* Defective T-helper cell function after T-cell-depleting therapy affecting naive and memory populations. Blood 2002; **99**:4053–62.
27 Lewin SR, Heller G, Zhang L *et al.* Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. Blood 2002; **100**:2235–42.
28 Geenen V, Poulin JF, Dion ML *et al.* Quantification of T cell receptor rearrangement excision circles to estimate thymic function: an important new tool for endocrine-immune physiology. J Endocrinol 2003; **176**:305–11.
29 Zhang L, Lewin SR, Markowitz M *et al.* Measuring recent thymic emigrants in blood of normal and HIV-1-infected individuals before and after effective therapy. J Exp Med 1999; **190**:725–32.
30 Hug A, Korporal M, Schroder I *et al.* Thymic export function and T cell homeostasis in patients with relapsing remitting multiple sclerosis. J Immunol 2003; **171**:432–7.
31 Yasunaga J, Sakai T, Nosaka K *et al.* Impaired production of naive T lymphocytes in human T-cell leukemia virus type I-infected individuals: its implications in the immunodeficient state. Blood 2001; **97**:3177–83.
32 Clave E, Rocha V, Talvensaari K *et al.* Prognostic value of pretransplantation host thymic function in HLA-identical sibling hematopoietic stem cell transplantation. Blood 2005; **105**:2608–13.
33 Petridou E, Klimentopoulou AE, Moustaki M *et al.* Recent thymic emigrants and prognosis in T- and B-cell childhood hematopoietic malignancies. Int J Cancer 2002; **101**:74–7.
34 Sfikakis PP, Gourgoulis GM, Moulopoulos LA *et al.* Age-related thymic activity in adults following chemotherapy-induced lymphopenia. Eur J Clin Invest 2005; **35**:380–7.
35 Hargreaves M, Bell EB. Identical expression of CD45R isoforms by CD45RC+ 'revertant' memory and CD45RC+ naive CD4 T cells. Immunology 1997; **91**:323–30.
36 Harris JM, Hazenberg MD, Poulin JF *et al.* Multiparameter evaluation of human thymic function: interpretations and caveats. Clin Immunol 2005; **115**:138–46.
37 Hazenberg MD, Borghans JA, de Boer RJ *et al.* Thymic output: a bad TREC record. Nat Immunol 2003; **4**:97–9.
38 Badley AD, Parato K, Cameron DW *et al.* Dynamic correlation of apoptosis and immune activation during treatment of HIV infection. Cell Death Differ 1999; **6**:420–32.
39 Gougeon ML, Lecoeur H, Sasaki Y. Apoptosis and the CD95 system in HIV disease: impact of highly active anti-retroviral therapy (HAART). Immunol Lett 1999; **66**:97–103.
40 Johnson N, Parkin JM. Anti-retroviral therapy reverses HIV-associated abnormalities in lymphocyte apoptosis. Clin Exp Immunol 1998; **113**:229–34.
41 Douek DC, McFarland RD, Keiser PH *et al.* Changes in thymic function with age and during the treatment of HIV infection. Nature 1998; **396**:690–5.
42 Nobile M, Correa R, Borghans JA *et al. De novo* T-cell generation in patients at different ages and stages of HIV-1 disease. Swiss HIV Cohort Study. Blood 2004; **104**:470–7.
43 Steffens CM, Smith KY, Landay A *et al.* T cell receptor excision circle (TREC) content following maximum HIV suppression is equivalent in HIV-infected and HIV-uninfected individuals. AIDS 2001; **15**:1757–64.
44 Hakim FT, Cepeda R, Kaimei S *et al.* Constraints on CD4 recovery postchemotherapy in adults: thymic insufficiency and apoptotic decline of expanded peripheral CD4 cells. Blood 1997; **90**:3789–98.
45 Dion ML, Poulin JF, Bordi R *et al.* HIV infection rapidly induces and maintains a substantial suppression of thymocyte proliferation. Immunity 2004; **21**:757–68.
46 Dion ML, Bordi R, Zeidan J *et al.* Slow disease progression and robust therapy-mediated CD4+ T-cell recovery are associated with efficient thymopoiesis during HIV-infection. Blood 2007; **109**:2912–20.

 