Calcineurin and mTOR inhibitors have opposing effects on regulatory T cells while reducing regulatory B cell populations in kidney transplant recipients

Irene Latorre, Ana Esteve-Sole, Dolores Redondo, Sandra Giest, Jordi Argilaguet, Sara Alvarez, Cristina Peligero, Isabelle Forstmann, Marta Crespo, Julio Pascual, Andreas Meyerhans

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Title:
Calcineurin and mTOR inhibitors have opposing effects on regulatory T cells while reducing regulatory B cell populations in kidney transplant recipients

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FOOTNOTES

ABBREVIATIONS (list alphabetically)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BFA</td>
<td>Brefeldin A</td>
</tr>
<tr>
<td>Breg cells</td>
<td>Regulatory B cells</td>
</tr>
<tr>
<td>B10</td>
<td>IL10-secreting B cells</td>
</tr>
<tr>
<td>CNI</td>
<td>Calcineurin inhibitors</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>KT</td>
<td>Kidney transplant</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian target of rapamycin</td>
</tr>
<tr>
<td>PBMCs</td>
<td>Peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>Treg cells</td>
<td>Regulatory T cells</td>
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</table>
ABSTRACT

Background

Regulatory B (Breg) and T (Treg) cells represent a biomarker for tolerance in transplant patients. Despite the importance of Treg and Breg in transplantation and the suggested crosstalk between both suppressive cell populations, little is known on how they are influenced by long-term immunosuppressive treatment. The aim of the present study was to investigate the effect of different immunosuppressive drugs used in routine clinical practice on Treg and Breg cell numbers.

Methods

Thirty-six kidney transplant recipients with stable graft function were recruited and classified according their concomitant therapy: 22 patients received calcineurin inhibitors (CNI) and 14 patients mammalian target of rapamycin (mTOR) inhibitors. A group of 8 healthy untreated subjects was included as control. Absolute numbers of peripheral blood-derived IL10-producing B cells (CD19^IL10^), CD19^CD24^hiCD38^hi transitional B cells and Treg cells (CD4^CD25^FOXP3^) were quantified in all KT patients and controls by flow cytometry.

Results

CD19^CD24^hiCD38^hi transitional B cells increased over time and seem to be related with long-term therapeutic graft survival irrespective of the treatment regimen. CNI and mTOR inhibitors significantly reduced numbers of Breg cells when compared with healthy individuals, whereas mTOR inhibitors expanded Treg cells in comparison with CNI drugs.

Conclusions

Bridging the drug-mediated reduction of Breg cell numbers in vivo with the requirements of Breg cells for long-term transplant success remains an as yet unresolved task for therapeutic intervention. Further larger cohort studies that evaluate the effect of different treatment regimen on defined lymphocyte subpopulations are warranted.

Keywords: regulatory B cells, regulatory T cells, immunosuppressive drugs, transplantation
1. INTRODUCTION

Organ transplantation is the treatment of choice for patients with end-stage organ failure. Its success critically depends on efficient immunosuppressive drug regimen to avoid allograft rejection. Effector T cells have long been recognized as critical mediators of rejection and regulatory T cells (Treg) as mediators of tolerance (1). However, literature suggests that B cell mediated immunity has a major contribution in the prevention of transplant rejection as well (2). This is especially evident during spontaneous tolerance (also called operational tolerance), defined as the development of tolerance after the complete withdrawal of immunosuppressants in a rare group of transplanted individuals. Spontaneous tolerant individuals present a B cell gene expression signature and preserve numbers of total B cells similar to healthy individuals (3-5). Within this B cell population, there is a subgroup of so-called regulatory B cells (Breg) that mediate tolerance by down-regulating effector immune responses via secreting IL10 or cell-to-cell contacts (6-11). Although there is no specific cell marker for such IL10-secreting B cells, certain B-cell subsets such as CD19^+CD24^hiCD38^hi transitional B cells have been shown to be enriched for IL10 expression (7, 10).

Studies on B cell depletion during transplant induction therapy and transplant tolerance suggest a suppressive function and a crosstalk between Treg and Breg subpopulations in transplantation (11). However, little is known on how both cell types are influenced by long-term treatment with immunosuppressive drugs. A very recent study has prospectively followed a cohort of kidney transplant (KT) recipients under tacrolimus for over one year post transplantation. Patients with high transitional B cell rates experienced reduced rejection episodes (12). Furthermore, across-sectional study covering 10 years post transplantation demonstrated that Breg cells contribute to the maintenance of graft survival in patients treated with calcineurin inhibitors (CNI) (13). Treatment of KT patients with belatacept, a novel T cell co-stimulation blocker, led to an increase of Breg and Treg cell numbers when compared to a standard CNI treatment (14, 15). It is unclear however how other
immunosuppressive drugs in clinical practice affect both regulatory cell subsets and whether these contribute to tolerance during very long time periods after transplantation.

2. OBJECTIVE

The present study investigated the effect of CNI and mammalian target of rapamycin (mTOR) inhibitor therapies on Breg and Treg cell numbers in KT recipients with stable graft function. Breg cells described as IL10-secreting B cells (7) or CD19^+CD24^hiCD38^hi transitional B cells (7, 10) as well as CD4^+CD25^-FOXP3^- Treg cells were quantified by flow cytometry and data were correlated with clinical parameters. A significant positive correlation of CD19^+CD24^+CD38^hi transitional B cell numbers with long-term (up to 28 years) graft survival under therapy was observed, irrespective of the drug-regimen. Moreover, a reduction of Breg cell numbers by mTOR inhibitors seemed to be compensated by an expansion of Treg cells. CNI treatment led as expected to a reduction of both regulatory cell subsets. These interesting observations derived from our pilot study with 36 patients call for longitudinal large cohort studies addressing the Treg and Breg crosstalk during diverse immunosuppressive drug regimen and their respective predictive values as biomarker of transplant success.

3. MATERIALS AND METHODS

3.1. Patients and inclusion criteria

We recruited KT recipients with stable graft function who attended the Nephrology Department of the Hospital del Mar (Barcelona, Spain) between December 2012 and February 2014. For immune phenotyping by flow cytometry, a total of 20 mL blood was drawn from each patient into lithium-heparinized tubes. Complete blood counts (Haemogram) were determined in parallel to derive absolute cell numbers. The collected patients’ data were: gender, age, kidney origin, CMV disease, drug-therapeutic profile, years after transplantation, estimated glomerular filtration rate (eGFR) and proteinuria/creatinine ratio. Ethical approval for this study was obtained from the corresponding Ethics Committee. All the study participants gave written informed consent before entering the study.
3.2. Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated by Ficoll-Histopaque (Sigma-Aldrich, St. Louis, MO, USA) density gradient centrifugation. Remaining red blood cells were lysed with ammonium chloride (NH₄Cl, 0.15M). PBMCs were washed with complete medium [RPMI 1640 with L-glutamine (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS; Sigma-Aldrich, St. Louis, MO, USA), 1μl/ml penicillin and 1μl/ml streptomycin (Invitrogen, Grand Island, NY, USA)]. Viable cells were counted with an inverted microscope using trypan blue.

3.3. Quantification of IL10-secreting B cells (B10), CD19⁺CD24⁺⁺CD38⁺⁺ transitional B cells and regulatory T cells (Treg)

Transitional B cells and Treg were quantified directly from PBMCs by flow cytometry as CD19⁺CD24⁺⁺CD38⁺⁺ B cells and CD4⁺CD25⁺FOXP3⁺ T cells, respectively. As the frequency of B10 cells in humans is low (i.e. around 0.6% of B cells), they were quantified after in vitro stimulation of PBMCs using a slightly modified published method (7). Briefly, a total of 10⁶ PBMCs were cultured for 48 hrs in a B cell maturation mix containing CD40L (1μg/ml; Insight genomics, Falls Church, VA, USA) and lipopolysaccharide (10μg/ml, LPS; Escherichia coli serotype 0111:B4; Sigma-Aldrich, St. Louis, MO, USA) in a final volume of 1ml complete medium within a humidified incubator at 37°C with 5% CO₂. Cells were subsequently stimulated for 5h with CLIP, consisting of ODN-2006 CpG (10μg/ml, phosphothiorated 5’ tcgtctttgtcggtttgtc 3’, Metabion, Martinsried, Germany), LPS (1μl/ml), ionomycin (1μg/ml; Sigma-Aldrich, St. Louis, MO, USA) and PMA (50ng/ml; Sigma-Aldrich, St. Louis, MO, USA). Brefeldin A (10μg/ml, BFA; Sigma-Aldrich, St. Louis, MO, USA) was added in order to stop cytokine secretion via Golgi transport. B10 cells were then quantified by flow cytometry as CD19⁻IL10⁻ B cells.
To exclude the possibility that the 48 hrs culture for activation of IL10-producing B cells induces cell proliferation and thus changes the cell population composition in vitro, PBMCs from two donors were labeled with Carboxyfluorescein Succinimidyl Ester (CFSE; Invitrogen, Grand Island, NY, USA) and stimulated during 2 and 5 days at 37°C with: (i) CD40L (1μg/ml) and LPS (10μg/ml), (ii) Bmix as positive control [pokeweed mitogen (0.01 µg/ml; PWM; Sigma L877, St. Louis, USA), protein A from Staphylococcus aureus (0.12 µg/ml; StaphA; P7144, Sigma, St. Louis, USA), Proleukin 2 (0.1 µg/ml; IL2; Novartis, Basel, Switzerland), Interleukin 10 (0.025 µg/ml; IL10; HiSS Diagnostics GmbH, Freiburg, Germany) and CpG ODN-2006 (1µg/ml)], or (iii) medium alone as negative control. Proliferation is then analyzed by CFSE-dilution with flow cytometry.

3.4. Antibody staining and flow cytometry

PBMCs were stained with the following fluorochrome-conjugated anti-human surface and intracellular antibodies: CD4 PerCPCy5.5 (SK3; BD Bioscience, San Jose, CA, USA), CD19 PECy7 (HIB19; BioLegend San Diego, CA, USA), CD24 PerCPCy5.5 (ML4; BD Bioscience, San Jose, CA, USA) CD25 APCH7 (M-A251; BD Bioscience, San Jose, CA, USA), CD38 APC (HIT-2; BD Bioscience, San Jose, CA, USA), IL10 PE (JES3-19F17; BD Bioscience, San Jose, CA, USA), TNF-α PE (6401.1111; BD Bioscience, San Jose, CA, USA) and FoxP3 Alexa 647 (259D/C7; BD Bioscience, San Jose, CA, USA). PBMCs were first washed with FACS buffer (phosphate buffered saline with 5% FCS, 0.5% BSA and 0.07% NaN₃) and then incubated during 30min at room temperature with surface antibodies. For intracellular cytokine staining, cells were incubated with Fix/Perm buffer (eBiosciences, San Diego, CA, USA) and then washed with Perm Buffer (eBiosciences San Diego, CA, USA) in order to fix and permeabilize cells. After washing, cells were incubated during 30min at room temperature with antibodies against intracellular antigens. Cells were finally fixed in FACS FIX buffer (0.9%NaCl, 1% paraformaldehyde) and acquired with a LSR II flow cytometer (BD Bioscience, San Jose, CA, USA) within 24 hours after cell staining. Absolute numbers of the different cell subsets were calculated on the basis of total blood lymphocyte counts of the
haemograms. Flow cytometry data analysis was performed using FlowJo 9.44 software (TreeStar, Inc., Ashland, OR, USA) following the gating strategy shown in Figure 1A-B.

3.5. Statistical data analysis
Comparison of different cell populations between groups of patients was performed using the Mann-Whitney U-test analysis. Differences were considered significant when p-values were lower than 0.05. Association between CD19^-^CD24^hi^-^-CD38^hi^-^ immature transitional B cells and years post-transplantation was assessed using Spearman correlation coefficient. A multivariate linear regression analysis was performed for CD19^-^CD24^hi^-^-CD38^hi^-^ cells and Treg cells adjusting for treatment regimen, years after transplantation and eGFR (mL/min). All statistical analyses were done using SPSS statistical software (SPSS version 15.0; SPSS, Chicago, IL, USA). The graphical representations were made with GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA).

4. RESULTS
4.1. Patient characteristics
KT patients were classified according to their concomitant therapy: (i) 22 patients were receiving CNI, of which 16 (72.7%) were under cyclosporine regimen and 6 (27.3%) under tacrolimus; (ii) 14 patients were taking mTOR inhibitors, of which 6 (42.8%) were under rapamycin regimen and 8 (57.2%) under everolimus. Regarding the induction therapy: (i) 16 KT patients (44.5%) received anti-CD25 monoclonal antibody, (ii) 17 KT patients (47.2%) received anti-lymphocyte therapy and (iii) 3 KT patients (8.3%) did not receive any induction therapy. A group of 8 healthy, untreated blood donors was included as control. The main demographic characteristics of all individuals included in the study and their treatments are summarized in Table 1. No significant differences on age and gender were observed between KT patients and healthy controls. Due to the inclusion of patients under classical CNI therapies, the time frame since transplantation was higher in this group than the one receiving mTOR inhibitors (p=0.082).
4.2. Transitional B cells correlate with long-term therapeutic graft survival irrespective of CNI or mTOR inhibitor treatment

In order to study whether IL10-secreting B cells and CD19^CD24^{hi}CD38^{hi} transitional B cells were influenced by long-term immunosuppressive therapy, we collected blood from KT recipients with stable graft function and correlated both regulatory B cell subsets with time after transplantation. Both B cell populations were quantified from PBMCs by flow cytometry as shown in Figure 1A and B. There was a moderate positive correlation between transitional B cells and years after transplantation, which is supported by a significant correlation coefficient (Spearman’s rho = 0.437; p=0.007) (Figure 1C). In order to explore whether the type of treatment has influenced the statistical evaluation of the observed correlation, a multivariate regression analysis for transitional B cells was performed adjusting for years post transplantation and treatment regimen. It revealed that CD19^CD24^{hi}CD38^{hi} transitional B cells significantly correlated with years post transplantation irrespective of CNI or mTOR inhibitor treatment (Table 2). Thus, the data taken together are concordant with previous claims on the importance of CD19^CD24^{hi}CD38^{hi} transitional B cells for transplantation success and extend observations with CNI inhibitor treatment to mTOR inhibitors (13).

4.3. CNI and mTOR inhibitors differentially affect Breg and Treg cell numbers in long-term immunosuppressed KT recipients

In order to investigate the effect of commonly used immunosuppressive drugs on Breg and Treg cell numbers in vivo, we measured Bregs as IL10-secreting B cells or CD19^CD24^{hi}CD38^{hi} transitional B cells and Tregs as CD4^CD25^{hi}FOXP3^{+} cells in our patient cohort. Since the quantitation of IL10-secreting Breg cells required a 48hrs stimulation with CD40L and LPS, we excluded that this would induce proliferation of B cells and thus a distortion in the B cell compartment (Figure 1D). The numbers of IL10-producing B cells and transitional B cells were significantly decreased in CNI- and mTOR inhibitor-treated patients with respect to healthy controls (Figure 1E; p=0.001 and p=0.011 for IL10-producing B
cells and p=0.041 and p=0.002 for CD4⁺CD25⁺FOXP3⁺ transitional B cells, respectively). Interestingly, the number of Treg cells in patients taking mTOR inhibitors was comparable to those of healthy controls (p=0.109), in line with previous observations (16-19). The number of CD19⁺CD24⁺CD38⁺ transitional B cells and Treg cells was significantly different between patients taking CNI and mTOR inhibitors (Figure 1E; p=0.022 and p=0.0003, respectively). However, these differences between the patient groups may be attributed to the time frame since transplantation, which was higher in the CNI group with respect to the one receiving mTOR inhibitors. Thus, in order to test whether the observed significant differences of transitional B and Treg cell numbers between the treated groups were influenced by years post transplantation, a multivariate analysis was performed for these two subsets adjusting for years post transplantation, treatment regimen and eGFR (mL/min). It revealed that the number of Breg cells correlated with years’ post transplantation irrespective of CNI or mTOR inhibitor treatment. Nevertheless, only mTOR inhibitor treatment rather than CNI treatment resulted in high Treg cell numbers (Table 2). Thus, during mTOR inhibitor treatment, a reduction of Breg cells may be compensated by maintenance or even an increase of Tregs.

5. DISCUSSION

Given the increasing evidences of a beneficial effect of regulatory lymphocyte subsets for transplantation success (5, 12, 13, 20, 21), the impact of different immunosuppressive drug regimen on these cells is insufficiently studied. Especially simultaneous evaluations of Breg and Treg cells are rare. Here we analyzed IL10-secreting B cell, CD19⁺CD24⁺CD38⁺ transitional B cell and Treg cell numbers in KT patients with stable graft function who received the classical CNI or mTOR inhibitor therapies. We found an increase of CD19⁺CD24⁺CD38⁺ transitional B cells over time post transplantation irrespective of the treatment regimen used. Thus a long-term transplant success of up to 28 years, which is the longest graft survival time within our patient cohort, seems to be associated with this regulatory B cell subset. Furthermore, a differential effect on Breg and Treg cell numbers was observed with CNI and
mTOR inhibitors when compared with healthy controls, supporting the need for a detailed evaluation of different drug regimen for transplant recipients.

The half-life of a living donor kidney transplant has been described to be around 12 years (22). The observations that successful transplantations far beyond 12 years are possible and that they correlate with regulatory B cell subsets clearly demonstrate that this time frame is not an inevitable expiry date of an organ but an immunologically manipulable condition. However, both CNI and mTOR inhibitor treatment reduce Breg cell numbers in vivo and in vitro (data not shown) implying that they are not the direct cause of the long-term success. Indeed, a major patient benefit of immunosuppressive treatment after transplantation is the reduction of acute graft rejection (23, 24) and rather little is known about how to actively pursue long-term perspectives. Clearly, fundamental studies on how to promote Breg development even in the presence of suppressive therapy is urgently needed to make targeted progress. Two recent studies evaluating the T cell co-stimulation inhibitor belatacept follow along these lines and show the first promising clinical results (14, 15). This drug, approved 2011 by the FDA, led to a significant increase of the frequency of transitional B cells relative to CNI treatment and concomitant graft acceptance. Results from long-term studies are now impatiently awaited.

Limitations of our study should be mentioned. First, the groups of patients according to their treatment regimen (CNI versus mTOR inhibitors) are different in terms of years after transplantation. This is due to the inclusion of patients under the classical drugs targeting calcineurin activity. Nonetheless, consistent results were obtained performing a multivariate analysis. Second, the number of patients included is limited. Third, our cohort of KT patients is not prospectively followed over time and does not included individuals with chronic rejection. However, as a pilot study it provides sufficient evidence that it would be interesting to move forward in this matter. In line with this, two of our patients lost their grafts and presented very high B effector/Breg ratios (Beff/Breg ratio: 141.8 and 201.2; median: 20.53) (25). These observations call for studying and following-up larger matched case-
control cohorts to better evaluate lymphocyte subsets as biomarker for individualized therapies and outcome.

5.1. Conclusions

In conclusion, (i) a differential effect of CNI and mTOR inhibitor treatment after KT on Breg and Treg lymphocyte subsets is described and (ii) transitional B cells and long-term transplantation success seem to correlate for both treatment regimens. However, the knowledge gap between the direct drug effect of lowering Breg cells with transplantation success that correlates with high Breg cell numbers remains, and requests further investigations with larger patient cohorts. Eventually this will lead to a better definition of personalized transplantation therapies.

ACKNOWLEDGMENTS

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REFERENCES

FIGURE LEGEND

Figure 1. CNI, mTOR inhibitors as well as years after transplantation are three independent parameters that have a differential effect on regulatory B and T cell subsets. (A) Dot plots from one representative donor showing gating strategy of (A) IL10-secreting B cells, CD19<sup>-</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> transitional B cells and (B) Treg cells (defined as CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup>). First, lymphocytes were gated using characteristic forward scatter (FSC-A) and side scatter (SSC-A). Then aggregates were taken off by gating on the diagonal that appears with FSC-A (area) and FSC-H (height). For B and T cell gating, CD19<sup>-</sup> cells and CD4<sup>+</sup> T were selected, respectively. IL10-secreting B cells are calculated using the difference between CLIP-B stimulated samples and non-stimulated samples. (C) Spearman correlation of years after transplantation with CD19<sup>-</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> immature transitional B cells. (D) Histograms showing CD19<sup>-</sup> cell proliferation within 2 and 5 days using CD40L+LPS, Bmix (positive control) or medium alone (negative control) as stimuli in one representative donor. The mean ± SEM of CD19<sup>-</sup> proliferation is represented for each condition. (E) Comparison of absolute numbers of IL10- producing B cells (B10), CD19<sup>-</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> immature transitional B cells and CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Treg cells between transplant patients under different immunosuppressive drug-regimens and healthy subjects. The horizontal line represents the median from each group. Statistical analysis was performed using Mann-Whitney test. Only significant p values less than 0.05 are shown in the graphs. Numbers of IL10- secreting B cells, CD19<sup>-</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> immature transitional cells and Treg cells were not significantly different between cyclosporine and tacrolimus individuals of the CNI group, or between rapamycin and everolimus individuals of the mTOR inhibitors group.

Breg, regulatory B cells; Treg, regulatory T cells; CNI, calcineurin inhibitors; mTOR, mammalian target of rapamycin; HC, healthy controls; SR: Spearman Rho; SEM, standard error of the mean.
Figure 1
Table 1. Demographic and clinical characteristics of renal transplant patients and healthy controls included in the study

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>All patients n=36 (%)</th>
<th>Calcineurin inhibitors n=22 (%)</th>
<th>mTOR inhibitors n=14 (%)</th>
<th>Healthy controls n=8</th>
<th>Significance between groups (p)*</th>
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<tr>
<td><strong>Gender</strong></td>
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<td>29/44 (65.9)**</td>
<td>14 (63.6)</td>
<td>10 (71.4)</td>
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<td>Female</td>
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<td>8 (36.4)</td>
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<td><strong>Age (years), median (IQR)</strong></td>
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<td>44.5 (54.0-33.0)</td>
<td>45.0 (53.2-29.0)</td>
<td>33.5 (58.0-28.0)</td>
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<td>Mycophenolate</td>
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<td>Years after transplantation, median (IQR)</td>
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<td>217 (402-134)</td>
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</tbody>
</table>

*Significance on age difference between the three groups of individuals was calculated using the Kruskal Wallis test. Significance on kidney origin was calculated using Fisher’s Exact test, since the total number of living donor recipients was 3. Significance on years after transplantation, eGFR and proteinuria/creatinine ratio was calculated using Mann-Whitney test.

**Transplant patients (n=36) and healthy controls (n=8) together

P values less than 0.05 were considered significant.

IQR, interquartile range; N/A, not applicable; CNI, calcineurin inhibitors; mTOR, mammalian target of rapamycin; eGFR, estimated glomerular filtration rate.
Table 2. Multivariate linear regression analysis for T and B cell subsets (dependent variable) adjusted for drug-therapeutic profile, years after transplantation and eGFR (mL/min)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CD19&lt;sup&gt;+&lt;/sup&gt;CD24&lt;sup&gt;hi&lt;/sup&gt;CD38&lt;sup&gt;hi&lt;/sup&gt; cells</th>
<th>Treg cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ) (CI95%)</td>
<td>p-value</td>
</tr>
<tr>
<td>Drug-therapeutic profile (mTor)</td>
<td>-1.65 (-3.70 – 0.391)</td>
<td>0.109</td>
</tr>
<tr>
<td>Time after transplantation (years)</td>
<td>0.17 (0.04 – 0.297)</td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td>eGFR (mL/min)</td>
<td>0.08 (-0.01 – 0.17)</td>
<td><strong>0.064</strong></td>
</tr>
</tbody>
</table>

\( \beta \) indicates the inclination of the slope. A positive \( \beta \) value indicates a positive correlation. A negative \( \beta \) value indicates a negative correlation.

Values for drug-therapeutic profiles are referred to mTor regimen.

* Significant p values

mTor, mammalian target of rapamycin; Beff, effector B cells; Breg, regulatory B cells; Treg, regulatory T cells; CI, confidence interval; eGFR, estimated glomerular filtration rate
HIGHLIGHTS

- Regulatory B cells increase over time after transplantation, suggesting that they might be related with graft survival.
- Immunosuppressive drugs have a differential effect on regulatory lymphocyte subsets.
- KT transplant recipients under CNI treatment show reduced levels of regulatory B and T cells.
- KT transplant recipients under mTOR treatment show reduced levels of regulatory B cells while regulatory T cells numbers are maintained.