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Research Article

Rise of a CD27⁻ IgD⁻ CD11c⁺ B cells population in kidney recipients achieving long-term graft stability under immunosuppression

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The use of immunosuppressive treatment is required to prevent rejection events, even a long time after kidney transplantation despite rare recipients achieving long-term graft stability without the need for immunosuppressive treatment, called operationally tolerant patients (TOLs). We comprehensively investigate the immune system of long-term IS recipients (LTTs) and TOLs to highlight their shared and unique immune features. Blood immune cell phenotyping was performed by spectral cytometry. Samples from 34 individuals were analyzed, including 6 LTTs, 8 TOLs, 10 stable patients at 1 year posttransplantation (STAs), and 10 healthy volunteers. B cells differed between LTTs and TOLs with a decreased total B-cell frequency and the acquisition of a memory phenotype in LTTs whereas a naive phenotype is maintained in TOLs. The frequencies of IgD⁻CD27⁻ B cells and CD11c⁺ memory B cells are increased in LTTs, with an exhausted phenotype, evoked by a significant decrease in CD25 expression. These CD11c⁺ B cells display an exhausted phenotype similar to those found in several chronic immune diseases in which they have been shown to participate in their pathophysiology, suggesting active chronic inflammation in LTTs. Altogether, these data indicate that precautions should be taken to minimize IS use.

Keywords: Atypical B cells · B cells · CD11c · Kidney transplantation · Long term stable patient



Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

Kidney transplantation is the treatment of choice for end-stage renal disease, but its management still needs to be improved to increase long-term graft survival [1]. The requirement of life-long immunosuppressive drugs (ISs) is responsible for important side

effects, including infection, malignancy, and nephrotoxicity, which may contribute to chronic allograft dysfunction [2–5]. Operational tolerance, defined as graft stability without requiring ISs, represents an end goal in the field of solid organ transplantation. Although numerous trials to reduce the use of ISs in kidney transplantation have been attempted, ISs remain indispensable to

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prevent graft rejection in the majority of recipients [6–8]. The study of immune cell homeostasis in patients achieving long-term graft stability under IS may provide important insights into understanding the immune conditions that prevent rejection events. We previously performed a phenotyping analysis focused on T- and B cells in a cohort of stable recipients under IS with middle-, long-term, and very long-term posttransplantation times. This work highlighted that a prolonged graft stability period under IS was associated with a reduced B-cell frequency and acquisition of a memory phenotype [9]. However, more work is needed to further explore regulatory or inflammatory immune cell populations, which may be of interest to understanding the immune profile of these patients.

Operationally tolerant patients (TOLs) are rare kidney recipients who achieve long-term stable function without IS treatment and represent the archetype of kidney transplant patients with a low risk of rejection. We and others identified several specificities in immune cells from TOLs, especially in their B cells. TOLs exhibit increased numbers of total B cells [10–14], associated with decreased frequencies of plasma cells (CD20⁺ CD38⁺ CD138⁺) and increased frequencies of transitional (CD24^{high} CD38^{high}) and naive (IgD⁺ CD27⁻) B-cell populations [10, 12–15]. Several studies also revealed an increased frequency of Granzyme B⁺ (GZMB) B cells and regulatory CD4⁺ CD127⁻ CD25^{high} FOXP3⁺ T cells with enhanced suppressive properties [16–18], a decrease in the frequency of PD-1^{high} CCR7^{low} follicular helper T cells [19] and a reduced expression level of CD16, perforin, and GZMA in CD56^{dim} NK cells, impairing their functional capacities [20] in the blood of TOLs. To this end, we compared a group of LTTs to TOLs, stable patients at 1-year posttransplantation (STAs), and healthy volunteers (HVs), in which we performed exhaustive phenotyping of their peripheral blood mononuclear cells (PBMCs) by spectral cytometry allowing the study of B cells. While the TOL B-cell phenotype reflects a relative immune regulation profile, those from LTTs may conversely trigger chronic immune system activation, in which IS maintenance is by far highly recommended.

Materials and methods

Study population

This research project was noninterventional and included 34 adult kidney transplant recipients who were monitored at the Angers, Nantes, Necker, and Saint-Louis hospitals. Reported clinical and research activities are consistent with the principles of the declarations of Istanbul and Helsinki and in line with the good practice recommendations of the University Hospital of Nantes. Written consent was obtained from all patients, and data were deposited prospectively into the multicenter DIVAT database approved by the French Ministry of Higher Education and Research (file 13.334-cohort DIVAT RC12_0452, www.divat.fr) and the CNIL (DR-2025-087 N°914184, 15/02/2015; ClinicalTrials.gov record-

ing NCT02900040). Normal renal function was defined as proteinuria below 0.5 g/24 h and a glomerular filtration rate (Modification of Diet in Renal Disease) over 40 mL/min. DSA monitoring was performed according to the center's standard of care. LTTs were defined as patients with normal renal function for more than 5 years posttransplantation. STAs were defined as patients with normal renal function at 1 year posttransplantation. TOLs were defined as kidney transplant patients with normal renal function and no IS for more than 1 year [21]. HVs from the French Blood Institute were included thanks to the agreement CPDL-PLER-2018 180.

Spectral cytometry

All resources and reagents are listed in Supporting Information Table S1. Antibodies of Panel 1 (T, B, NK, DC, monocytes) and 2 (T cells) are listed in Supporting Information Tables S2A and S2B, respectively. The global design of the study is described in Fig. 1A. Frozen PBMCs were thawed using a serum-free thawing solution and then incubated for 1 hour before staining. Cells were analyzed using a Cytex AURORA spectral flow cytometer.

Data analyses were performed using OMIQ software (Dotmatics, www.dotmatics.com) according to guidelines for the use of flow cytometry (Supporting Information Fig. S1A) [22]. After data compensation, the same gating strategy was applied for each individual to remove debris, doublets, dead cells, and autofluorescent cells (Supporting Information Fig. S1A). The PeacoQC algorithm was used as a quality control data process, removing the remaining aberrant events in the dataset [23]. An internal HV control was applied to the CytoNorm normalization process to correct potential batch effects [24]. Complete methods are described in the Supplementary Information.

Statistical analysis

Group comparisons were performed using nonparametric Kruskal–Wallis tests with Dunn's multigroup comparisons tests, and intragroup comparisons were performed using nonparametric Wilcoxon paired tests. Analyses were performed using GraphPad Prism v.9 (GraphPad Software).

Results

Patient description

The clinical characteristics of the 34 individuals are displayed in Supporting Information Table S3. Whereas posttransplantation times were not different between TOLs and LTTs (median = 148.5, CI 95% = [77.0–410.0] and 228.5 [70.0–389.0] months, respectively), they were significantly lower in the STAs ($p = 0.0068$ and 0.0004 compared with LTTs and TOLs, respectively).

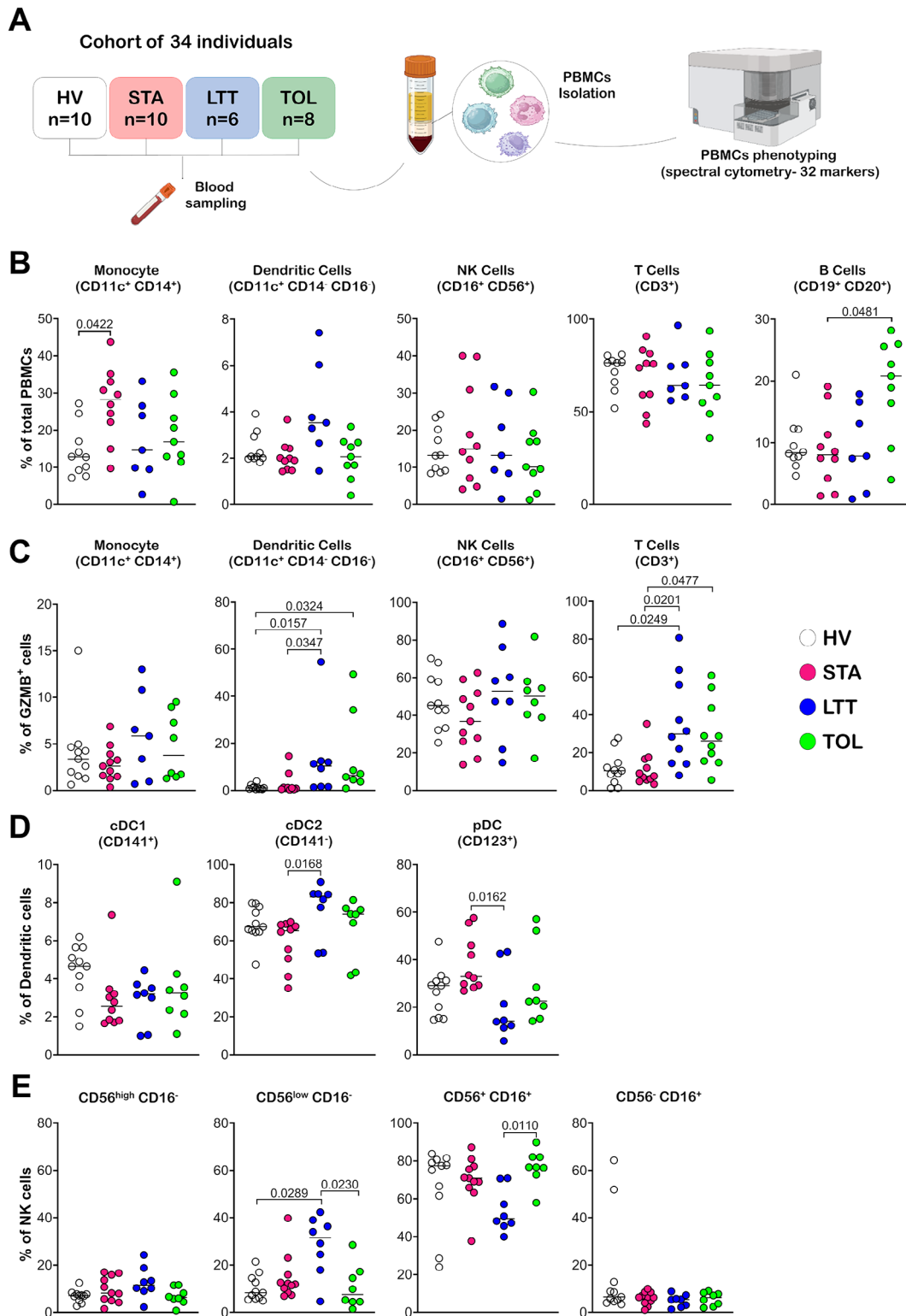


Figure 1. PBMCs populations phenotyping in kidney transplanted patients. (A) Schematic representation of methods and materials used in this study. (B) Representation of frequency of monocytes (CD11c⁺ CD14⁺), dendritic cells (DCs) (CD3⁻ CD19⁻ CD11c⁺ CD14⁻ CD16⁻), NK cells (CD16⁺ CD56⁺), T cells (CD3⁺) and B cells (CD19⁺ CD20⁺) in total PBMCs. (C) GZMB⁺ cells frequency (%) in total monocytes, DCs, NK, and T-cell populations. (D) Frequency (%) of conventional DC1 (CD141⁺) and DC2 populations (CD141⁻) and plasmacytoid DC (CD123⁺) in total DCs. (E) Frequency (%) of CD56^{high} CD16⁻ NKs, CD56⁺ CD16⁻ NKs, CD56⁺ CD16⁺ NKs and CD56⁻ CD16⁺ NKs in total NKs. In each graph, the bars indicate the median, and each point represents a single individual. The *p*-values were calculated using nonparametric Kruskal–Wallis test with the Dunn multiple comparisons tests.

Recipient age was not different between TOLs, LTTs, and STAs, while the ages of HVs were significantly lower than those of LTTs ($p = 0.0303$) and TOLs ($p = 0.0346$). At the time of sampling, LTTs were all treated with calcineurin inhibitors (cyclosporine A/tacrolimus: 2/4), five out of six received antiproliferative agents, and none had received steroids or mTOR inhibitors. STAs were all treated with CNIs (cyclosporine A/tacrolimus: 2/8) at the time of sampling, none received mTOR inhibitors, five STAs received only anti-proliferative agents, two only steroids, and three both antiproliferative agents and steroids.

LTTs show a B-cell imbalance in favor of differentiated B-cell subpopulations

We performed an extensive PBMC phenotyping of each group (Fig. 1A; Supporting Information Fig. S1–S2 and Table S2). Excepted for B cells, we did not find any significant difference in the frequency and absolute number of the main peripheral blood cells, e.g. CD11c⁺CD14⁺ monocytes, CD3⁻CD19⁻CD14⁻CD16⁻CD11c⁺ dendritic cells, CD16⁺CD56⁺NK cells, and CD3⁺ T cells, for the LTT and TOL groups compared with the other groups (Fig. 1B; Supporting Information Fig. S3A). We only observed a significant increase in total monocytes' frequency ($p = 0.0422$) and absolute number ($p = 0.0035$) in the blood of STAs compared with HVs (Fig. 1B; Supporting Information Fig. S3A). Further characterization of these cells revealed an increase in the frequencies of GZMB⁺ DCs and GZMB⁺ T cells in TOLs compared with HVs ($p = 0.0324$ and 0.0447 , respectively) and in LTTs compared with STAs ($p = 0.0347$ and 0.0201 , respectively) and HVs ($p = 0.0157$ and 0.0249 , respectively; Fig. 1C). We also found an increase of CD141⁻ DCs and a decrease of CD123⁺ plasmacytoid DCs frequencies in LTTs compared with STAs ($p = 0.0168$ and 0.0162 , respectively; Fig. 1D), aside with an increase in the CD56^{low} CD16⁻ NK cell population in LTTs compared with HVs and TOLs ($p = 0.0289$ and 0.0230 , respectively; Fig. 1E). A study of monocyte subpopulations revealed an increased frequency of CD11c⁺CD14⁺ monocytes in STAs compared with HVs, and conversely an increased frequency of CD14⁺CD16⁺ monocytes in HVs compared with the other groups (Supporting Information Fig. S3B). No significant variation in T-cell subpopulation frequency was observed between groups (Supporting Information Fig. S4).

The B cells compartment showed the largest differences in LTTs and TOLs. First, in agreement with our previous studies, we found a significant increase in the frequency and absolute number of total CD19⁺CD20⁺ B-cells in blood from TOLs compared with STAs ($p = 0.0481$; Fig. 1B; Supporting Information Fig. S3A). Within the B-cell compartment, we found a decreased frequency of IgD⁺CD27⁻ naive B cells in LTTs compared with STAs and TOLs ($p = 0.0328$ and 0.0336 , respectively) which was associated with an increased frequency of differentiated B cells in LTTs compared with STAs and TOLs, including IgA⁺ ($p = 0.0512$ vs. STAs, 0.0621 vs. TOLs) and IgG⁺ B cells ($p = 0.0093$ vs. STAs, 0.0127 vs. TOLs; Fig. 2A). Despite low levels in peripheral blood (~5%), we report

here a significantly increased frequency of IgD⁻CD27⁻ B cells, considered as double negative (DN) memory B cells [25], in LTTs compared with STAs and TOLs ($p = 0.0107$ and 0.0383 , respectively; Fig. 2A) and a significant increase in plasmablasts-plasma cells (CD24⁻CD38^{high}) frequency in LTTs compared with TOLs ($p = 0.0316$; Fig. 2A). These data show a more differentiated B-cell phenotype in the blood from LTTs compared with the other groups of transplanted recipients, particularly TOLs.

LTTs display a higher frequency of CD11c⁺ B cells with an IgD⁻ CD27⁻ DN memory phenotype, class switch BCR expression, and exhausted phenotype

We then analyzed the expression of markers associated with the homeostasis and activation of B cells, including PD-1, CD25 (IL2RA), GZMB, and the integrins CD11b and CD11c (Fig. 2B). We observed a more activated phenotype in B cells from STAs compared with the other groups and in particular TOLs, with a significantly increased frequency of B cells expressing PD-1 ($p = 0.0022$ in STAs vs. TOLs; Fig. 2B) and CD25 ($p = 0.0104$, 0.0088 vs. HVs and TOLs, respectively; Fig. 2B). We observed a higher frequency of B cells expressing GZMB in TOLs, in accordance with our previous results [16], and LTTs than in HVs ($p = 0.0732$, $p = 0.0534$; Fig. 2B). LTTs displayed a significantly increased frequency of CD11b⁺ B cells ($p = 0.0112$ and 0.0451 compared with HVs and TOLs, respectively) and a higher frequency of CD11c⁺ B cells compared with the other groups ($p = 0.0110$, 0.0458 , 0.0116 compared with HVs, STAs and TOLs, respectively; Fig. 2B).

Given this increase in CD11c⁺ B cells in LTTs (median of 36.55% vs. 10.85%, 10.34%, and 10.50% in HVs, STAs, and TOLs, respectively), we looked at their distribution in the different B-cell subpopulations. This increased CD11c⁺ B-cell population in LTTs was mainly composed of IgD⁻CD27⁻CD11c⁺ DN memory B cells, IgD⁻CD27⁺CD11c⁺ switch memory B cells, and, to a much lesser extent, IgD⁺CD27⁻CD11c⁺ naive B cells (Fig. 3A and B). No difference was found of CD11c⁺ B-cell frequency in transitional B cells or plasmablasts-plasma cell compartments between groups (Supporting Information Fig. S5).

In all groups, we observed in CD11c⁺ B cells an equivalent distribution of switch IgA⁺, switch IgG⁺, and unswitch IgA⁻ IgG⁻ B cells, with a significant increase of switch IgA⁺ CD11c⁺ and IgG⁺ CD11c⁺ B cells in LTTs compared with the other groups (Fig. 3C). We also found a significantly higher level of unswitch IgA⁻ IgG⁻ CD11c⁺ B cells in LTTs compared with HVs ($p = 0.0267$; Fig. 3C). Unswitch CD11c⁺ B cells in LTTs were mostly represented in the IgD^{low}IgM^{low} and IgD^{low}IgM^{high} compartments (Supporting Information Fig. S6). Together these results indicate that CD11c⁺ B cells in LTTs were mainly in the switch class B-cell compartment and that CD11c⁺ unswitch class B cells mostly exhibited down-regulated expression of IgD.

We further characterized the CD11c⁺ B cells in the different groups of patients and HVs, looking at the GZMB⁺, CD11b⁺, PD-1⁺, and CD25⁺ cell frequencies in CD11c⁺ B cells. No differences

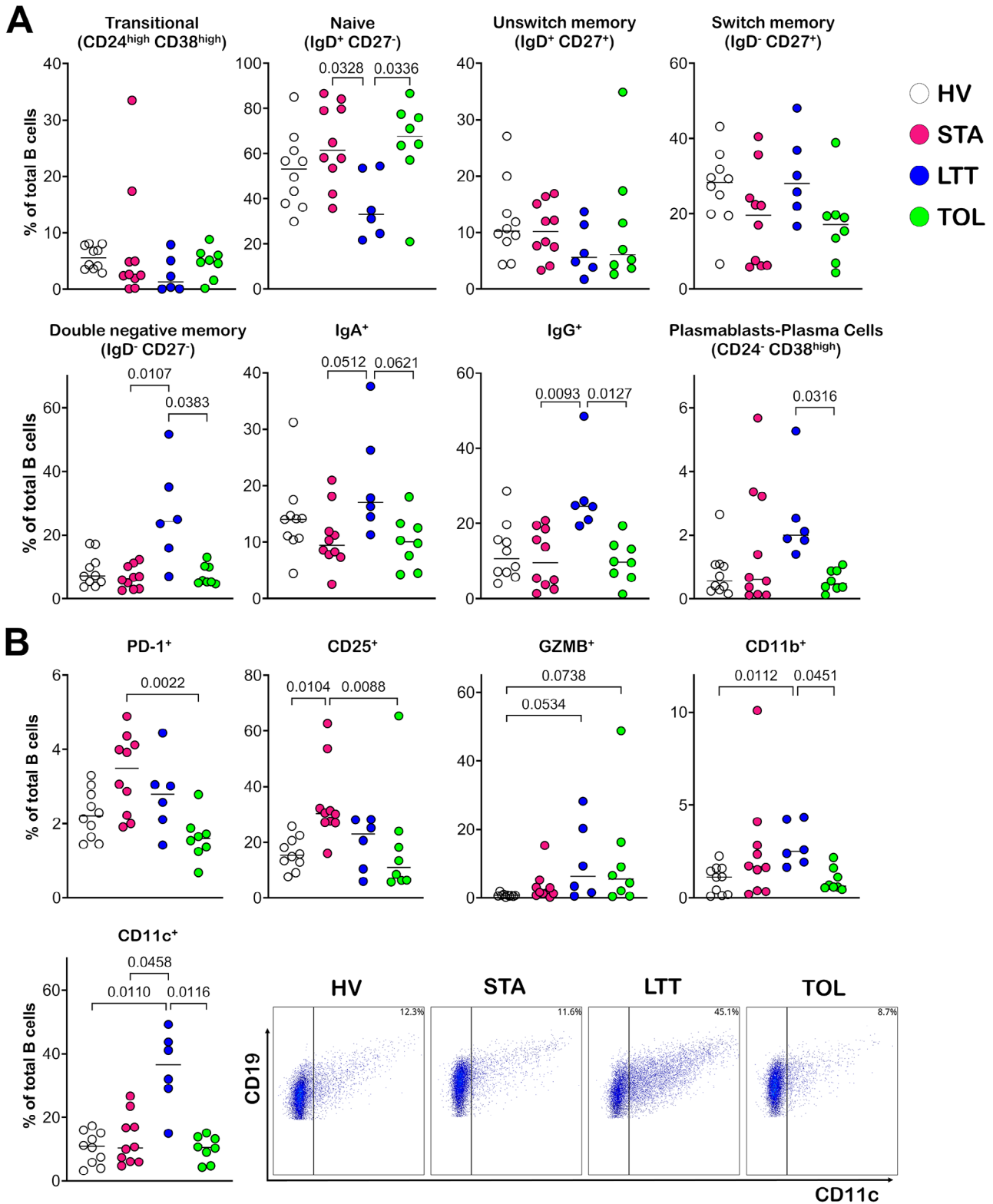


Figure 2. A switch toward a memory phenotype of LTTs B cells. (A) Frequency (%) in total B cells for each group of transitional (CD24^{high} CD38^{high}), naive B cells (IgD⁺ CD27⁻), unswitch memory B cells (IgD⁺ CD27⁺), switch memory B cells (IgD⁻ CD27⁺), double negative memory B cells (IgD⁻ CD27⁻), IgA⁺ B cells, IgG⁺ B cells and plasmablasts-plasma cells (CD24⁻ CD38^{high}). (B) Frequency (%) in total B cells for each group of PD-1⁺, CD25⁺, GZMB⁺, CD11b⁺, and CD11c⁺ B cells. Scatterplots of CD11c expression using four representative individuals of each group are represented. The bars indicate the median, and each point represents a single individual. The *p*-values were calculated using nonparametric ANOVA (Kruskal–Wallis) with the Dunn multiple comparisons test.

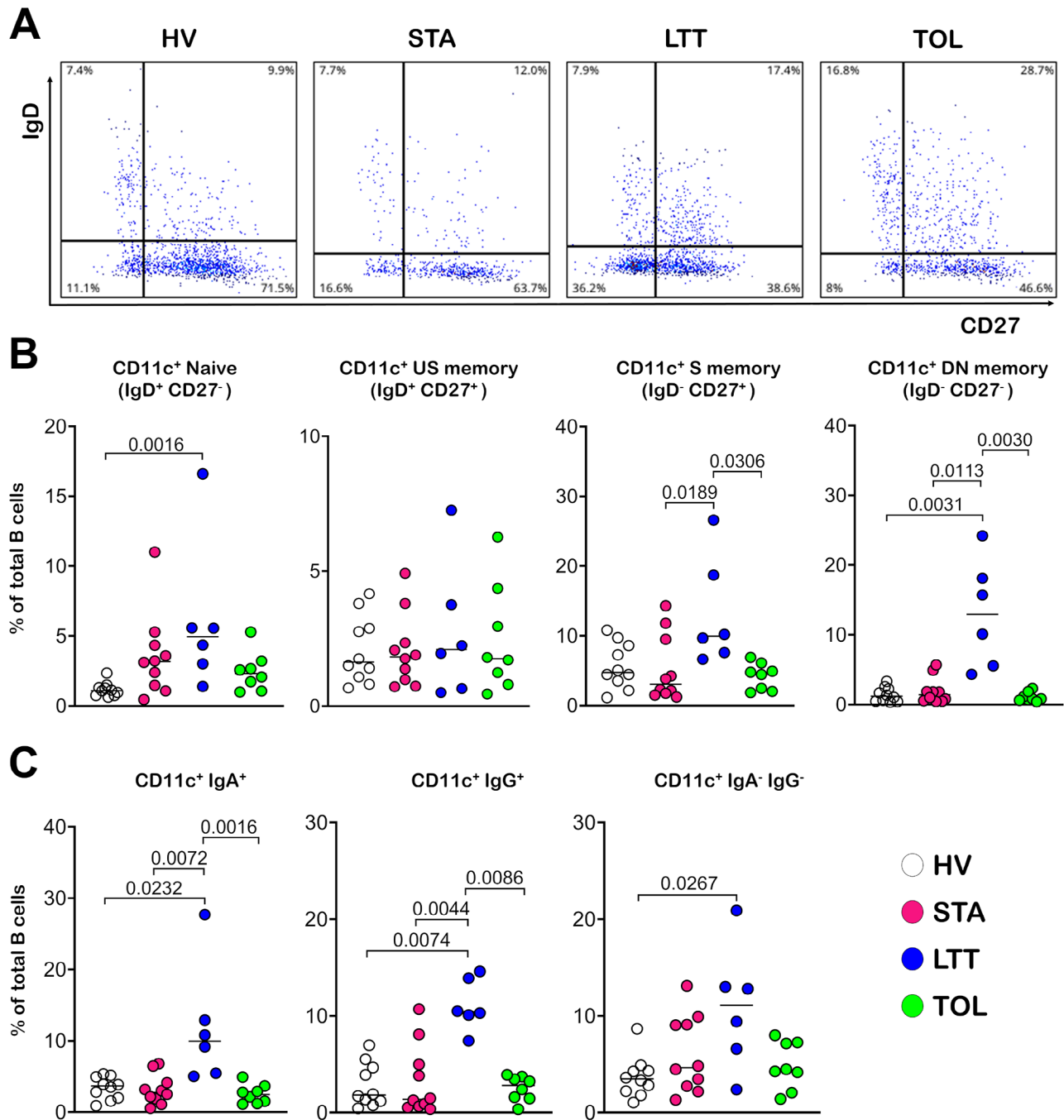


Figure 3. CD11c⁺ B cells repartition in B-cell subpopulations. (A) A scatterplot of IgD and CD27 expression in CD11c⁺ B cells in each group using four representative individuals. (B) Frequency (%) of CD11c⁺ B cells in total B cells for each group in naive (IgD⁺ CD27⁻), unswitch memory (IgD⁺ CD27⁺), switch memory (IgD⁻ CD27⁺), and double negative memory (IgD⁻ CD27⁻) B cells. (C) Frequency (%) of CD11c⁺ B cells in total B cells for each group in IgA⁺ B cells, IgG⁺ B cells, and IgA⁻ IgG⁻ B cells. In (B) and (C) the bars indicate the median, and each point represents a single individual. The p-values were calculated using nonparametric Kruskal–Wallis test with the Dunn multiple comparisons test using the LTTs group as control.

were observed in the PD-1⁺, CD11b⁺, and GZMB⁺ cell frequencies between LTTs and the other groups (Fig. 4A). In contrast, CD11c⁺ B cells from LTTs displayed a lower frequency of CD25⁺ cells than those from STAs ($p = 0.0078$; Fig. 4A).

To complete this analysis, we also compared CD11c⁺ with CD11c⁻ B cells in each group. We found that the GZMB⁺ cell fre-

quency was increased in CD11c⁺ (vs. CD11c⁻) B cells in each group but not significantly in LTTs (Fig. 4A), suggesting that the increase in GZMB⁺ B-cell frequency observed in the total B cells of LTTs may also be due to CD11c⁻ B cells. CD11b⁺ cells were mainly present in the CD11c⁺ B-cell compartment, representing approximately 5% of CD11c⁺ B cells compared with 1%

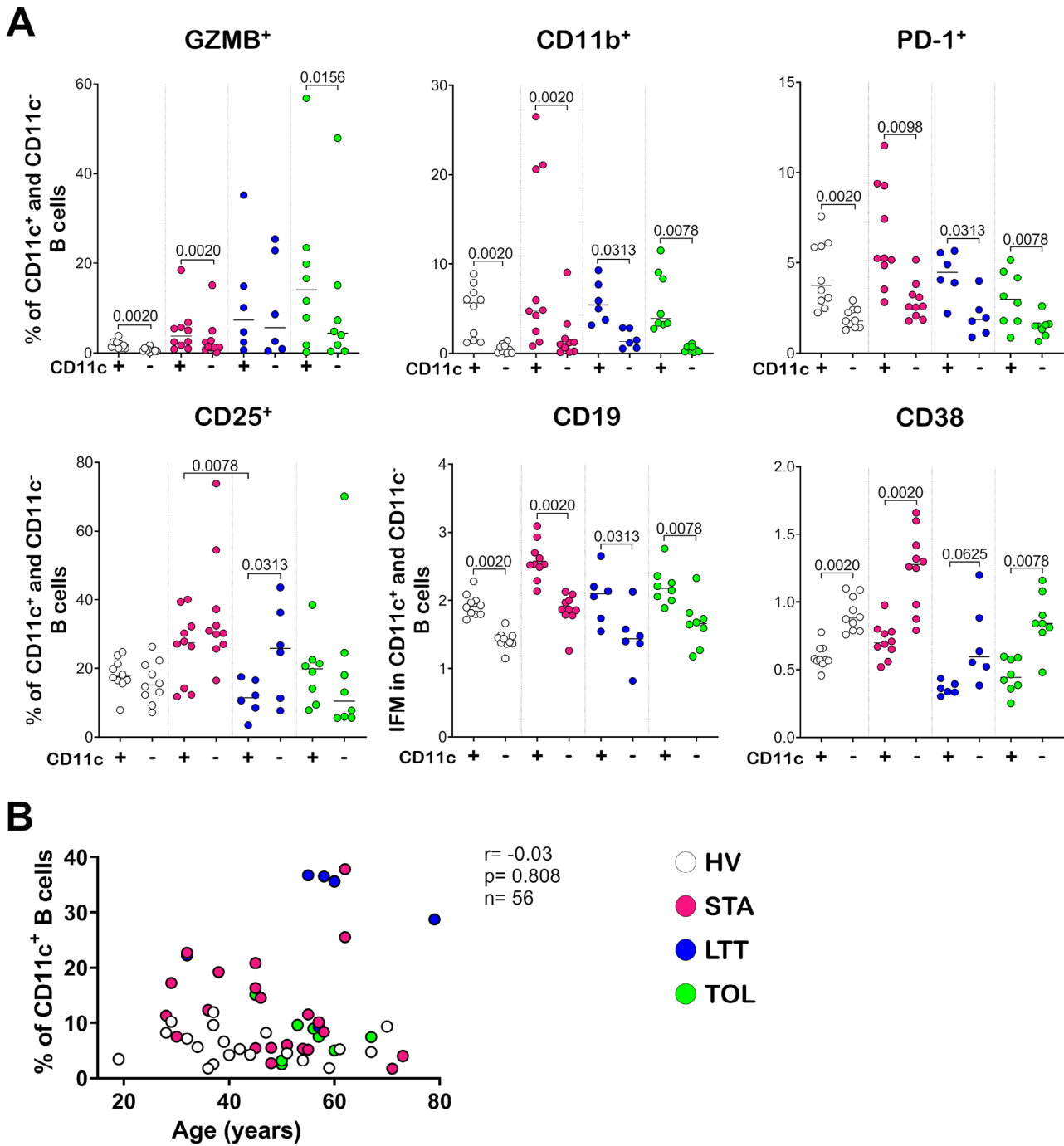


Figure 4. Expression of markers of B-cell activation and regulation in CD11c⁺ B cells and correlation between CD11c⁺ B cells frequency and individual's ages. (A) Median of CD11c⁺ and CD11c⁻ B cells in each group of GZMB⁺ cells frequency (%), CD11b⁺ cells frequency (%), PD-1⁺ cells frequency (%), CD25⁺ cells frequency, CD19 expression as induced fluorescence mean (IFM), CD38 expression as IFM. Each value is indicated by the points, and medians are indicated by the bars. Intergroup *p*-values were calculated using the nonparametric Kruskal–Wallis test with the Dunn multiple comparisons tests using the LTTs group as control. Intragroup *p*-values were calculated using a nonparametric paired Wilcoxon test. (B) Patients age at sampling in function of CD11c⁺ B cells frequency. A total of 56 individuals are presented including 20 HVs, 22 STAs, 6 LTTs, and 8 TOLs. Spearman correlation has been used to evaluate relationship between age and CD11c⁺ B-cell frequency.

of CD11c⁻ B cells (Fig. 4A). We found a significantly higher frequency of PD-1⁺ cells in CD11c⁺ (vs. CD11c⁻ B cells) in all groups of patients. This higher frequency is associated with a significant decrease in the frequency of CD25⁺ cells in CD11c⁺ (vs.

CD11c⁻ B cells) only in LTTs (Fig. 4A). Altogether, these data show that LTTs possess a higher frequency of CD11c⁺ B cells with an IgD⁻CD27⁻ DN memory phenotype, class switch BCR expression and an exhausted CD25⁻PD-1⁺ phenotype. We also reported

upregulated expression of CD19 and downregulated expression of CD38 in the CD11c⁺ (vs. CD11c⁻) B cells from all groups (Fig. 4A). Based on literature [26–28], we also analyzed the potential correlation between this increased CD11c⁺ DN memory B-cell population and age of individuals. On the entire cohort of individuals ($n = 34$) plus 22 independent samples to increase statistical power (12 STA, 10 HVs), we found no correlation between age at blood sampling and CD11c⁺ B cells frequency in blood ($r = -0.03$, $p = 0.808$; Fig. 4B).

Discussion

We previously reported that very long-term stable recipients (>25 years posttransplantation) displayed a reduced frequency of total and naive B cells and an increased frequency of switch memory (CD27⁺IgD⁻IgM⁻) B cells compared with patients with a shorter posttransplantation time [9]. In the present study, we confirmed this phenotype in LTTs compared with the other groups. Accordingly, we investigated the B-cell compartment in LTTs. Whereas all the groups of patients and HVs display comparable frequencies of unswitch (IgD⁺CD27⁺) and switch memory (IgD⁻CD27⁺) B cells, only LTTs exhibited an increased frequency of IgD⁻CD27⁻ DN memory B cells and CD11c⁺ B cells. Moreover, although CD11c⁺ B cells largely belong to the switch memory compartment (IgD⁻CD27⁺) in other groups, CD11c⁺ B cells in LTTs belong mainly to the DN (IgD⁻CD27⁻) compartment and only secondarily to the switch memory B-cell compartment. The origin and role of DN memory and CD11c⁺ B cells are still incompletely understood but have been increasingly described in recent years as expressing the T-bet marker and associated with chronic inflammatory disorders, including autoimmunity, infections, and immunosenescence [25, 29]. Interestingly, these CD11c⁺ B cells display high expression of CD19 and low expression of CD38, which makes this population comparable with the CD19^{high} CD21⁻ CXCR5⁻ CD11c⁺ DN population predominantly found in blood from patients with systemic lupus erythematosus (SLE) and with IgD⁻CD27⁻, CD21⁻CD27⁻, CD21⁻CD38⁻ DN B cells reported in blood from patients with primary Sjögren's syndrome [30, 31]. Not surprisingly, we found a general increase in the frequency of GZMB⁺ B cells in CD11c⁺ cells compared with CD11c⁻ B cells from the different groups of patients. Indeed, GZMB is a marker of differentiated B cells, which is consistent with the increased frequency of differentiated B cells in LTTs [32, 33]. As demonstrated in TOLs, GZMB⁺ B cells in LTTs may have some regulatory properties involved in long-term graft stability [16]. Further investigation would be needed to evidence the functional roles of CD11c⁺ and CD11c⁺ GZMB⁺ B cells in LTTs and in TOLs. LTTs also display a higher frequency of IgA⁺ and IgG⁺ CD11c⁺ B cells than other groups. Since CD11c⁺ B cells with a switch class represented approximately 2/3 of total CD11c⁺ B cells, they likely contribute to the increased frequency of IgA⁺ and IgG⁺ total B cells also found in LTTs. Regarding unswitch class B cells, the distribution of CD11c⁺ B cells in LTTs was higher in

the IgD^{low} compartment, suggesting an IgM memory phenotype of CD11c⁺ unswitch B cells in these patients, which also coincides with the increase in differentiated B cells in these patients. Thus, the frequency of CD11c⁺ B cells harboring a DN class switch memory phenotype was increased in LTTs, in accordance with the activated phenotype of CD11c^{high} T-bet⁺ B cells in SLE patients [34].

Interestingly, while CD11c⁺ B cells from LTTs present a decreased frequency of CD25⁺ (IL2RA) cells compared with their counterparts CD11c⁻ B cells, CD11c⁺ B cells from LTTs also displayed an even lower frequency of CD25⁺ cells compared with STAs CD11c⁺ B cells. In addition, more PD-1⁺CD11c⁺ B cells than PD-1⁺CD11c⁻ B cells were observed in LTTs. These results suggest a progressive exhaustion of CD11c⁺ B cells over a long period of IS treatment, which is consistent with several studies in which CD11c⁺ B cells appear to have an exhausted phenotype [30, 31, 35, 36]. Notably, upregulated expression of PD-1 and downregulated expression of *IL2RA* mRNA were observed in the CD11c⁺ B cells of SLE patients [30, 31]. This finding also coincides with the fact that CD11c⁺ B cells fail to express CD25 *in vitro* in response to BCR or CD40 triggering [30, 35, 36] in HVs [36] and SLE [30], common variable immunodeficiency (CVID) or rheumatoid arthritis (RA) patients [35]. CD11c⁺ B cells have also been found to be associated with the expression of several inhibitory molecules, including CD22, CD32, CD85, CD72, PDL1, and CD95 (Fas) [30, 31, 34–36]. This finding supports the fact that CD19^{high} CD11c⁺ memory B cells from patients with HIV, RA, or CVID are more susceptible to FasL-induced apoptosis or more prone to spontaneous apoptosis than their CD19^{low} CD11c⁻ counterparts [35, 37]. Altogether, CD11c⁺ B cells from LTTs share a common profile with those observed in the blood of patients with chronic inflammatory diseases, suggesting that CD11c⁺ B cells from LTTs are exhausted cells.

Interestingly, despite their exhausted profile, CD11c⁺ B cells in the literature are also responsive to TLR7 and TLR9 innate stimuli and are able to proliferate and secrete antibodies both in mice and humans [26, 27, 30, 35]. In mice, these CD11c⁺ B cells that are induced following chronic activation of TLR7 also express CD11b [26]. This observation is consistent with our findings that CD11b expression is restricted to CD11c⁺ B cells also observed in SLE patients [34]. We also report an increased frequency of plasmablasts-plasma cells in the blood from LTTs. Additionally, CD11c⁺ DN memory B cells in SLE patients express more *PRDM1* and *IRF4*, 2 genes involved in plasma cell generation, and were also associated with active disease and autoantibody secretion [30, 34]. Similarly, the frequency of DN CD11c⁺ memory B cells is increased in primary Sjögren's syndrome patients and expresses high levels of autoreactive antibodies [38]. CD11c⁺ have also recently been identified as clearly involved in the immune response against recrudescence malaria infection, with a central role in optimal T_{FH} cell response in this context [39]. Moreover, a recent study showed that kidney-transplanted patients who had experienced episodes of humoral rejection in the presence of DSA within the first 24 months of transplantation,

had a larger population of CD11c⁺T-bet⁺ atypical B cells [40]. Sequencing and cross-match tests realized on CD11c⁺ B cells from patients suffering from chronic inflammatory diseases, revealed that these cells were related to the development of DSA and the allo-immune response [30, 35, 40]. While we cannot rule out a regulatory role of CD11c⁺ B cells in LTTs, the association of this population with chronic allo-immune response suggests that its measure in clinical routine could help to stratify LTTs' patients according to their risk of rejection and avoid risky IS minimization.

CD11c⁺ B cells are also named "age-associated B cells" as they have been found to increase with age in mice and humans in physiological and pathological situations [26–28]. Atypical CD11c⁺ B are also found to be involved during childhood in the same chronic autoimmune and infectious disorders described in adults [41]. A recent study revealed the frequency of CD11c⁺ B cells in a cohort of kidney transplant patients before and after 3 years of transplantation [42]. The CD11c⁺ B-cell frequency at 3 years posttransplantation was similar to the frequency found in HVs and higher than that before transplantation. This finding is consistent with our analysis of the similar frequency of CD11c⁺ B cells in STAs (1 year) and HVs. They also show that this increased frequency is more important in recipients older than 50 years [42]. Nevertheless, no such correlation was found between CD11c⁺ B-cell frequency and age in our cohort regardless of their clinical status, suggesting another explanation for the increased frequency of CD11c⁺ B cells only in LTTs.

In conclusion, we report an increase in the CD11c⁺ memory B-cell population in immunocompromised patients with long-term graft stability, whereas this population is three times lower in TOLs and patients with short-term graft stability. Further analysis is obviously needed to elucidate the role of this particular B-lymphocyte population in the blood of LTT patients, but the current findings clearly suggest the maintenance of a chronic inflammatory immune response is contained by immunosuppressive drugs. Studies carried out on malaria and HIV patients with impaired immune responses support this hypothesis since CD11c⁺ B-cell frequency is increased in these pathologies and drops after malaria resolution or after the initiation of effective antiretroviral therapy in HIV patients [43, 44]. In liver transplantation, studies have shown that the beneficial effect of IS withdrawal was differently correlated with posttransplant time [45, 46]. In renal transplantation, studies have clearly shown failure of IS withdrawal in patients less than 6 years posttransplantation [6–8, 47]. The results of these studies and ours do not suggest better IS weaning capacities in long-term kidney recipients but rather support previous studies highlighting immunological differences in liver and kidney transplant patients [48, 49]. Our study also shows that these LTTs are different from TOLs from an immunological point of view and that it might be wise to monitor these B-cell populations with an exhausted phenotype, which are indicative of a chronic inflammatory state and can be reactivated in the event of treatment modification.

Data limitation and perspectives

This work is a descriptive study that highlights specific features of the immunology of long-term stable kidney transplant patients, particularly in comparison with patients who tolerate their graft, the grail in immunology. These results need to be validated on a larger number of patients. The role of this CD11c⁺ B-cell population in LTTs will need to be demonstrated, in particular through in vitro functional studies. Finally, the potential role of this B population as a biomarker for monitoring long-term stable patients, particularly with regard to immunological risk and adjustment of IS drugs, will also need to be demonstrated and validated on large cohorts of patients.

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Abbreviations: **CVID:** common variable immunodeficiency · **DN:** double negative · **HVs:** healthy volunteers · **IS:** immunosuppressive therapies · **LTTs:** stable patients over 5 years posttransplantation · **PBMCS:** peripheral blood mononuclear cells · **RA:** rheumatoid arthritis · **SLE:** systemic lupus erythematosus · **STAs:** stable patients at 1-year posttransplantation · **TOLs:** tolerant patients

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