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Brief communication

Immunological profiles of HIV-positive recipients of liver transplant

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ABSTRACT

Background: Scarce data are available about immune cell frequencies in HIV-positive recipients of liver transplant. Alterations in immune subsets can lead to persistent immune activation and disease progression or reduced HIV-specific responses. In liver transplantation, impaired immune tolerance can lead to organ rejection.
Methods: HIV-positive subjects with undetectable HIVRNA and CD4 > 100/mm^3 were included. Control groups were non-transplanted HIV-positive patients with similar immunovirological parameters and healthy subjects. B cells (memory, transitional, and mature subsets), T cells (effector TH1, nonclassic TH1, TH17, TH1/17; T regulatory naïve and effector subsets and CD8$^+$ T regulatory cells), and NK cells (CD56^{dim} and CD56^{bright} subsets) were analyzed by flow cytometry.
Results: A total of 56 patients, including 14 HIV-positive transplant recipients (HIV-LT), 14 HIV-positive controls, and 28 healthy controls were included. Median age of HIV-LT patients was 54.9 years with median time from transplant of 7.6 years. Eleven (79%) were HIV/HCV coinfected. Compared to nontransplanted patients, HIV-LT displayed significantly increased frequency of T CD8$^+$ cells, lower percentage of T CD4+ cell, and lower number of nonclassic TH1, TH1/17 cells and naïve T CD4$^+$ regulatory cells (Tregs). Healthy controls showed increased numbers of B cell subsets and decreased percentage of T effector subpopulations compared to HIV-LT. Compared to HIV-positive patients, healthy controls had higher B cells, NK cells, CD4$^+$ T cells, naïve CD4+ Tregs but lower CD8$^+$ T cells, effector Tregs, CD8$^+$ Tregs, and all T effector cell subsets.
Conclusions: Immune cell subpopulations potentially associated with HIV progression and organ rejection were detected in HIV-positive transplant recipients. We confirmed altered frequencies of B, T, and NK cell populations in HIV-positive liver transplant recipients compared to healthy controls. The imbalance among immune cell subsets deserves further studies to identify markers of transplant outcome and potential therapeutic targets.

1. Introduction

Prior to the introduction of HAART, HIV positivity was an absolute contraindication for solid organ transplantation (SOT) [1] due to the reduced life expectancy of HIV-infected patients and the concern that immunosuppressants could have favored post-transplant opportunistic infections (OI). Prolonged survival due to HAART, however, has increased the prevalence of non-OI related causes of death. Among these, end-stage liver disease related to HCV coinfection and drug toxicity caused an increase in the demand for liver transplant (LT) among HIV-infected individuals [2,3]. SOT currently represents a valid therapeutic option for HIV patients with stable viral infection and end-stage organ disease [4]. In this cohort, mortality rates comparable to non-HIV patients and limited occurrence of OI have been reported, especially for renal transplant recipients [4,5]. Conversely, clinicians managing HIV-positive liver transplant recipients (LTR) have faced worse outcomes compared to the HIV-negative counterpart, especially among coinfected HIV/HCV patients [4–7].

The immune system plays a pivotal role in graft survival, causing organ rejection if not controlled or, on the other side, favoring the occurrence of infections. In HIV-positive individuals, T cell alterations such as reduction of CD4$^+$ T cell absolute number and decreased CD4:CD8 ratio appear to be key immune disorders for disease progression [8]. Although the distribution of B, T, and NK cells has been extensively studied in HIV-positive patients and in HIV-negative transplant recipients, the analysis of immunological profiles in HIV-positive LTR has not been previously documented. Data on T cell function and cytokine production among HIV-positive patients

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Table 1
Clinical characteristics of HIV-positive liver transplant recipients.

N, sex	CDC stage	HAART	Timing from LT (years)	Rejection episodes	CD4 count (N/mm^3)	CD4 (%)	Immunosuppressants
1, F	A2	TDF + FTC + RAL	7.9	No	740	49	Tacrolimus
2, M	A2	3TC/ABC + DTG	9.2	No	524	31	Tacrolimus
3, M	B3	TAF + FTC + RAL	0.5	No	138	31	Tacrolimus + MMF
4, M	B2	3TC/ABC + RAL	1.6	No	169	15	Tacrolimus
5, M	B3	DRV/COBI+RAL	7.2	No	140	15	Tacrolimus
6, M	B3	TDF + 3TC + RAL	9.0	No	333	32	Tacrolimus
7, M	B2	TDF + FTC + FVP	9.3	No	380	26	Tacrolimus
8, M	A2	3TC + DTG + MVC	7.2	No	814	37	Tacrolimus
9, M	A3	ATV + DTG	9.1	No	300	27	Tacrolimus
10, M	A2	ABC + 3TC + RAL	8.9	No	857	36	Tacrolimus
11, M	B3	ABC + 3TC + DTG	1.5	No	110	16	Tacrolimus
12, M	B2	3TC/ABC + RAL	3.5	No	737	29	Tacrolimus
13, M	A2	3TC + FVP + RAL	9.0	No	142	29	Tacrolimus
14, M	B3	TAF + FTC + DTG	0.4	No	490	32	Tacrolimus + MMF

LT = liver transplant; MMF = mycophenolate mofetil.

Table 2
Characteristics of HIV-positive liver transplant recipients and controls.

Characteristics	HIV-LT (*N* = 14)	HIV-CTRL (N = 14)	P
Male sex (%)	13 (93)	13 (93)	NS
Caucasian race (%)	14 (100)	13 (93)	NS
Mean age (years; IQR)	54.9 (52.3–59.9)	51.6 (44.4–59.4)	NS
HIV transmission risk			
IVDU	12 (86)	12 (86)	NS
Sex	2 (14)	2 (14)	
Timing from HIV diagnosis (years)	30.4 (25.5–31.5)	25.0 (16.0–30.0)	0.01
CDC stage			
A2	5 (36)	4 (29)	NS
A3	1 (7)	2 (13)	
B2	3 (21)	4 (29)	
B3	5 (36)	4 (29)	
HCV coinfection	12 (86)	12 (86)	NS
HCV treatment with SVR	11/12 (92)	11/12 (92)	NS
HCV-related chronic liver disease	2 (14)	4 (29)	NS
Median CD4 (cells/mm3)	357 (142–524)	391 (152–829)	NS
HAART (%)			
Integrase inhibitors	13 (93)	7 (50)	0.04
PIs	3 (21)	8 (57)	NS

NS = not significant, IQR = interquartile range, IVDU = intravenous drug user, HAART = .
highly active antiviral therapy, PI = protease inhibitor.

undergoing SOT are also limited to few reports [9–11].

Among T cell populations, decreased T regulatory cell (Tregs) absolute counts have been described in HIV-patients and could be linked to immune hyper-activation [12]. Natural killer (NK) cells could play an important role in controlling viral expansion through cell antibody-dependent cell cytotocity, but their number and function is often impaired, especially among viremic HIV-patients [13]. Finally, B-cell abnormalities that are still not completely clarified, including defects of IgM$^+$ memory B cells and paucity of HIV-specific IgA responses, likely prevent the occurrence of an effective antibody response against HIV [14].

We have analyzed the frequencies of peripheral subpopulations of lymphocytes by flow cytometry in a cohort of stable HIV-positive LTR and compared them with non-transplanted HIV-positive patients and healthy controls to observe if numerical alterations were present.

2. Material and methods

2.1. Patients

Fourteen HIV-positive patients who received liver transplantation from a deceased donor between 2007 and 2017 were included in the study. Inclusion criteria were: patients transplanted for at least 5 months, stable immunovirological parameters (e.g., undetectable HIVRNA and CD4 > 100/mm^3) and receiving HAART for at least 12 months. Twelve patients were HIV/HCV coinfected. Control groups included 14 non-transplanted HIV-positive patients with stable immunovirological parameters and 28 healthy subjects.

Data were prospectively collected during the period from June 2017 to June 2018. Demographic data, immunovirological parameters, type of immunosuppressive regimen, previous episodes of organ rejection and occurrence of opportunistic infections were electronically recorded. HIV and HCV viral loads were quantified using the polymerase chain reaction-based COBAS AMPLICOR HIV and HCV Monitor assays [Roche]. Immunosuppressive regimen included either tacrolimus or cyclosporine with or without mycophenolate mofetil, while corticosteroid therapy was withdrawn by the end of the fourth postoperative month. The immunosuppressive dosage was adjusted according to clinical requirements and controlled by whole blood trough levels. Cyclosporine was dosed to obtain serum levels between 600 and 800 μg/l (2 h after drug administration), while tacrolimus predose levels ranged between 4 and 8 μg/l. The local institutional review board approved the study and informed consent was obtained from all individuals.

2.2. Immunological analysis

Mononuclear cells were isolated from the peripheral blood by Ficoll gradient centrifugation [Lympholyte-H, Cedarlane]. Flow cytometry analysis was performed using the BD FACSCanto™ II flow cytometer and FACSDiva 7 Software. For the analysis of immune subpopulations, the following anti-human antibodies [Becton Dickinson, Franklin Lakes, NJ] were used: for T cells, anti-CD3 (BV500), anti-CD4 (APC), anti-CD8 (PercCP-Cy5.5), anti CD161 (B421), anti-CXCR3 (PE), anti-CD28 (PE), anti-CD25 (PECy7), anti-CCR4 (PECy7), anti-CCR6 (APC), anti-CD127 (BV421), and anti-CD45RA (APC); for B cells, anti-CD19 (PECy7), anti-CD24 (PE), and anti-CD38 (APC); for natural killer (NK) cells, anti-CD3 (V500), and anti-CD56 (BV421), and anti-CD16 (PerCp-Cy5.5) [15]. Results were expressed as percentage or absolute cell count/ml. A total of 250,000 events were collected.

2.3. Statistical analysis

Continuous and categorical data were reported as mean ± SEM. Mann-Whitney test was used to determine the significance of differences between groups of patients. Categorical variables were evaluated using chi-square or, when appropriate, the two-tailed Fisher's exact test. Analyses were performed using SPSS v. 20.0 (IBM, SPSS, Chicago, Illinois). All tests were two-tailed, and a *P* value <.05 was determined

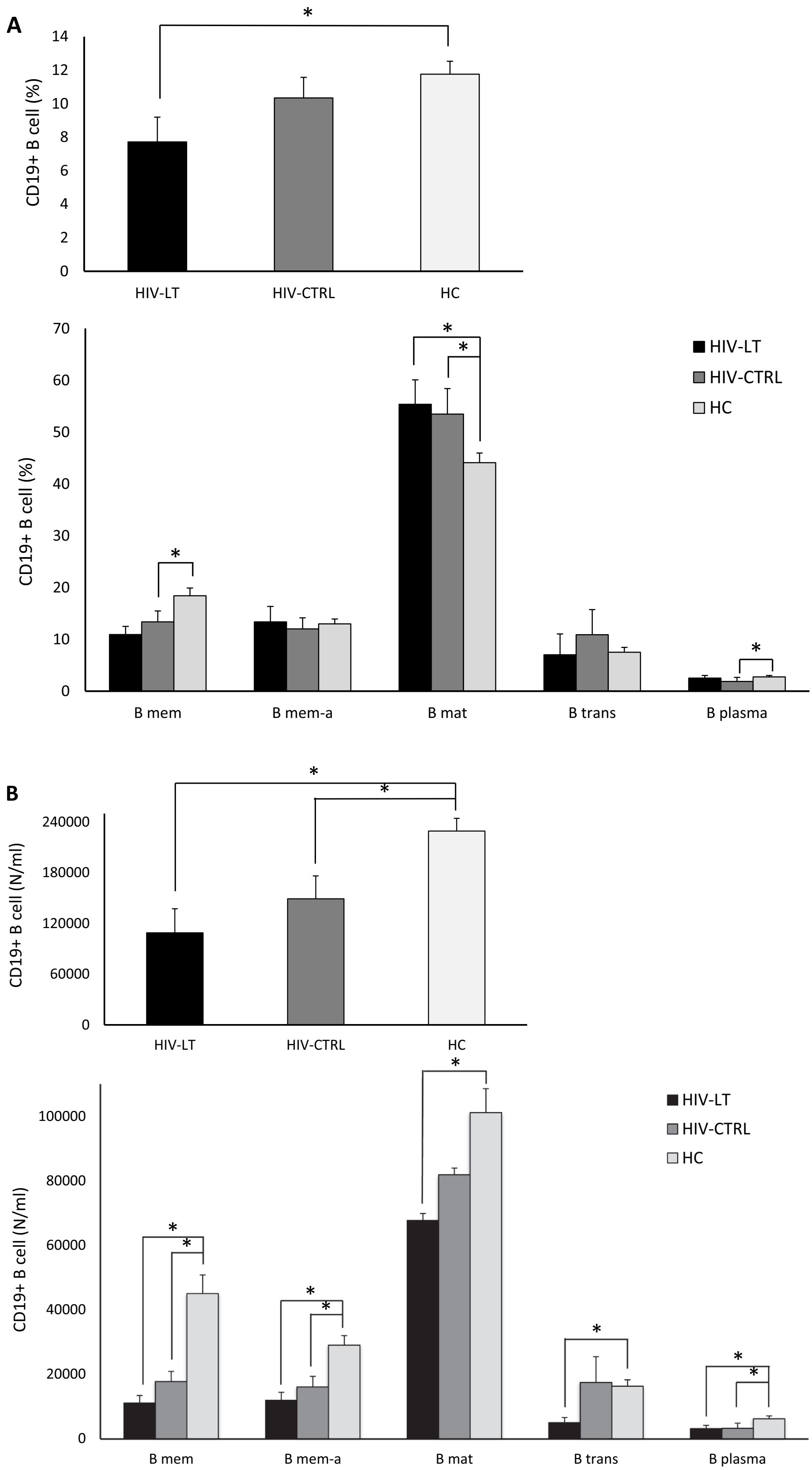

Fig. 1. B cell population distribution as percentage (A) and total number (B) in HIV-positive liver transplant recipients, HIV-positive controls, and healthy controls. B cells were identified as $CD3^{-}CD14^{-}CD19^{+}$. $CD19^{+}$ B cell subpopulations were assessed as B memory (B mem, $CD24^{high}CD38^{-}$), B memory atypical (B mem-a, $CD24^{+}CD38^{-}$), B mature (B mat, $CD24^{+}CD38^{low}$), B transitional (B reg, $CD24^{+}CD38^{high}$), and plasma B cells (B plasma, $CD24^{-}CD38^{high}$). Total number of cells is expressed per ml. Data are presented as mean ± SEM. $*p \le .05$.

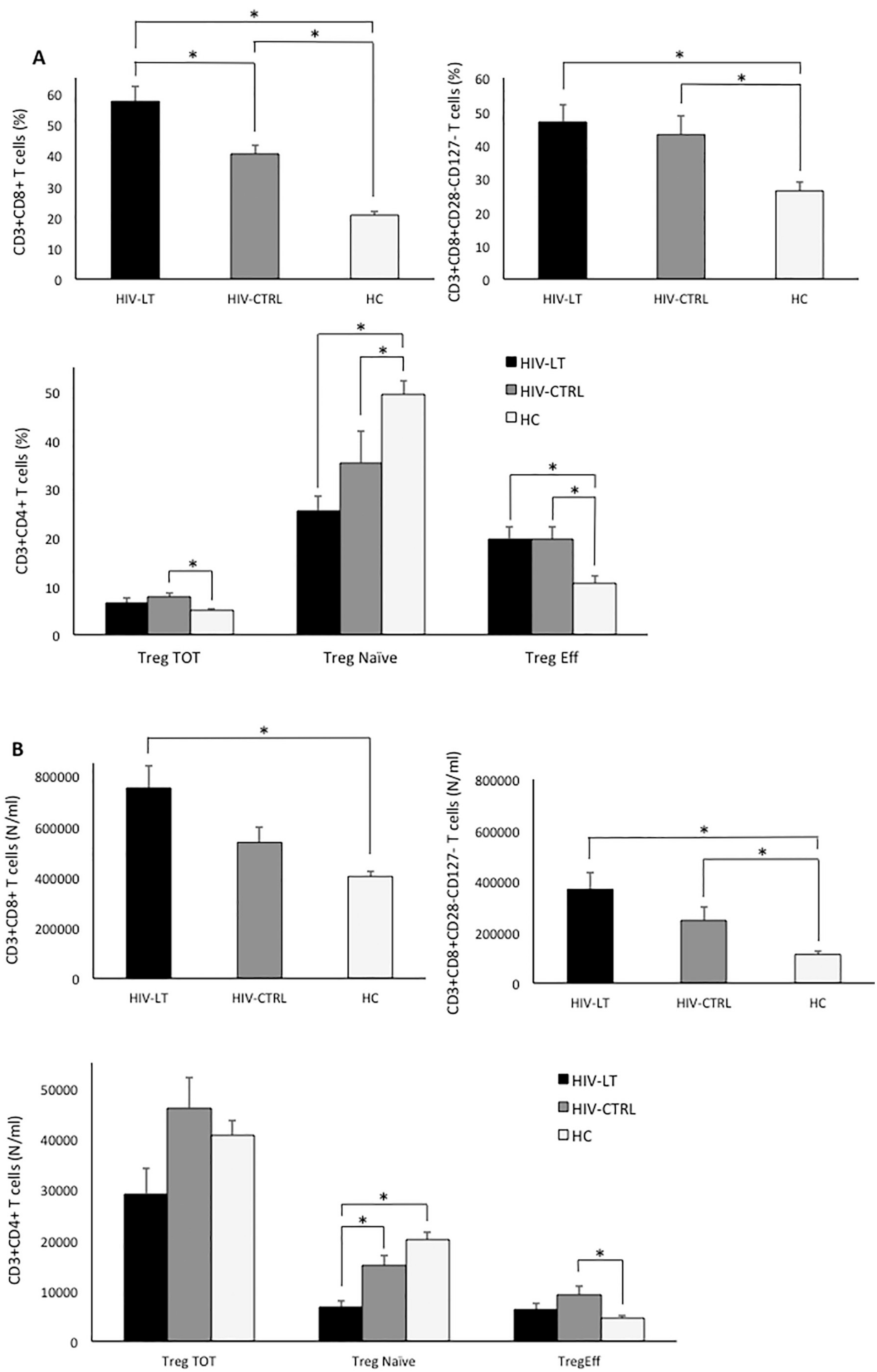

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Fig. 2. T regulatory cell distribution among HIV-positive liver transplant recipients, HIV-positive controls, and healthy controls. Percentage (A) of CD3$^+$CD4$^+$ T regulatory cells and CD3$^+$CD8$^+$ T regulatory cells and total number (B) of CD3$^+$CD4$^+$ T regulatory cells and CD3$^+$CD8$^+$ T regulatory cells are reported.
Tregs were assessed as CD3$^+$CD4$^+$, including naïve Tregs (CD45RA$^+$CD25^{low}) and effector Tregs (CD45RA$^-$CD25^{high}), and CD3$^+$CD8$^+$ T regs (CD3$^+$CD4$^+$CD28$^-$CD127$^-$). Total number of cells is expressed per ml. Data are presented as mean ± SEM. *p ≤ .05.

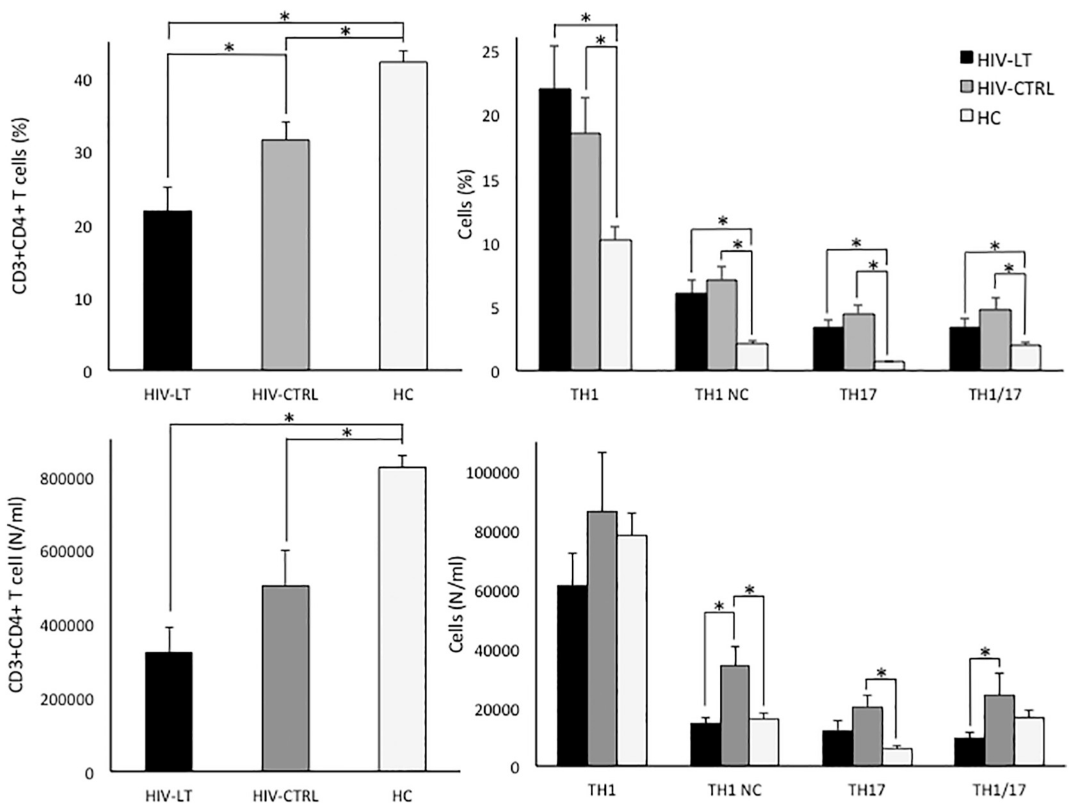

Fig. 3. T effector population distribution reported as percentage (upper panel) and total number (lower panel) of cells in HIV-positive liver transplant recipients, HIV-positive controls, and healthy controls.
CD3$^+$CD4$^+$ effector T cell populations were assessed as T-helper 1 (TH1, CCR6$^-$CD161$^-$), non-classical TH1 (TH1 NC, CCR6$^-$CD16$^+$CXCR3$^+$), TH17 (CCR6$^+$CD161$^+$), and TH1/17 (CCR6$^+$CD16$^+$CXCR3^{high}CCR4^{low}). Total number of cells is expressed per ml. Data are presented as mean ± SEM. *p ≤ .05.

to represent statistical significance.

3. Results

3.1. Patient populations

A total of 56 patients, including 14 HIV-positive LTR (HIV-LT), 14 HIV-positive nontransplanted controls (HIV-CTRL), and 28 healthy controls (HC) were included in the study. All patients were Caucasians except for one patient who was of African origin in the HIV-CTRL group. None of the HIV-positive patients enrolled in the study experienced previous acquired immunodeficiency syndrome (AIDS) events, and all were successfully treated with HAART showing undetectable (<50 copies/mL) HIV viral load.

HIV-LT were predominantly males (93%) and received liver transplant for decompensated cirrhosis (n = 8) or hepatocellular carcinoma (n = 6). Median age was 54.9 years (IQR 52.3–59.9) and median time from LT was 7.6 years (IQR 1.6–9.0). Twelve patients (86%) were HIV/HCV coinfected. Of these, 10 (90%) received HCV treatment after LT achieving sustained virological response, while one patient relapsed during treatment with directly acting antivirals. Two patients with HCV reinfection after OLT progressed to chronic liver disease. None of the patients in the HIV-LT group experienced organ rejection or liver decompensation after liver transplant. Patients' clinical and transplant-related characteristics are detailed in Table 1.

HIV-CTRL showed similar characteristics compared with HIV-LT (Table 2). Four patients from this group had HCV-related chronic liver disease; none experienced liver decompensation. Healthy controls included 17 (60%) men and 11 (40%) women with median age 48.3 years (IQR 33.8–56.7).

3.2. Immune cell frequencies

Percentage and absolute numbers of T, B, and NK cells are summarized in Supplementary Table 1.

3.3. B cells

No differences were observed in total B cell frequencies between HIV-LT and HIV-CTRL, while HC had higher total B cell percentage and numbers compared to HIV-LT (P = 0002 and P = .01, respectively). Among B cell subpopulations, transitional B cell number was lower in HIV-LT compared to healthy controls (P = .03), while HIV-CTRL showed an increased trend compared to HC (difference not significant). Healthy controls showed significantly increased B memory and plasma cell percentage compared to HIV-CTRL (P = .02) and lower percentage of mature B cells compared to HIV-LT and HIV-CTRL (P = .01 and P = .02, respectively). All B cell subset numbers were increased in HC compared to the HIV-positive groups (Supplementary table 1). Fig. 1 summarizes the distribution of B cells among groups.

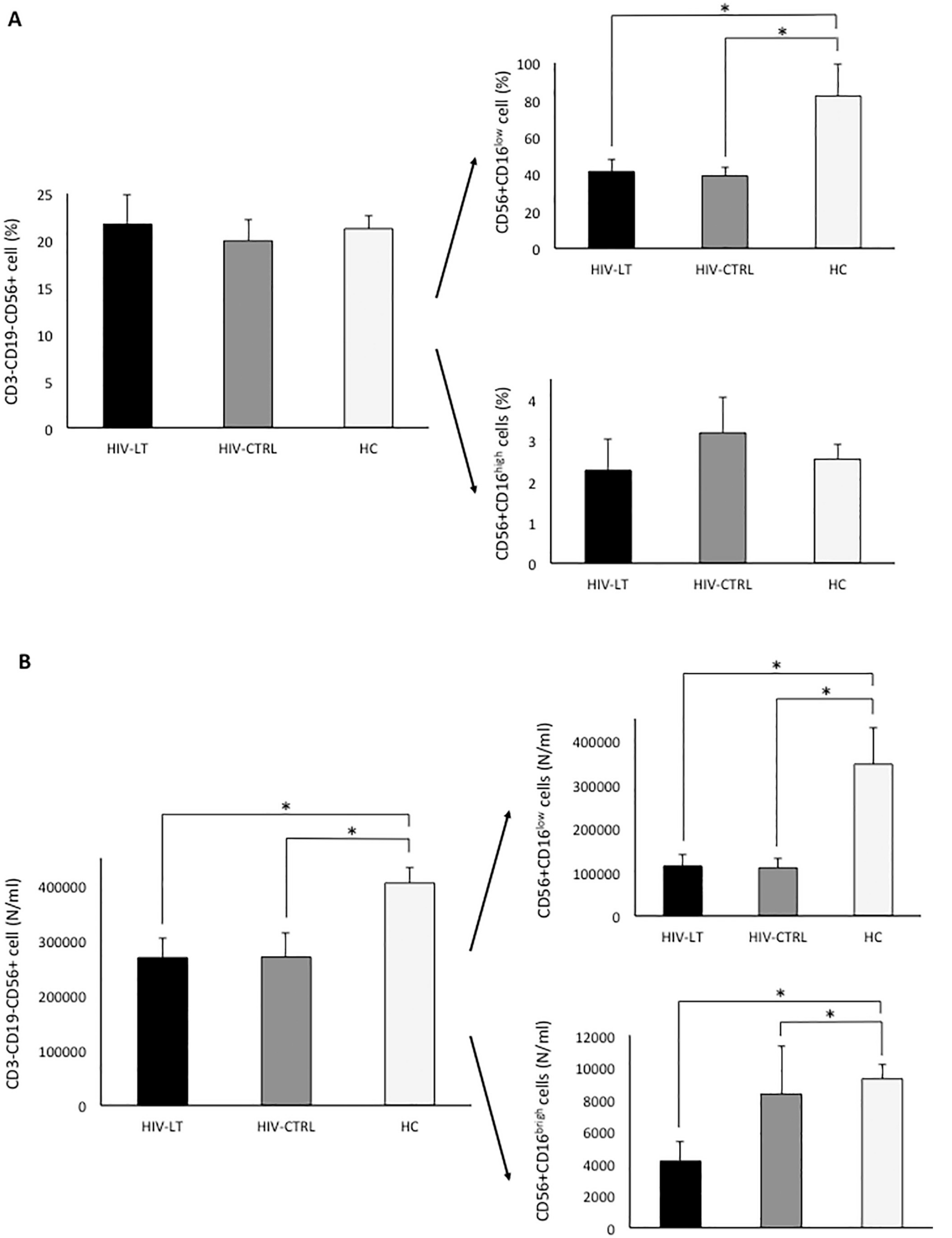

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Fig. 4. NK cell subsets in HIV-positive liver transplant recipients, HIV-positive controls, and healthy controls. Percentage (A) and total number (B) of NK cells are reported.
NK cells were assessed as $CD56^{dim}$ ($CD3^{+}CD14^{+}CD56^{low}$) and $CD56^{bright}$ ($CD3^{+}CD14^{+}CD56^{high}$) cells. Total number of cells is expressed per ml. Data are presented as mean ± SEM. *p ≤ .05.

3.4. T effector cells

$CD4^{+}$ T cell percentage was lower in HIV-LT compared to HIV-CTRL ($P = .03$, Fig. 2). As expected, healthy controls had increased percentage and absolute $CD4^{+}$ T cell number compared to HIV-positive patients. $CD3^{+}CD8^{+}$ number and percentage were higher in HIV-LT compared to HIV-CTRL and HC. Significantly lower numbers of non-classical T-helper 1 (TH1 NC) and TH1/17 were detected in the HIV-LT group compared to HIV-CTRL ($P = .04$ and $P = .05$, respectively). Healthy controls showed lower percentage of all T effector cell subsets compared to HIV-positive patients (Fig. 2) and lower number of TH1 NC and TH17 compared to HIV-CTRL ($P = 0{,}01$ and $P = .002$).

3.5. T regulatory cells

Total $CD4^{+}$ regulatory T cells (Tregs) did not differ between HIV-LT and HIV-CTRL (Fig. 3), while a lower percentage was reported in HC vs. HIV-CTRL ($P = .03$). Lower number of naïve Tregs was shown by HIV-LT compared to HIV-CTRL ($P = .005$) and HC ($P < .0001$), while effector Tregs were similar between the two HIV-positive groups. HC displayed significantly lower percentage of effector Tregs compared to HIV-LT ($P = .001$) and HIV-CTRL ($P = .002$) and lower numbers compared to HIV-CTRL ($P = .02$). $CD8^{+}$ Tregs were similar between HIV-positive groups, while healthy controls showed decreased numbers and percentage of $CD8^{+}$ Tregs (Fig. 3).

3.6. NK cells

Overall, $CD56^{+}$ NK cells distribution was similar between HIV-LT and HIV-CTRL, while HC displayed higher numbers of NK cells compared to HIV-LT ($P = .005$) and HIV-CTRL ($P = .02$). Healthy controls had higher number and percentages of $CD56^{dim}$ and increased number of $CD56^{bright}$ NK cells compared to the HIV-positive counterpart (Fig. 4).

4. Discussion

HIV-positive LTR have shown higher rates of acute graft rejection, rapid progression of HCV disease, and increased multiorgan failure compared to HIV-positive kidney transplant recipients and HIV-negative LTR [6,16]. Reports analyzing immune disorders and cytokine signatures in HIV-positive SOT recipients are scarce, but appear useful to investigate potential immunological predictors of graft outcomes. A study analyzing the ex vivo production of IFN-gamma by T cell stimulated with various antigens in a cohort of 14 HIV/HCV positive LTR showed that the responses did not change over time, suggesting that LT does not impair specific immune responses in HIV/HCV coinfected patients [9]. In kidney transplant recipients, increased immune activation (defined by the proportion of $CD3^{+}HLA\text{-}DR^{+}$ cells) was demonstrated compared to HIV-negative patients and, interestingly, appeared inversely correlated with an increased risk of organ rejection [10]. A study including 69 HIV/HCV coinfected LTR showed that markers of monocyte activation (sCD14) were significantly lower in patients with graft loss, whereas IL-10 levels correlated with rejection [11].

To our knowledge, studies investigating T and B lymphocyte and NK cell profiles in HIV-positive LTR have not been performed.

Overall, we observed frequencies of B, T, and NK cells in healthy controls and nontransplanted HIV patients comparable to those previously reported in the literature. Expected differences in immunological profiles between HIV-infected patients and healthy controls were displayed, including the reduction of B, NK cells and T $CD4^{+}$ lymphocytes and the increase in the proportion of T $CD8^{+}$ lymphocytes in the HIV cohort. Interestingly, similar frequencies of B cells, NK cells, total T effector and total Treg cells between transplanted and nontransplanted HIV-positive patients were detected. While this seems to confirm that liver transplantation may not significantly affect quantitative cell immunity in patients with HIV infection, finer differences and higher differentiation in cell subpopulations were observed and should be taken into consideration.

Table 3 summarizes the characteristics of immune cell subsets in different patient populations and their potential implications in clinical practice.

B cells play essential roles in transplant alloimmunity and, as we observed in LTR, their proportion tends to decrease over time [17]. A lower number of memory B cells was observed in HIV-positive compared to HIV-negative patients, as previously reported also for patients receiving HAART [17]. In SOT, pathogen-specific memory B cells are critical to protect the graft and contribute to long-term patient survival [18]. The proportion of B cells in HIV patients is also altered by over-represented aberrant B-cell subpopulations (e.g., immature transitional B cells and activated mature B cells) characterized by limited proliferation in response to B cell stimuli [14,19]. As previously reported, immature transitional B cells were more frequent among HIV-positive controls compared to healthy controls [14]. HIV-LT, however, showed lower transitional B cells compared to HC. This could be relevant since transitional B cells possess IL-10-mediated regulatory capacity [20,21], although contrasting results were reported for HIV transplant recipients who showed decreased rejection rates in presence of IL-10 production [11]. The proportion of atypical memory B cells, characterized by diminished ability to proliferate and secrete cytokines or antibodies [22], remained similar among the HIV groups. Hypergammaglobulinaemia, usually reversed by HAART, was not evident among HIV-LTR and HIV-CTRL compared to healthy controls.

In conclusion, clinicians should be aware that a reduction of memory B cells as well as the increase of immature transitional B cells, that occur during uncontrolled HIV infection, could have an impact on HIV-LT graft survival. In our study, that included only stable patients, B cell subpopulations among HIV-LT were similar to HC. The use of an optimised HIV regimen, that is based on patients' pre-transplant history and avoids drug-drug interactions, is key to maintain a favourable post-transplant immune-virological response.

Tregs have been extensively analyzed in HIV patients and are considered targets and potential reservoirs of HIV infection [23]. Tregs exert a dual, complex effect: they can be beneficial to control HIV infection by suppressing chronic inflammation but also detrimental in reducing HIV-specific responses [24,25]. Tregs have a key role in immune tolerance after transplant and, as we observed, their proportion can be similar to HC in stable transplant recipients [26,27]. In HIV-positive viremic patients, Tregs percentages among $CD4^{+}$ T cells increase while their absolute numbers decrease, indicating reduced suppression capacity on targets other than CD4 (e.g., $CD8^{+}$ T cells, NK cells); during HAART, Tregs percentage and the suppressive capacities against $CD4^{+}$ T cells are reduced, while Tregs count is restored [25]. We observed similar proportions of Tregs between HIV-LT and HIV-CTRL, indicating that total Tregs distribution is not significantly affected by HIV transplantation in stable patients. Among Tregs subpopulations, naïve Tregs correlate with CD4 count and are mainly affected in the acute phase of the infection, while effector Tregs are reduced in chronically HIV-infected patients and have potential

Table 3
Proportion and main functions of immune cell subsets among different patient population and potential implications for clinical practice.

Immune cell subset	SOT recipients	HIV-infected patients	Our study	Potential implications and interventions
B cells				
Memory B cells	Decrease over time associated with alloimmunity and low pathogen-specific activity [17,18]	Reduction and abnormality favor infections and poor outcomes [45]; partially restored by HAART	Lower in HIV-LT and HIV-CTRL vs. HC	Defects may cause impaired response to immunisations and affect graft survival; optimization of HAART is important
Immature transitional B cells	May possess IL-10 regulatory activity [20,21]	Increase leads to reduced cytokines and antibodies secretion [14]	Lower N in HIV-LT vs. HC	IL10 production associated with rejection in HIV-LT [11]
Gamma-globulin	Reduction associated with infections [46]	Increase/abnormality cause defective antigen responses [47]; reversed by HAART	Lower % and N in HIV-LT vs. HC (both HIV groups)	Optimization of HAART is important; benefits of IVIG use in LT still unclear [48]
T cells				
T CD4	Reduction by immunosuppressants associated with opportunistic infections	Reduction associated with opportunistic infections; improve with HAART [32]	Lower % and N in HIV-LT vs. HC; lower % for HIV-LT vs. HIV-CTRL	HAART and CD4 monitoring important; tailor immunosuppressants
T CD8	Can be associated to allograft dysfunction or rejection [49]	Increase associated with chronic inflammation and non-AIDS-related events; partially reverted by HAART [50]	Increased % in HIV-LT vs. HIV-CTRL and HC; increased N (HIV-LT vs. HC)	Optimization of HAART is important; manipulation of CD8 pathway under investigation in transplant [49]
T regs	Increase associated with immune tolerance [27]	Vary according to disease stage. Suppress inflammation but reduce specific responses [26,28]	Similar for HIV-LT vs. HIV-CTRL and HC; increased % HIV-CTRL vs. HC	Tregs manipulation under investigation in HIV [51]
Naïve T regs	Increase associated with immune tolerance	Affected in the acute phase of the infection; correlate with CD4 proportion [29]	Lower % and N for both HIV groups vs. HC	Less relevant involvement in HIV infection
Effector Tregs	Increase associated with immune tolerance	Suppression of HIV-specific CD8 responses; decrease over time partially reverted by HAART [29]	Higher % for both HIV groups vs. CTRL; higher N for HIV-CTRL vs. HC	Relevant for disease progression and chronic immune cell activation in HIV-LT recipients
$CD8^+$ T regs	Associated with allograft tolerance [49]	Correlate with chronic immune cell activation [31]	Higher % and N for both HIV groups vs. HC	Relevant for HIV progression and inflammation
Non classic TH1, TH17 and TH1/17	Associated proinflammatory cytokines, Tregs resistance, rejection [36,37]	Can act as HIV reservoirs; proinflammatory effect [33,34]	Higher % for both HIV groups vs. HC	May contribute to organ rejection, increase warrants vigilance after transplantation [10,11]
NK cells	Reduction associated to worse outcomes [41,43]	Reduction of cytolysis and antibody-dependent cellular cytotoxicity associated with HIV progression [39]	Lower N for both HIV groups vs. HC; reduced NK^{dim} % for both groups vs. HC	Decrease may be associated with worse outcome for HIV-LT; studies on immune tolerance ongoing

deleterious effect in HIV pathogenesis by suppressing specific CD8$^+$ T cell responses [29,30]. In our case, effector Tregs were higher among HIV-LT compared to non HIV patients. HIV infection has also been associated with remarkable expansion of CD8$^+$ Tregs that have been correlated with clinical disease and chronic immune cell activation even in patients receiving HAART [31]. Although we did not observe any difference in CD8$^+$ Tregs between transplanted and non-transplanted HIV-positive patients, the proportion of CD8$^+$ Tregs was significantly higher compared to HC. These data highlight the importance of Tregs subpopulations among HIV-LT. Monitoring, and potentially targeting, selected Tregs populations could be important to address disease progression and the risk of organ rejection in this cohort (Table 3).

We observed CD4$^+$ T cell depletion among HIV-LT patients compared to HC and HIV-CTRL, while the number and percentage of T CD8$^+$ cells were increased, as typically reported in HIV-positive patients [32]. Among T effector subpopulations, HIV transplanted patients displayed lower number of non classic TH1 and TH1/17 compared to the nontransplanted counterpart, while all T effector populations were higher in the HIV groups compared to HC. T cells with TH17 and TH1/17 polarization have a role as long-term HIV reservoirs during HAART [33,34] and, even if their number is often preserved, higher levels of integrated HIVDNA have been associated with TH1/17 cell subgroups. In transplant recipients, organ rejection was associated with increased numbers of TH17 and TH1/17 cells [35,36]. Furthermore, non classic TH1 have been linked to increased amounts of proinflammatory cytokines and resistance to suppression by regulatory T cells [37].

The impact of T effector populations in HIV-LT is still not well understood. Furthermore, immune activation appeared counter-intuitively protective in HIV-positive patients undergoing SOT [10,11]. In this cohort, the imbalance between different types of effector T cells may represent a key mechanism involved in organ rejection and deserves further analysis.

NK cells were reduced in HIV-positive patients compared to healthy controls [38,39], while we did not observe any difference in their distribution between the two HIV groups. NK cells remain critical against HIV infection through cytolysis and antibody-dependent cellular cytotoxicity [40], and their alteration has beens associated to HIV/HCV coinfection and disease progression [41,42], Furthermore, immunosuppressants can affect NK cell number and function. Recently, the involvement of NK cells in immunoregulatory functions has been hypothesised [43]. For these reasons, monitoring the proportion of NK cells in HIV-LT patients could be useful to identify cell depletion that has been previously associated with worse outcomes [44].

From a clinician's perspective, knowledge of immune cell proportions and their changes over time may help stratify HIV-positive LT recipients who may require additional attention after transplantation.

Our study has several limitations. Firstly, T cell function and cytokine production were not explored, thus the frequency of cells reported may not clearly reflect cell immune activation or tolerance. Secondly, although the two HIV groups had similar characteristics, they could differ in terms of HIV and/or HCV disease progression and may not allow a conclusive direct comparison with HIV-positive LTR, also because of a limited number of patients enrolled. Finally, none of the patients experienced OI or graft rejection, limiting the analysis of potential adverse events associated with different immune profiles.

In conclusion, an imbalance of certain immune cell populations that is known to be associated with HIV progression and immune senescence was found among HIV-positive transplant recipients, including altered frequencies of T cells (e.g., reduced T CD4$^+$ cells and increased T CD8$^+$ cells, effector CD8$^+$ Tregs, and T effector subpopulations). Furthermore, we observed the presence of immunological profiles that could have a negative impact on graft survival and favor organ rejection, such as decreased B and NK cell frequency, reduced memory B cells and increased effector CD4$^+$ Tregs among transplanted patients compared to healthy controls.

The impact of altered cell frequencies on HIV progression and organ rejection deserves further studies in order to identify immune signatures that can be predictive or protective of graft outcomes and potential therapeutic targets to prevent organ rejection in this patient population.

Conflict of interests

The authors had no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trim.2019.05.001.

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