Premature immunosenescence in HIV-infected patients on highly active antiretroviral therapy with low-level CD4 T cell repopulation

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Objectives: To analyse the role of thymic function and its association with cellular immunosenescence markers in patients with low-level CD4 T cell repopulation, despite complete HIV RNA replication control on highly active antiretroviral therapy (HAART).

Methods: Cellular immunosenescence markers comparing patients with CD4 T cell counts ≤250 cells/mm³ for ≥48 weeks ($n=11$) and patients with a CD4 T cell count ≥500 cells/mm³ ($n=11$) were investigated. Both groups were also compared with 11 healthy volunteers of similar age. Naive CD4 T cell counts, β- and δ-T cell rearrangement excision circles, recent thymic emigrants, replicative senescence marker, cell activation, and rate of apoptosis were analysed. The Mann–Whitney *U*-test was used to compare parameters between both low-level and high-level CD4 T cell repopulation groups, and healthy volunteers.

Results: Our results showed a lower thymic activity in patients with low-level CD4 T cell repopulation, leading to a decline in CD4 T cell production. On the other hand, a higher activation along with a higher replicative senescence of CD4 T cells contributed to a higher rate of apoptotic CD4 T cells in this group of patients.

Conclusions: We propose a model with several different related mechanisms involved in premature immune senescence in HIV-infected patients with low-level CD4 repopulation on HAART. The understanding of such different mechanisms could help find effective strategies to prevent immune decay.

Keywords: CD4 T cells, HIV, activation, apoptosis, thymic function, senescence, HAART

Introduction

CD4 T cell increases have been shown to be associated with the level of virus suppression in HIV-infected patients on highly active antiretroviral therapy (HAART), although with a substantial variability among them.[1–3] However, some patients show only a low CD4 T cell level, despite HIV RNA replication control on HAART.[4–6] Moreover, HIV-infected patients with an altered T cell homeostasis are of special interest because they may be more susceptible to opportunistic infections.[7]

The low CD4 T cell repopulation could be due either to low cell reconstitution or high cell destruction. Several mechanisms have been reported to be involved, such as apoptosis of uninfected cells,[8,9] lack of cell redistribution from secondary lymphoid organs,[10,11] and poor proliferative capacity of senescent CD4 T cells.[6] Another mechanism to explain a higher impairment in the immune system is the presence of thymotropic virus.[5] However, the importance of thymic dysfunction in HAART-associated T cell restoration remains controversial.[12] Although Delobel *et al.*[5] reported a similar frequency of recent

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thymic emigrants in patients with low and high CD4 T cell repopulation, we have previously demonstrated that thymic volume is the best predictor of the speed and degree of CD4 T cell recovery in patients receiving HAART.[13–15] Moreover, it has been recently observed that patients with low CD4 T cell repopulation had a quantitative and functional hematopoietic progenitor cell defect.[16] In addition, a slower CD4 T cell repopulation correlated with lower thymic volume in patients with an increase in CD4 T cell count of <100 cells/mm^{3} after 48 weeks on HAART.[17]

Some characteristics found in HIV-infected patients with low CD4 T cell repopulation could show similarities with factors associated with immunosenescence in the elderly. This ageing is involved in many processes affecting the functionality of the immune system, i.e. thymic involution, alteration of the T cell repertoire, cellular activation, apoptosis, and also decline of the frequency of CD4 T cells that is a consequence of interleukin (IL)-2 and IL-2 receptor down-regulation.[18–20] Therefore, our aim was to analyse the role of thymic function and its association with cellular immunosenescence markers in patients with low-level CD4 T cell repopulation, despite complete HIV RNA replication control on HAART.

Materials and methods

Patients

A cross-sectional study was performed in a cohort of HIV-1-infected patients from the Infectious Diseases Service at Virgen del Rocío University Hospital, Spain. Eleven consecutive patients who had undetectable viral load since they started HAART ($\geq$48 weeks) and had $\leq$250 cells/mm[3] at the time of study were included as the low-level CD4 T cell repopulation group. In addition, another 11 consecutive patients who had undetectable viral load since they started HAART ($\geq$48 weeks) and had $\geq$500 cells/mm^{3} at the time of study were included as the high-level CD4 T cell repopulation group. Both groups were compared with 11 healthy volunteers of similar age. A written informed consent was obtained from all the patients and the ethics committee of the hospital approved the study.

HIV RNA quantification and hepatitis C virus (HCV) co-infection

Plasma HIV-1 RNA was measured by quantitative PCR (HIV Monitor[TM] Test kit version 1.5, Roche Molecular System, Hoffman-La Roche, Basel, Switzerland) according to the manufacturer's instructions. This assay has a detection limit of 50 HIV-1 RNA copies/mL. HCV RNA was quantified using plasma samples by a commercially available PCR procedure (COBAS Amplicor, Roche Diagnostics, Barcelona, Spain).

Immunophenotypic analysis

Fresh samples were used to determine absolute numbers of CD4 T cells and percentages of naive CD4 T cells (CD4-PerCP, CD45RA-FITC, CD27-PE) by flow cytometry, as previously described.[21,22] Cells were analysed using a BD FACSCalibur cytometer and CellQuest Software. Using multiparametric flow cytometry, percentages of naive CD4 T cells that also expressed CD57 were analysed in frozen samples. CD57 was used to determine the immune system exhaustion. The monoclonal antibodies used for the analysis of lymphocyte subsets were CD57-FITC, CD45RA-ECD, CD27-PC5

and CD4-PC7 (Immunotech, Marseille, France). Cells were analysed using the Cytomics FC 500 Flow Cytometer (Beckman Coulter, Fullerton, CA, USA) and CXP software (Beckman Coulter) acquisition program immediately after cell staining.

δ-/β-T cell receptor excision circle (TREC) ratio level quantification in isolated CD4 T cells

TRECs are generated by the rearrangement of T cell receptor genes during intrathymic maturation.[23] DNA from isolated CD4 T cells was used for the quantification of β- and δ-TRECs. CD4 T cells were isolated from peripheral blood mononuclear cells (PBMCs) by immunomagnetic-positive separation (Dynabeads, Dynal) using monoclonal anti-CD4 antibodies according to the manufacturer's instructions.

The determination of β- and δ-TREC contents was carried out using the following protocol: (i) two first-round amplifications were performed in parallel, one amplifying the six fragments of β-TRECs using 80 nM of each of the six sense primers (T3A, T3B, T3C, T3D, T3E, T3F) and 280 nM of antisense AS primer, and the other amplifying the δ-TRECs using 100 nM of primers DTF6 and DTR61; and (ii) one second-round amplification (real-time PCR) of both β- and δ-TRECs together using 200 nM of primers T3R, T2, DTF7 and DTR66, and probes TCRFL/TCRLC and P1/P2 (Table 1).

Both first rounds were performed in triplicate with an initial denaturation at 95°C for 10 min, followed by 20 cycles of 20 s at 95°C, 45 s at 57°C and 30 s at 72°C, with a final elongation at 72°C for 5 min. The second-round amplification was run using the LightCycler® FastStart DNA MasterPLUS (Roche Molecular Biochemicals) at 95°C for 10 min, followed by 45 cycles of 95°C for 10 s, 57°C for 20 s and 72°C for 15 s. Fluorescence was acquired at the end of the annealing phase using hybridization probes coupled to the acceptor fluorophores LC640 or Red705.

To perform the standard curve, amplification products of both the β- and δ-TREC first-round PCRs were cloned into the pGEM T-EASY vector according to the manufacturer's instructions (Promega, Madison, WI, USA). The concentrations of the different plasmids were measured by quantitative real-time PCR with SP6/T7 primers using the LightCycler® FastStart DNA MasterPLUS SYBR Green I (Roche Molecular Biochemicals, Mannheim, Germany). PCR conditions were 10 s at 95°C followed by 40 cycles of 95°C for 10 s, 50°C for 15 s and 72°C for 20 s. The standard curve for the ratio determination was performed by mixing the δ-TREC plasmid with an equimolecular mix of all four cloned β-TRECs at a ratio of 25:1. Serial dilutions of the standard curve were used to refer the amplification products from unknown samples of both the β- and δ-TRECs. Thus, known concentrations of the cloned fragments, the crossing point of β- and δ-TREC molecules, and known CD4 T cell DNA concentration were used to calculate β- and δ-TRECs per 10[6] CD4 T cells.

Thymic volume

Six patients with low-level CD4 T cell repopulation and seven patients with high-level CD4 T cell repopulation had previously participated in other studies and their thymic volume measurements had been recorded before starting HAART. Mediastinic computed tomography was performed with a modified method previously described.[24] Briefly, 5 mm thick contiguous mediastinic sections at 5 mm intervals were always measured by the same radiologist using a 3000 GE Sytec Scanner in coded samples. Thymic tissue was carefully delimited in all the slices between the first and the last to exclude mediastinic fat infiltration (high density for soft tissue and

Table 1. Primers and probes used for the quantification of δ- and β-TRECs

Name	Sequence (5′→3′)
First round of amplification A	
T3A	CGGCCGTAGCGACGTACCCTTTCGATGGACCCTCACAG
T3B	CGGCCGTAGCGACGTACCGACAAGGCACCAGACTCACAG
T3C	CGGCCGTAGCGACGTACCAAGCTCTGGAAGGGAACACAG
T3D	CGGCCGTAGCGACGTACCCCGTTTCTCTCCCTCACACAG
T3E	CGGCCGTAGCGACGTACCGGGCAGAAACTGAGAACACAG
T3F	CGGCCGTAGCGACGTACCCTTGCGCCTTATGCTGCACAG
AS	TGAACCAAATTGCATTAAGACC
First round of amplification B	
DTR61	TCTGACATTTGCTCCGTG
DTF6	AGAAGGCTCTGTCTAGTGTG
Second round of amplification	
DTF7	AGGCTCTGTCTAGTGTGATAAC
T2	CCCAGGAGGAAAGAAGAGGAC
T3R	CGGCCGTAGCGACGTACC
DTR66	TGACATGGAGGGCTGAAC
Probes	
P1	CTGGGAGTTGGGACCGCCAGAGAGGT-FL
P2	Red705-TTTGTAAAGGTTTCCCGTAGAGTTGAATCATTGTG-PH
TCRFL	AGGGATGTGGCATCACCTTTGTTGACA-FL
TCRLC	LC640-GGCACCCCTCTGTTCCCCACAGGA-PH

low density for surrounding fat). CT Sytec software (version 4.0, General Electrics Medical Systems, Milwaukee, WI, USA) integrated all the defined thymic areas along the slices (the first and last slice volumes being automatically halved to account for partial volume averaging) to calculate thymic volume.[25]

Analysis of cell activation

Activated CD4 T cells from fresh samples were analysed using CD4-PerCP in combination with CD38-FITC and HLA-DR-PE (Becton Dickinson monoclonal antibodies) by flow cytometry. The level of β_2-microglobulin in serum samples was determined using a turbidimetric microparticle enzyme immunoassay (Tina-quant, β_2-microglobulin, Roche Diagnostics, USA), according to the manufacturer's recommendations.

Apoptosis level

Apoptotic rate was analysed in fresh CD4 T cells with vital dye 7AAD and annexin-V PE (Becton Dickinson), according to the manufacturer's instructions. Four types of cells can be identified: (i) viable cells (annexin-V$^-$ 7AAD$^-$); (ii) cells in the early stage of apoptosis (annexin-V$^+$ 7AAD$^-$); (iii) apoptotic dead cells (annexin-V$^+$ 7AAD$^+$); and (iv) necrotic dead cells (annexin-V$^-$ 7AAD$^+$). In addition, intracellular activated caspase-3 was analysed in permeabilized CD4 T cells using anti-cleaved caspase-3 polyclonal antibody (Becton Dickinson).

For the detection of Bid protein, equal amounts of total extracted protein (50 μg) from PBMCs, as determined by using a BCA protein assay kit (Pierce, Rockford, IL, USA), were separated by SDS–PAGE on 12% polyacrylamide gels (Invitrogen, Carlsbad, CA, USA) and electroblotted onto nitrocellulose membranes (Amersham, Little Chalfont, UK). Membranes were then blocked with 1% blocking reagent (Roche, Mannheim, Germany) in 0.05% Tween 20–PBS and incubated with anti-Bid polyclonal antibody (dilution 1:1000; Cell Signaling Technology, Danvers, MA, USA) in blocking buffer overnight. Immunological complexes were revealed with horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:1000 dilution; Cell Signaling Technology) in blocking buffer for 1 h at room temperature. Bid protein was detected by chemoluminescence following film exposure. Scanning densitometry was performed with the Scan Analysis software (Biosoft, Cambridge, UK). Arbitrary densitometric units of the protein of interest were corrected for those of β-actin.

Statistical analysis

All continuous variables were expressed as a median for each variable [interquartile range (IQR)], and categorical variables as number of cases and percentage. The Mann–Whitney U-test was used to compare parameters between both low-level and high-level CD4 T cell repopulation groups, and healthy volunteers. Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS 15, Chicago, IL, USA). A P value <0.05 was considered statistically significant.

Results

Characteristics of the patients

Comparisons between the low-level and high-level CD4 T cell repopulation groups are shown in Table 2. Only two variables were statistically different between the two groups: total CD4 T cell count ($P<0.001$), since this was a selection criterion; and nadir CD4 T cell count ($P<0.001$). No other statistical differences were found.

Table 2. Comparison between low-level and high-level CD4 T cell repopulation patients at the time of study

	Low-level CD4 repopulation patients (*n*=11)	High-level CD4 repopulation patients (*n*=11)	*P*
Age (years)	45 (42−49)	50 (36−61)	0.43
Male	10 (90.9)	9 (81.8)	0.53
Injecting drug users	4 (36.3)	1 (9.2)	0.13
HCV co-infection	4 (36.3)	2 (18.2)	0.34
Protease inhibitor-based treatment	4 (36.3)	3 (27.3)	0.65
CDC clinical stage C	4 (36.3)	2 (18.2)	0.34
CD4 count (cells/mm^3)	169 (112−203)	587 (546−635)	**<0.001**
Weeks on HAART	124 (57−224)	202 (154−284)	0.22
Time since HIV diagnosis (years)	4.4 (2.8−11.1)	3.5 (2.4−5.7)	0.37
Nadir CD4 count (cells/mm^3)	23 (4−44)	239 (189−289)	**<0.001**

Continuous variables are expressed as median (IQR) and categorical variables are expressed as number of cases (%). Statistically significant results are shown in bold.

Thymic function

Percentage of naive CD4 T cells (CD4+ CD45RA+ CD27+) was significantly lower in the low-level CD4 T cell repopulation group compared with both the high-level CD4 T cell repopulation group (*P*=0.023) and healthy donors (*P*<0.001), as shown in Figure 1(a).

The median δ-TRECs per million CD4 T cells was significantly lower in the low-level CD4 T cell repopulation group compared with both the high-level CD4 T cell repopulation group (*P*=0.034) and healthy donors (*P*<0.001), as shown in Figure 1(b). In contrast, β-TREC quantification in low-level CD4 T cell repopulation patients was below the detection limit.
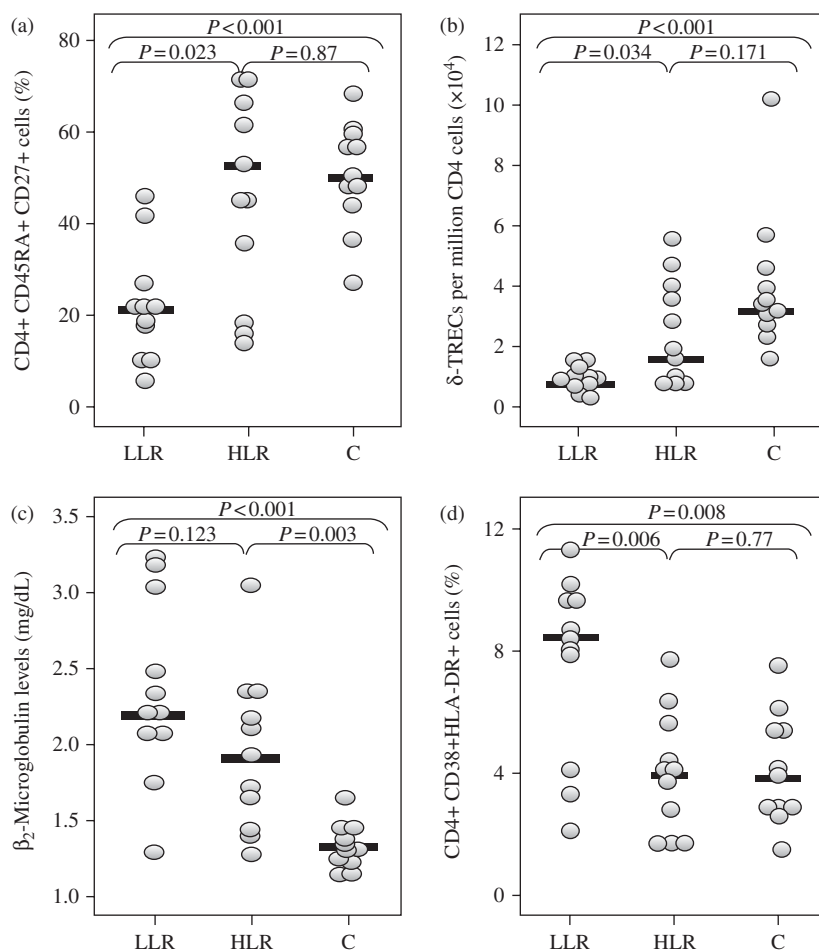

Figure 1. (a) Percentage of naive CD4 cells. (b) δ-TRECs per million cells. (c) Levels of β$_2$-microglobulin in serum samples. (d) Proportion of HLA-DR and CD38 expression in CD4 cells. LLR, low-level CD4 repopulation; HLR, high-level CD4 repopulation; C, healthy controls.

Table 3. Quantification of δ-/β-TREC ratios in CD4 T cells in the different groups of studied patients

Patients	δ-TRECs[a]	β-TRECs[a]	δ-/β-TREC ratio
Low-level CD4 repopulation			
164	10211	—	—
372	9964	—	—
48	6523	—	—
328	11769	—	—
403	5018	—	—
356	8103	—	—
392	7838	—	—
347	12309	—	—
351	9045	—	—
74	5668	—	—
184	10900	—	—
High-level CD4 repopulation			
416	15480	620	25
354	9102	72	126
297	38078	—	—
371	27463	—	—
368	8196	—	—
121	6625	—	—
231	42401	—	—
330	10800	—	—
410	51095	—	—
370	8226	—	—
334	35682	166	214
Healthy donors			
1	15802	92	171
2	27698	—	—
3	23372	377	62
4	48328	—	—
5	28763	454	63
6	39748	506	78
7	103056	849	121
8	24558	369	67
9	31973	272	120
10	15881	661	24
11	33486	393	85

—, TREC levels below detection limits.
[a]TRECs per million CD4 T cells.

The quantification of β-TRECs in high-level CD4 T cell repopulation patients was only possible in three patients (27.2%), but in nine healthy donors (81.8%). Hence, the δ-/β-TREC ratio could not be measured in the low-level and high-level CD4 T cell repopulation groups (Table 3).

Before HAART, thymic volume was higher in patients with high-level CD4 T cell repopulation [median 3.07 cm^3 (interquartile range 2.01–6.26), $n=7$] than in low-level CD4 T cell repopulation patients [median 2.13 cm^3 (interquartile range 0.28–4.8), $n=6$], although with no statistical significance ($P=0.199$).

Activation analysis

As shown in Figure 1(c), the median level of β$_2$-microglobulin was higher in patients with low-level CD4 T cell repopulation compared with patients with high-level CD4 T cell repopulation ($P=0.123$), although with no statistical significance. Both levels were statistically higher than the levels found in healthy controls ($P<0.001$ and $P=0.003$, respectively). In addition, HLA-DR and CD38 were highly expressed by CD4 T cells in the low-level CD4 T cell repopulation group compared with the high-level CD4 T cell repopulation group ($P=0.006$) (Figure 1d).

Analysis of CD4 T cell senescence

To evaluate senescence, the relationship between CD57 expression and the percentage of naive CD4 T cells (CD4+ CD45RA+ CD27+) was analysed (Figure 2a). As shown in Figure 2(b), CD57 expression on naive CD4 T cells was statistically higher in the low-level CD4 T cell repopulation group compared with healthy controls ($P=0.006$), while differences were not significant when compared with the high-level CD4 T cell repopulation group ($P=0.2$).

Apoptotic rate in CD4 T cells

The expression of activated caspase-3 (Figure 3a) and annexin-V (Figure 3b) in CD4 T cells was significantly higher in low-level CD4 T cell repopulation patients ($P<0.001$) compared with the other groups. In addition, the expression of annexin-V ($P=0.023$) and active caspase-3 ($P=0.027$) was statistically higher in the high-level CD4 T cell repopulation group than in healthy controls.

Bid protein is a cytosolic member of the Bcl-2 family of apoptosis regulatory proteins[26] that is cleaved by caspase-8 to create a truncated form of Bid (tBid), which translocates to mitochondria and causes the release of cytochrome *c* into the cytosol.[27] The expression of tBid was higher in patients with low-level CD4 T cell repopulation (10 out of 11 patients, 90.9%) compared with high-level CD4 T cell repopulation patients (5 out of 11 patients, 45%) (Figure 3c). In healthy controls, only one out of 11 individuals (9%) had detectable tBid expression (data not shown).

Discussion

The present study shows that patients with a low-level CD4 T cell repopulation have a lower level of naive CD4 T cells and δ-TREC levels, and also a trend to have lower thymic volume. In addition, higher activation levels and CD57 expression in naive CD4 T cells (senescence marker) could be involved in the enhanced cellular apoptosis, directly affecting the total CD4 T cell pool.

One limitation of this study is the sample size; however, it is important to highlight that patients with low-level CD4 T cell repopulation were restricted to those with a CD4 T cell count of ≤250 cells/mm^3 and an undetectable viral load since they started HAART. There is a need to study these patients, since low-level CD4 T cell repopulation is becoming recognized as an important risk factor for developing many non-AIDS-related diseases and traditional AIDS-related events.[28]

Our results suggest deficits in *de novo* CD4 T cell production in patients with low-level CD4 T cell repopulation, since they have a lower naive CD4 T cell count, reduced levels of δ-TRECs in CD4 T cells and a trend to have a lower thymic
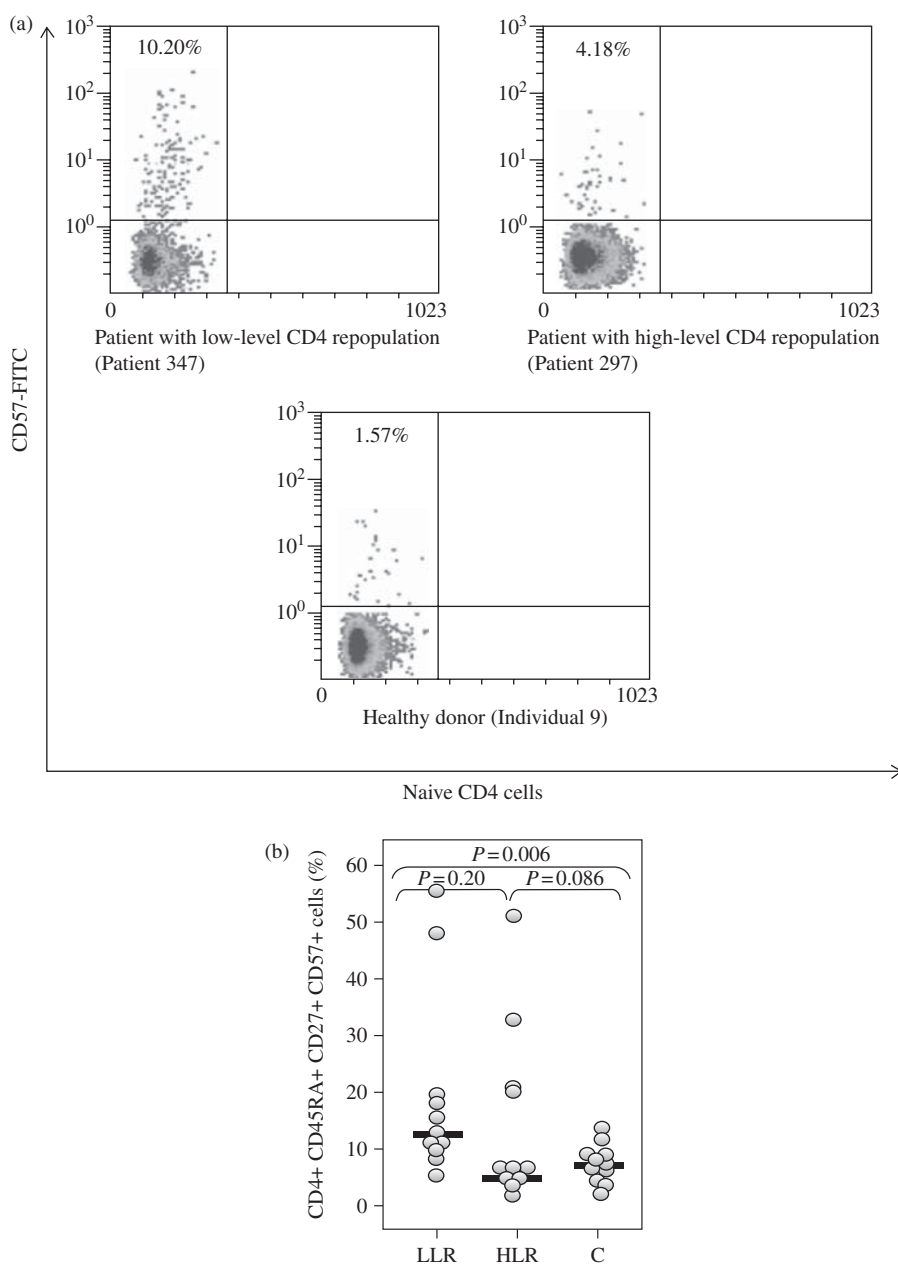
Figure 2. (a) Example of a density plot showing CD57 expression in naive CD4 T cells in three representative individuals of the three groups studied. (b) Comparison of the percentage of CD4+ CD45RA+ CD27+ CD57+ expression in the different groups. LLR, patients with low-level CD4 T cell repopulation; HLR, patients with high-level CD4 T cell repopulation; C, healthy controls.

volume (in those patients whose measurement was available). Measurement of the δ-/β-TREC ratio in CD4 T cells was not possible as β-TREC levels could not be quantified and, therefore, the effect of cell redistribution could not be avoided. Of note, β-TREC levels were only detected in 27.2% of patients with high-level CD4 T cell repopulation. We initially tried to measure the δ-/β-TREC ratio in naive CD4 T cells, but the poor rate of isolation in patients with low-level CD4 T cell repopulation resulted in insufficient extracted DNA to perform the analysis (data not shown). It cannot be excluded that thymic impairment, interfering with peripheral T cell homeostasis, could also be induced by thymotropic viruses, reducing an important source of naive T cell production,[29,30] or even by a specific host genetic characteristics. On the other hand, as expected, patients with low-level CD4 T cell repopulation also had a lower nadir CD4 T cell count. Moore and Keruly[31] have recently reported that when HAART is initiated at low CD4 T cell counts (≤350 cells/mm³), normal CD4 T cell levels are not achieved despite sustained HIV RNA load suppression. Moreover, it has been determined that the nadir CD4 T cell count is a determinant factor of suboptimal CD4 T cell repopulation and clinical outcome.[4,32–37] It is possible that the low-level CD4 T cell repopulation of our patients was simply the consequence of a lower CD4 cell count starting point. However, it is important to highlight that our study was performed ≥48 weeks after HAART was initiated. Furthermore, since we
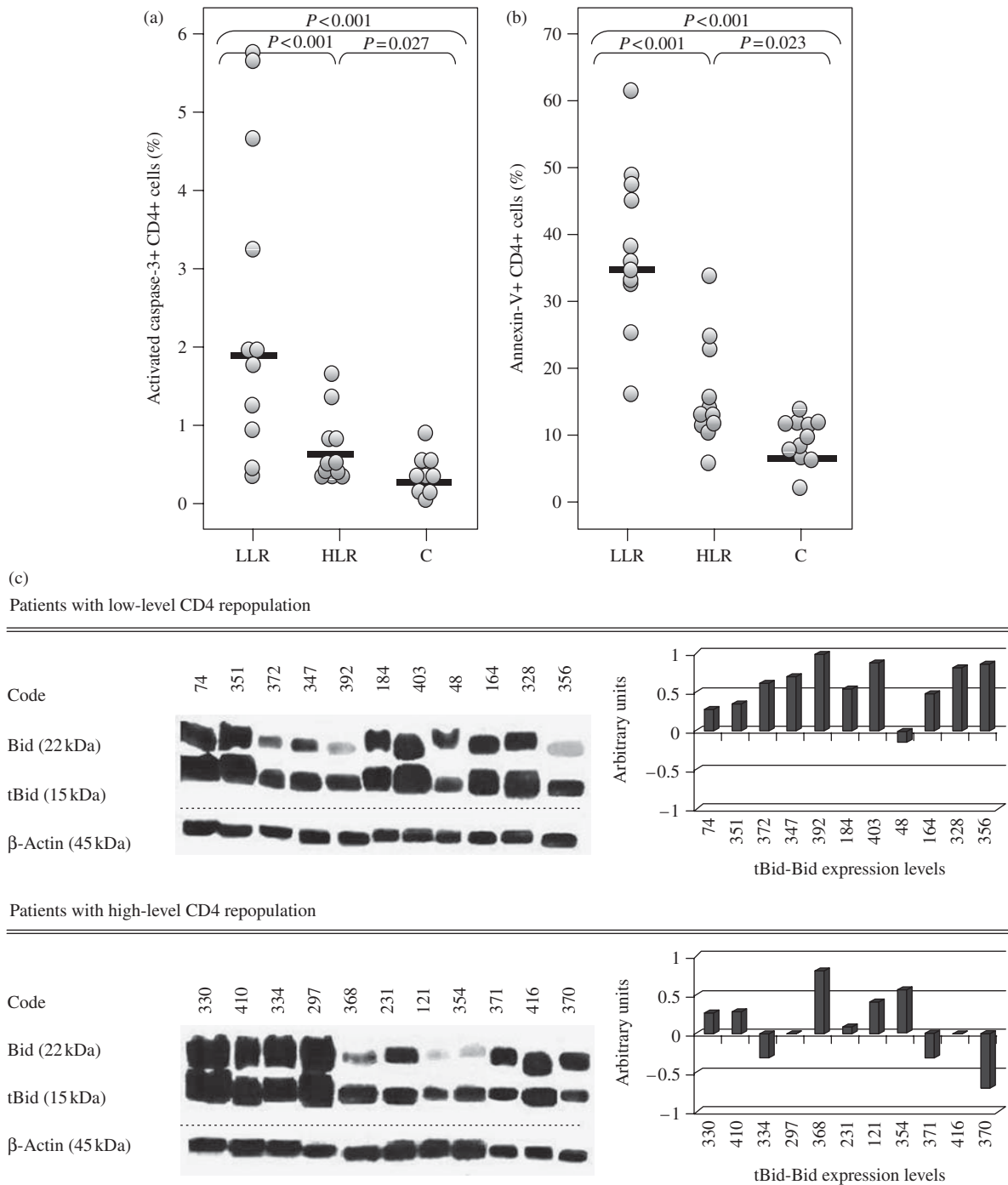
Figure 3. Apoptotic rate of CD4 T cells. (a) Expression of activated caspase-3 in CD4 T cells in the different groups of patients. (b) Expression of annexin-V in CD4 T cells in the different groups of patients. LLR, patients with low-level CD4 T cell repopulation; HLR, patients with high-level CD4 T cell repopulation; and C, healthy controls. (c) Expression of Bid and tBid forms in patients with low-level and high-level CD4 T cell repopulation; densitometric analysis of the difference between tBid expression levels and Bid expression levels normalized by β-actin expression levels is shown next to each western blot.

also had a greater follow-up of all patients, we confirmed that these patients did not reach a CD4 T cell level of >250 cells/ mm^3, even after the additional 2 years on HAART since this study was performed (data not shown). Therefore, it is possible that the lower nadir CD4 T cell count and also the persistent immune dysfunction could be the consequence of impairment in thymic function. The thymus is crucial for the reconstitution of the T cell compartment following lymphodepletion;[13,14] thus, lower nadir CD4 T cell count is not inconsistent with a thymic impairment. There is evidence that thymic activity may increase after HAART and correlates with cell reconstitution.[38]

On the other hand, immunological activation may be an additional factor that contributes to immunological and clinical deterioration of HIV-infected patients,[6,39] despite sustained viral
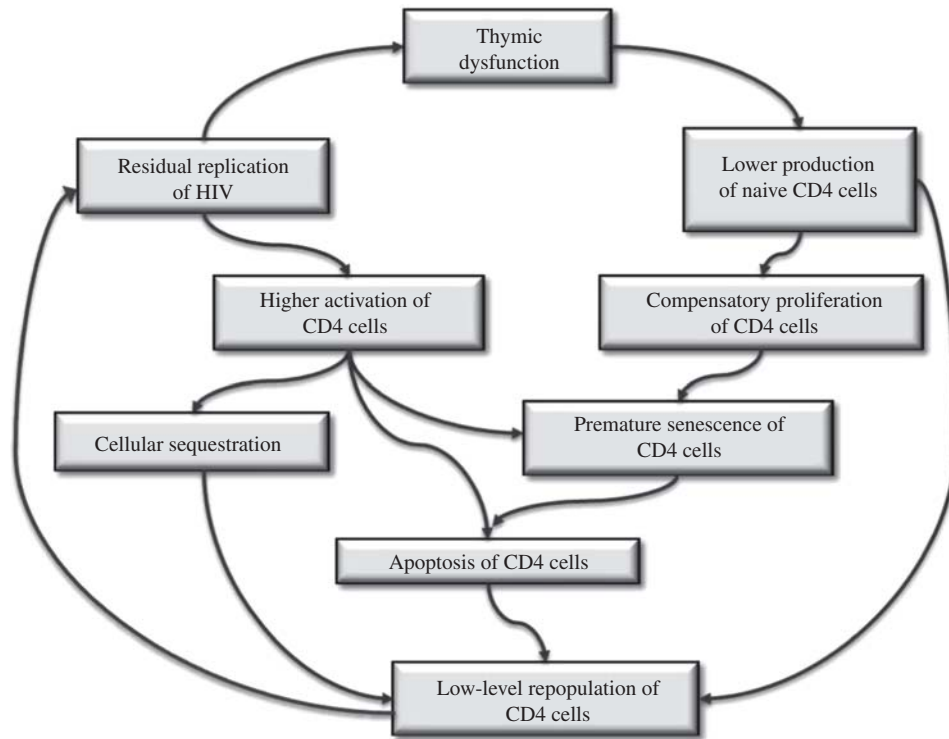
Figure 4. Proposed schematic model of premature senescence in patients with low-level immune repopulation despite undetectable HIV replication on HAART.

control under HAART. HIV infection has been associated with a high expression of activation markers on CD4 T cells, but these markers decrease after initiating HAART.[39–41] In this study, the high activation level of CD4 T cells found in patients with low-level CD4 T cell repopulation might be due to undetectable HIV replication or viral replication in inaccessible compartments.[42]

It has been previously reported that an increased number of CD4 T cells expressing CD57 is associated with poor CD4 T cell repopulation.[6] Specifically, our data showed a higher proportion of naive CD4 T cells expressing CD57 in patients with low-level CD4 T cell repopulation, despite a controlled virological response. The expression of the senescence marker CD57 on CD4 cells with a naive phenotype may be surprising. Probably, peripheral compensatory mechanisms would be necessary to reverse peripheral T cell lymphopenia in HIV-infected patients with poor immunological response.[43] Thus, an increased entry into cell cycle of peripheral T cells might induce a higher level of immunosenescence in patients with low-level CD4 T cell repopulation. In patients with high-level CD4 T cell repopulation, the CD57 expression on naive CD4 T cells was statistically lower due to the ability to generate sufficient numbers of CD4 T cells. However, more studies are necessary to establish this cell subset.

The higher rate of peripheral CD4 T cell apoptosis in patients with low-level CD4 T cell repopulation could also affect cell repopulation in patients under HAART. There are many reported data indicating that apoptosis plays a major role in this CD4 T cell depletion.[4,44] Moreover, we also analysed active caspase-3 since it has been recognized as a central key in mediating apoptosis and precedes phosphatidyl serine exposure on the external leaflet of the lipid bilayer, as indicated by annexin-V

binding.[45,46] A lower survival rate of CD4 T cells along with a higher expression of proapoptotic proteins was found in patients with low-level CD4 T cell repopulation.

In summary, thymic dysfunction alters peripheral T cell homeostasis in patients with low-level CD4 T cell repopulation. We propose that the deficit in naive CD4 T cell production causes a compensatory proliferation of peripheral cells, which may accelerate their senescence. In a parallel way, higher cell activation may also contribute to the premature senescence of CD4 T cells. For example, T cell immunosenescence has been described in rheumatoid arthritis and in HIV-infected donors without treatment, similar to that observed in elderly individuals, and it is probable that this premature immunosenescence occurs due to compensatory proliferation by a deficiency in the ability to generate a sufficient naive CD4 T cell count.[47,48] Premature ageing of naive CD4 T cells could be a risk factor for developing a proliferative inability and also a higher CD4 T cell apoptosis rate. Thus, CD4 T cell sequestration in lymphoid tissues induced by activation along with apoptosis may determine a lower CD4 T cell repopulation in these patients. This poor CD4 T cell repopulation probably favours the inability of T cell immunity to effectively suppress thymotropic virus, playing an important role in both thymic activity and peripheral T cell homeostasis, as thymocytes and naive CD4 T cells preferentially express the CXCR4 co-receptor. A proposed schematic model including all these related factors is shown in Figure 4. However, additional studies are necessary to prove this model. Since new therapeutic strategies are needed to specifically treat HIV-infected patients with low-level CD4 T cell repopulation, our study contributes to the global knowledge of the mechanisms involved in these patients.

Acknowledgements

Funding

Transparency declarations

None to declare.

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