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Peripheral CD19 + CD24^{high}CD38^{high} B-regulatory cells in lung transplant recipients

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ABSTRACT

Background: The role of CD19 + CD24^{high}CD38^{high} B-regulatory cells in solid-organ Transplant (Tx) in acceptance are still scarce. In previous studies on kidney transplant recipients may suggest a protective role of this cell subtype in graft tolerance and the existence of a cross talk between B-and T-regulatory clones. In lung transplantation, the role of B-regulatory cells has never been investigated. In a murine tracheal transplantation model, this subset seems able to prevent tracheal obliteration when in combination with rapamycin. Aim of this study is to analyze peripheral CD19 + CD24^{high}CD38^{high} B-reg cells counts in a cohort of lung recipients, their association with several clinical and pharmacological variables and their possible association with T regulatory cell.

Methods: From Jan 2009 to Dec 2014, 117 lung Tx recipients were submitted to an immunological follow up I-FU(median: 108.7 months (6.7–310.5)). Immunological follow up consisted of a complete blood peripheral immuno-phenotype, inclusive of CD19 + CD24^{high}CD38^{high} B-cells (globally 1106 determinations). We tested the association between B-reg and relevant variables by linear or regression models for repeated measures, adjusting for time from Tx.

Results: Among all variables analyzed at multivariate analysis: chronic rejection (OR − 0.19, $p = .039$), use of Mycophenolate (OR − 0.38, $p < .001$) and the presence of a concomitant pulmonary infection of *S. aureus* (OR 0.66, $p = .002$) and *A. fumigatus* (OR 0.50, $p = .009$) were significantly associated to B-reg cell.

No significant correlation between CD19 + CD24^{high}CD38^{high} B-reg cells and T-reg cells counts was found in our cohort.

Conclusions: Our present data highlight, for the first time, that this cell subset might participate in long-term lung graft acceptance mechanisms.

1. Introduction

Lung transplant (Tx) is the only therapy for patients with end-stage lung diseases. Long-term survival of the graft is hampered by the occurrence of chronic rejection, that occurs in nearly 50% of patients by the 5th post-Tx year [1]. According to the most recent classification, there are two major clinical phenotypes of chronic rejection: bronchiolitis obliterans syndrome (BOS) an obstructive form that accounts for nearly 70% of cases, [2] and the restrictive allograft syndrome (RAS), a restrictive form responsible of 18–30% of cases, characterized by a worse outcome with respect to BOS [3,4].

Both phenotypes are a result of several different injuries to the graft, entailing chronic airway/interstitial inflammation [5] and ultimately leading to an exuberant and unrelenting fibro-reparative process [6]. Although, recent evidence ascribes an important pathogenic role to non-specific inflammatory mechanisms, the relevance of specific immunity has been clearly established [7,8]. Effector T cells have long been recognized as critical mediators of rejection and can be regulated and inhibited by several population of regulatory lymphocytes [9]. Among these, CD4 + CD25^{high} regulatory T cells (T-reg) are the best-studied mediators of tolerance [9]. Experimental evidence in animals have strongly suggested that lung graft acceptance is associated to an

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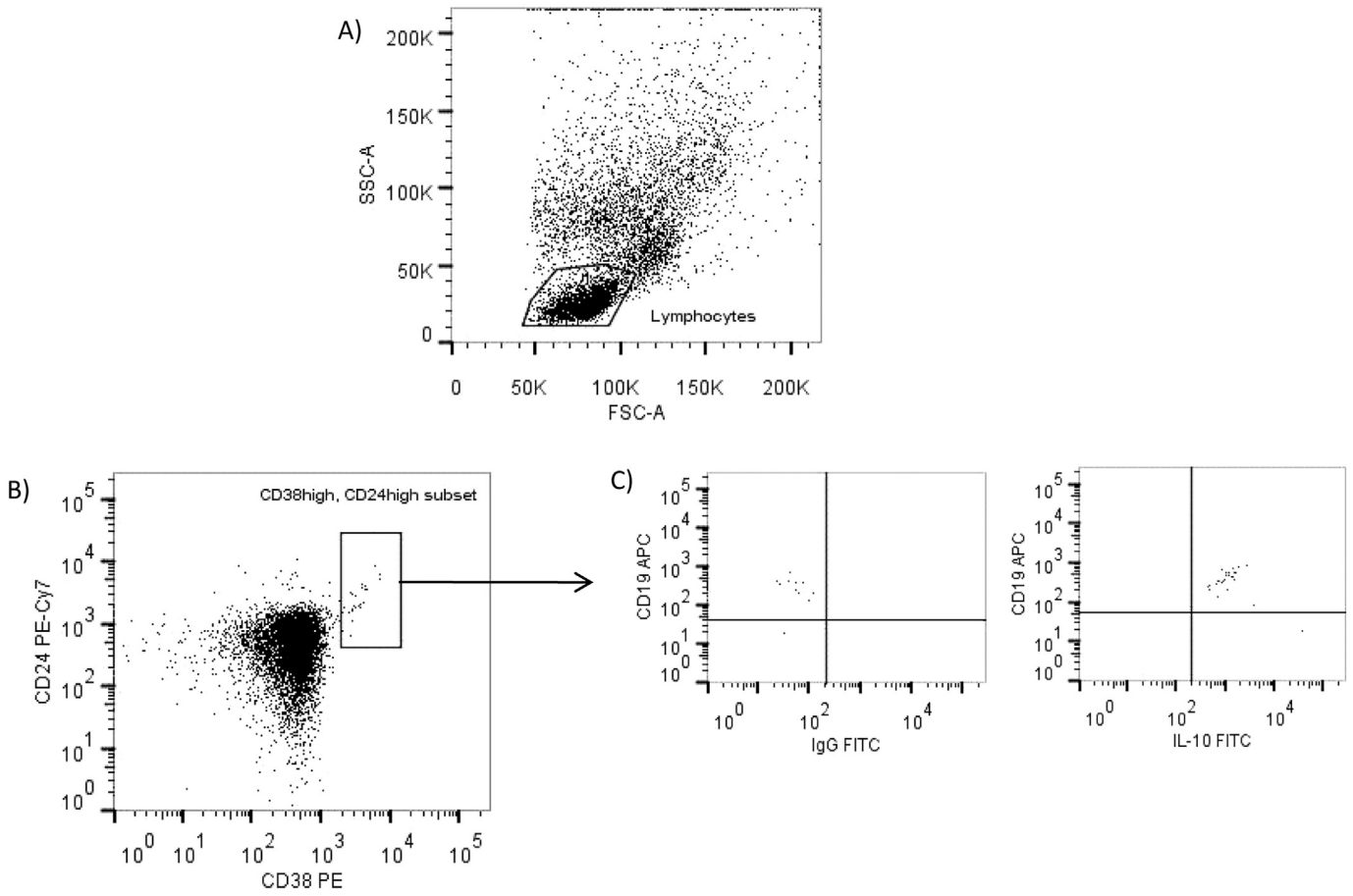

Fig. 1. Representative flow cytometry analysis of IL-10 producing Breg cells in isolated PBMCs. A) Lymphocytes were gated according to forward and site scatter; B) Double positive CD24^{high} and CD38^{high} were selected in the gate of lymphocytes; C) CD19 + CD24^{high}CD38^{high} IL-10 producing B-cells with isotype control.

intra-graft [10,11] and peripheral [12] CD4 + CD25^{high} T-reg cell expansion. In a previous work, we reported in a retrospective and longitudinal study that variation of peripheral CD4 + CD25^{high}CD127- T-reg cell counts can predict CLAD onset/progression [13] and other studies have confirmed our data [14].

Besides regulatory T cells, some recent reports highlighted the role of B-cell regulatory subsets as possible mediators of graft acceptance [15,16]. Within B-regulatory populations (B-reg): the subset of immature B-cell, phenotypically characterized as CD19 + CD24^{high}CD38^{high} has been described at peripheral level in healthy individuals [17,18]. This subset is characterized by the release of high amount of IL-10 (much higher than other B-reg cell subsets) and by the ability to suppress Th1 and Th17 differentiation [19] and convert CD4 + T cells into T-regs and Tr1 cells [18].

Evidences on the role of this B-reg cell subset in transplant acceptance are still scarce. A recent cellular analyses in kidney transplant recipients demonstrated a higher level of naive and transitional B-cells but not of memory B-cells (CD19 + IgD − CD38 + / − CD27 +) in the peripheral blood of tolerant patients without immunosuppressive treatment, compared with immunosuppressed patients with stable graft function, [20] thus suggesting a protective role of this cell subtype in graft tolerance.

This observation has been subsequently confirmed by Silva and coll. [21], while the role of induction treatment as well as calcineurin inhibitor –based regimen on the regulation of B-reg cell counts in kidney recipients has been hypothesized [22]. In addition, recent reports suggested the existence of a cross talk between B-and T-regulatory clones [23].

As for lung transplantation, the role of B-regulatory cells has not yet been investigated in vivo, the only evidence being a report on a murine tracheal transplantation model showing that rapamycin was able to prevent long-term tracheal obliteration through a massive tracheal infiltration of regulatory B-cells [24].

To our knowledge, the present study is the first that analyze longitudinally peripheral CD19 + CD24^{high}CD38^{high} B-reg cells counts in a cohort of lung recipients assessing their association with several clinical and pharmacological variables (in particular the occurrence of BOS and RAS, type of treatment, occurrence of infections, onset of kidney dysfunction or neoplasia) as well as their possible association with CD4 + CD25^{high} CD127^{dim} regulatory T cell rates.

2. Materials and methods

2.1. Patients

In our Center, LTx is performed since 1991. The study was conducted from 1st January 2009 to 31st December 2014. During this period, 137 lung recipients entered the immunological follow-up. All patients were submitted to the assessment of a complete peripheral immune phenotype at least twice a year. In 117 patients, we also performed the B-regulatory cell assessment.

Our immune suppression protocol has undergone some changes over time and has been described elsewhere [13]. No patients underwent lympho-depleting induction treatment at time of transplantation.

Our endoscopic surveillance protocol has been reported in previous papers [25]. BOS diagnosis and severity grades has been assessed according to published guidelines [6,27–29]. RAS has been retrospectively diagnosed for patients diagnosed before 2013, according to

radiological (CT scan showing a pattern of persistent interstitial/upper lobe fibrosis) and functional criteria (persistent decline in forced expiratory volume in 1 s (FEV 1) of > 20% compared to the best post Tx value and a decline in total lung capacity of > 10% compared to baseline) [27–29]. In case of a BOS 0p or early RAS diagnosis, patients were prescribed a 3-month course of chronic low-dose azithromycin. At the same time, patients underwent gastro-esophageal reflux assessment and maximization of anti-reflux medical treatment. In case of a further decline consistent with a BOS/RAS diagnosis, since 2003, patients are referred to the Apheresis Unit for compassionate ECP treatment [29]. Our cytomegalovirus surveillance protocol has been detailed in previous studies [30].

The ethics review committee of the IRCCS Policlinico San Matteo of Pavia approved the research n° ICS 30.4/RF00.65.

2.2. Phenotype analysis of B- and T-regulatory by flow cytometry

To characterize the phenotype of B lymphocyte subsets, 50 µl of heparinized venous blood was used for surface staining using antibodies against CD19 APC (Allophycocyanin; clone SJ25C1; BD Biosciences), CD24 PE-Cy7 (Phycoerythrin Cyanin 7; clone ML5; BD Biosciences) and CD38 PE (R Phycoerythrin; clone HIT2; BD Biosciences). After 15 min of incubation at room temperature in the dark, red blood cells were lysed with FACs Lysing Solution following the manufacture's protocol (BD Biosciences). Each sample was incubated with appropriate mouse immunoglobulin isotypes as a control. FACS analysis was performed by gating first on the lymphocyte and after on the CD19 B cell population. The gating strategy for determination of $CD19+CD24^{high}CD38^{high}$ Fig. 1. Acquisition and analysis were performed on a BD FACSCanto II (BD Biosciences).

To characterize the phenotype of T lymphocyte subsets, 50 µl of fresh whole blood was incubated with the appropriate amounts of fluorochrome-labeled monoclonal antibodies CD45 APC Alexa Fluor 750 (Allophycocyanin-Alexa Fluor 750; clone J33), CD4 APC (Allophycocyanin; clone 13B8.2), CD69ECD (R Phycoerythrin-Texas Red; clone TP1.55.3), CD25 PE (R Phycoerythrin; clone B1.49.9), CD127 PC5 (R Phycoerythrin-Cyanine 5.1; clone R34.34) for the T-reg and CD45 APC Alexa Fluor 750, CD4 APC, CD3 FITC (Fluorescein isothiocyanate; clone UCHT1), CD8 PE (R Phycoerythrin; clone B9.11), CD56 PC5 (R Phycoerythrin-Cyanine 5.1; clone N901), CD16 PC5 (R Phycoerythrin-Cyanine 5.1; clone 3G8), CD19 PC7 (Phycoerythrin Cyanin 7; clone J3–119) for the lymphocytic population (Beckman Coulter) at room temperature in the dark for 15 min using appropriate mouse immunoglobulin isotypes as a control. Following incubation, 1 ml erythrocyte lysing solution (VersaLyse, Beckman Coulter) was added to the samples and incubated under the same conditions for 20 min. In some samples, peripheral blood mononuclear cells (PBMC) were stained with CD4 APC, CD25 FITC (Fluorescein isothiocyanate; clone B1.49.9), and CD127 Alexa Fluor 647 (clone HIL-7R-M21) (BD Pharmingen), fixed and permeabilized, followed by intracellular staining with Foxp3 PE (R Phycoerythrin; clone PCH101) or control IgG1 (Human Regulatory T cell Staining kit, eBioscience) for 30 min. [13].

2.3. Intracellular stain for IL-10 detection

Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient-separation (Lymphoprep). For analysis of cytokine production, 1×10^6 cell/ml were stimulated with ODN 2006 Type B CPG (1 µg/ml; InvivoGen) and Recombinant Human CD40 Ligand/TNFSF5 (1 µg/ml; R&D Systems) for 72 h at 37 °C and 5% CO_2. For the last 5 h were added Brefeldin A (5 µg/ml), PMA (50 ng/ml) and ionomycin (1 µg/ml) (Sigma Aldrich). Cells were washed and stained for CD19 APC, CD24 PE-Cy7 and CD38 PE and incubated for 20 min at room temperature in the dark. After membrane staining, the cells were fixed and permeabilized using the Lyse/Fix (BD Biosciences) and stained with anti-human IL-10 FITC (Fluorescein isothiocyanate; clone B-N10; IQ Products) or FITC-conjugated IgG isotype control for 30 min at room temperature in the dark. Acquisition and analysis were performed on a BD FACSCanto II (BD Biosciences). We have confirmed on a separate experiment that $CD19^+CD24^{neg}CD38^{neg}$ B cells do not produce IL-10 (data not shown).

2.4. Statistical analysis

Descriptive statistics were produced for demographic, clinical and laboratory characteristics of patients. Mean and standard deviation (SD) are shown for normally distributed variables, and median and interquartile range (IQR) for non-normally distributed variables, numbers and percentages for categorical variables. Groups were compared with parametric or nonparametric tests, according to data distribution, for continuous variables, and with Pearson's $\chi 2$ test (Fisher's exact test where appropriate) for categorical variables. The association between B-reg and a number of predictors was explored by means of bivariate and multivariate linear mixed models, with patient and time since Tx as random effects, and predictors and time (also) as fixed effects. In all cases, test were two-tailed, and the *p*-value cut-off for significance was set at 0.05. Stata computer software version 14.0 (Stata Corporation, 4905 Lakeway Drive, College Station, Texas 77,845, USA) was used for statistical analysis.

3. Results

3.1. Patients

Overall, 117 patients were included in this retrospective study and followed-up for 108.7 ± 66.4 months (6.7–310.5 months from transplant). Demographics and clinical features of included patients are listed in Table 1, including gender, age at transplant, transplant indication, type of transplant, length of follow-up and type of immunosuppressive drugs used.

Being a retrospective immunological FU, the overall number of included samples is high: n° 1106 with a median of 11 samples/patient

Table 1
Demographic and clinical features of the patients enrolled in this study.

N° of Patients = 117	
Mean age at Tx (years ± SD)	45.8 ± 12.5
Sex (M:F)	74:43
Lenght of post-Tx follow-up (months/range, media)	108.7 ± 66.4 / 6.7–310.5
Tx Indications	
Emphysema / Alpha1 antitrypsin Deficiency	21
Primary graft disfunction	1
Bronchiectasis / Cystic Fibrosis	25
Interstitial lung disease	45
Pulmonary hypertension / Ebstein's disease / Eisenmenger Syndrome / Mounier-Kuhn Syndrome	23
Rare pulmonary conditions	3
Type of Tx	
Single lung Tx	38
Double lung Tx	71
Heart and lung Tx	8
Total number of determination = 1106	
Immunosuppression therapy	
Cyclosporine	134
Tacrolimus	948
Azathioprine	21
Mycophenolate mofetil	551
Rapamycin	230
Prednisone	1106
Extracorporeal photopheresis	697
Azithromycin	317

Table 2
bi- and multi-variate linear regression analysis for CD19 + CD24^{high}CD38^{high} Breg linear mixed models were fitted, with patient and time since Tx as random effects, and individual predictors and time as fixed effects. RAS-Restrictive allograft syndrome, BOS-Bronchiolitis obliterans syndrome.

		Bivariate			Multivariate		
Variable	Category	Coef	95% CI	P-value	Coef	95% CI	P-value
Chronic rejection (BOS + RAS)	Ever	-0,16	−0.35 to 0.01	0.073	−0.19	−0.37 to −0.01	**0.039**
	RAS	−0,48	−0.98 to 0.02	0,06			
	BOS	−0,05	−0.39 to 0.30	0,797			
BOS grade	0p	−0,17	−0.37 to 0.04	0,109			
	1	−0,11	−0.34 to 0.13	0,374			
	2	−0,16	−0.47 to 0.16	0,332			
	3	−0,30	−0.66 to 0.07	0,113			
Lymphocytic population	cd3	0	0 to 0	0			
	cd 3 cd4	0,001	0 to 0.01	0			
	cd 3 cd8	0	0 to 0	0,001			
	cd19	0,004	0.003 to 0.005	0			
	cd1 6 cd56	0	0 to 0	0,002			
	cd4cd25high	0,002	0 to 0.005	0,071			
Immunosuppresive therapy	Cyclosporine	−0,18	−0.61 to 0.26	0,420			
	Tacrolimus	0,04	−0.26 to 0.33	0,812			
	Azathioprine	0,59	0.01 to 1.20	**0,05**	0.50	−0.09 to 1.10	0.099
	Mycophenolate	−0,39	−0.55 to −0.22	**< 0.001**	−0.38	−0.54 to −0.21	**< 0.001**
	Rapamycin	−0,03	−0.22 to 0.28	0,814			
	Prednisone	−0,38	−0.97 to 0.22	0,215			
Azithromycin therapy		−0,09	−0.27 to 0.09	0,326			
Extracorporeal photopheresis		0,07	−0.16 to 0.30	0,564			
Kidney failure		−0,05	−0.27 to 0.17	0,652			
Infections							
	Bacterial	0,13	−0.07 to 0.33	0,209			
	Staph aureus	0,64	0.21 to 1.08	**0,003**	0.66	0.24 to 1.09	**0.002**
	Viral	−0,07	−0.22 to 0.08	0,363			
	Fungal	0,13	−0.16 to 0.42	0,378			
	Aspergillus spp	0,57	0.19 to 0.95	**0,003**	0.50	0.13 to 0.87	**0.009**

Bold statistically significant correlation

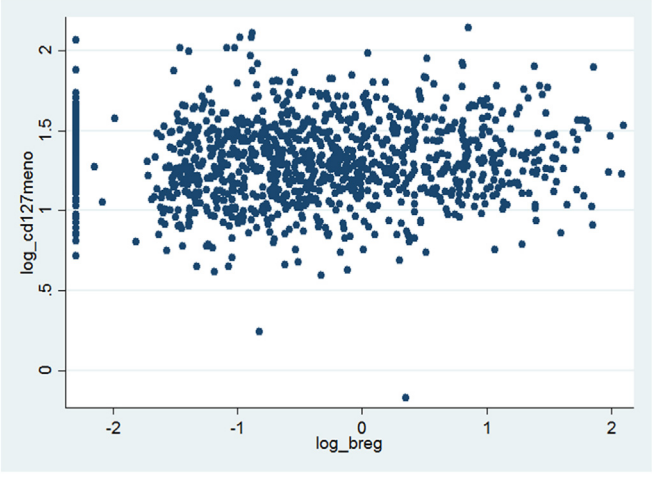

Fig. 2. Scatterplot of T-reg cells counts and B-reg cells counts showing no correlation between cells population.

(IQR 8–15).

3.2. Functional characterization of CD19 + CD24^{high}CD38^{high} B cells

On five blood samples of representative lung transplant recipients, functional characterization of B-reg cell subset has been performed showing that 96,4% of CD19 + CD24^{high}CD38^{high} B cell subset are IL-10 producing cells. (Fig. 1).

3.3. Variables associated to B-reg cell counts

Results of bivariate and multivariate analysis are shown in Table 2.

All tested immunological variables (CD3 +,CD4 +, CD8 +, CD19 +, CD16 + CD56 + and CD25^{high} cells) resulted positively associated to B-reg (Table 2) at the initial bivariate analysis. As for clinical variables, only four were significantly associated to CD19 + CD24^{high}CD38^{high} B-reg cells count: treatment with azathioprine (p = .041) and mycophenolate mofetil (p < .001), pulmonary infections such as *Staph. Aureus* (p = .005) and *A. fumigatus* (p = .003). Of note, we did not find any significant association with a specific phenotype of chronic rejection (BOS or RAS) or with BOS severity, as well as no association with acute cellular rejection was detected, although a low number of episodes was registered in this cohort. At multivariate analysis, all but one (azathioprine treatment) associations found in the univariate analysis were confirmed. Of note, the presence of CLAD (either BOS or RAS) was added to the multivariate analyses, even if the variable was not significant at the univariate analysis (Table 2).

3.4. B-reg and T-reg correlation

Since literature data suggest that a possible interaction between B and T-regulatory cells "in vivo" might bring to an expansion of T-regulatory clones, we aimed to assess whether a correlation between CD19 + CD24^{high}CD38^{high} B-reg and CD4 + CD25^{high}CD127- T-reg peripheral cell counts was detectable in lung transplant recipients. As shown in Fig. 2, we could not detect any statistically significant correlation between CD19 + CD24^{high}CD38^{high} B-reg cells and CD4 + CD25^{high}CD127- T-reg cells counts.

4. Discussion

B-regs have been reported within the CD19 + CD24^{high}CD38^{high} immature B-cell population in peripheral blood of healthy individuals [17-30]. Multiple studies have demonstrated that B-regulatory cells are able to suppress inflammatory response via the production of IL-10, an

anti-inflammatory cytokine that has been thus used as a marker of B-regulatory cells [18-31]. In present study we clearly demonstrated that CD19 + CD24^{high}CD38^{high} B cell subset, in lung recipients, is highly enriched with IL-10 producing B cells thus suggesting that this mechanism is relevant in driving their regulatory functions. By the paracrine IL-10 secretion, B-regs have been reported to inhibit Th1 responses via IL-10 and Th17 differentiation, in addition CD19 + CD24^{high}CD38^{high} B-regs could also convert "in vitro" CD4 + T effectors into T-regs and Tr1 cells [18-31]. Recent studies from chronic graft-versus-host disease (cGVHD) have also highlighted the role of CD19 + CD24^{high}CD38^{high} B-regs in establishing transplant tolerance by suppressing effector T-cell responses [32,33].

There is scanty experimental evidence that that CD19 + CD24^{high}CD38^{high} B-reg cells might be implicated in transplanted graft acceptance, mainly gained in the setting of kidney transplantation. A recent report on a small cohort of kidney recipient, showed that immunosuppressive regimen based on a combination of mTOR- and calcineurin inhibitors reduces CD19 + CD24^{high}CD38^{high} B-reg cell counts [22].

As for the lung transplantation, in a murine animal model of heterotopic tracheal transplantation, commonly accepted as surrogate BOS model, the use of mTOR inhibitor has been reported to increase acceptance and halt tracheal fibro-obliteration. This effect has been ascribed to a marked tracheal infiltration by CD19 + CD24^{high}CD38^{high}/IL-10-producing regulatory B-lymphocytes, thought to expand, via IL-10, T-reg clones [24]. Thus, the real role of CD19 + CD24^{high}CD38^{high} B-reg cell subset "in vivo" is still undefined. This regulatory aspect remains poorly characterized in human clinical transplantation. Thus for the first time, we aimed to analyze counts of CD19 + CD24^{high}CD38^{high} B-reg cells in the peripheral blood of lung transplant recipients and to find possible associations with a number of clinical and therapeutic variables.

On the basis of present study, we can infer a role for CD19 + CD24^{high}CD38^{high} B-reg cell subset in the long term acceptance of lung graft since we specifically found a slight but significant down-regulation in presence of chronic rejection irrespective of its phenotype. Of note, we did not find any association with grade > 2 acute cellular rejection or antibody mediated rejection episodes maybe due to the low number of samples obtained during these two specific immunological complications.

Furthermore, an interesting observation was that CD19 + CD24^{high}CD38^{high} B-reg cell counts are significantly high during specific bacterial and fungal infections (*S. aureus* and *A. fumigatus*). Even if the small number of samples obtained during *S. aureus* and *A. fumigatus* infectious episodes might have limited the strength of the statistical analysis. Recent experimental data highlighted that pro-inflammatory stimula induced by a specific gut microbiota were able to expand B-reg cell number and function and to restrain inflammatory reaction associated to antigen-induced arthritis in mice [34]. We could hypothesize that specific infective/inflammatory stimula might participate to B-reg cell expansion "in vivo", however this point must be addressed with specifically designed studies.

We also tested the association of B-reg cell counts with a number of clinical variables, including also kidney failure and different immunosuppressive strategies, such as ECP or azithromycin. Interestingly, only azathioprine and mycophenolate mofetil were found to be significantly associated to peripheral CD19 + CD24^{high}CD38^{high} B-reg cell counts at the univariate analysis; while, at the multivariate model, only the MMF effect was significant. Unlike previous evidence in literature, [22] we could not detect any significant variation of B-reg cell counts with respect to other specific immunosuppressive drugs such as calcineurin or m-TOR inhibitors, however, as for the latter, a limited number of patients could have been included in the study. Since induction treatment is not performed at our center, we could not assess the possible role of lympho-depleting strategies.

Finally, since an interaction between B and T-reg cells via IL-10 has been reported elsewhere, [24] we decided to verify this hypothesis in our cohort. As shown in the results section, we could not confirm this observation in humans and found no association between the CD19 + CD24^{high}CD38^{high} B-reg and the CD4 + CD25^{high}CD127- T-reg cell subset. However, we cannot exclude that other T-reg subset such as Tr1 (that were not addressed in our study) might have been regulated by CD19 + CD24^{high}CD38^{high} B-reg cell in our patients.

We must acknowledge a few limitations. First, we focused on the best characterized B-reg subset (CD19 + CD24^{high}CD38^{high} B-cells) but according to more recent papers many other subsets may coexist and exert different mechanism of immune-regulation/suppression [18]. Therefore, it will be crucial in the next future to address which subset of B-reg is more relevant in driving solid organ transplant acceptance. Second, patients started our immunological FU at different times from Tx; we cannot be certain that bias would not impact on results even if this particular aspect was taken into account in the analysis by multivariate linear mixed models, with patient and time since Tx as random effects, and predictors and time (also) as fixed effects. Finally, given the retrospective nature of this paper, the different samples were collected at different time point in different patients and sometimes even on clinical need (infection or suspect of chronic rejection), so there might be other variables affecting the results.

5. Conclusion

This report on peripheral CD19 + CD24^{high}CD38^{high} B-reg cell kinetics in lung recipients highlights, for the first time, that this cell subset might participate in long-term lung graft acceptance mechanisms and that the counts of these B-reg cells are significantly influenced not only by type of immunosuppressive regimen but also by specific infective complications. These data deserve further confirmation with specifically designed larger prospective studies.

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References

[1] R.D. Yusen, L.B. Edwards, A.Y. Kucheryavaya, et al., International Society for Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-first adult lung and heart-lung transplant report–2014; focus theme: retransplantation, J. Heart Lung Transplant. 33 (10) (2014 Oct) 1009–1024.
[2] M. Sato, K. Ohmori-Matsuda, T. Saito, et al., Time-dependent changes in the risk of death in pure bronchiolitis obliterans syndrome (BOS), J. Heart Lung Transplant. 32 (5) (2013 May) 484–491.
[3] M. Sato, D.M. Hwang, T.K. Waddell, L.G. Singer, S. Keshavjee, Progression pattern of restrictive allograft syndrome after lung transplantation, J. Heart Lung Transplant. 32 (1) (2013 Jan) 23–30.
[4] E. Ofek, M. Sato, T. Saito, et al., Restrictive allograft syndrome post lung transplantation is characterized by pleuroparenchymal fibroelastosis, Mod. Pathol. 26 (3) (2013 Mar) 350–356.
[5] S.S. Weigt, A. DerHovanessian, W.D. Wallace, J.P. Lynch 3rd, J.A. Belperio, Bronchiolitis obliterans syndrome: the Achilles' heel of lung transplantation, Semin. Respir. Crit. Care. Med. 34 (3) (2013 Jun) 336–351.
[6] S.E. Verleden, D.M. Vasilescu, J.E. McDonough, et al., Linking clinical phenotypes of chronic lung allograft dysfunction to changes in lung structure, Eur. Respir. J. 46 (5) (2015 Nov) 1430–1439, https://doi.org/10.1183/09031936.00010615.
[7] B.M. Vanaudenaerde, S.I. De Vleeschauwer, R. Vos, et al., The role of the IL23/IL17 axis in bronchiolitis obliterans syndrome after lung transplantation, Am. J. Transplant. 8 (9) (2008 Sep) 1911–1920.
[8] A. Evers, S. Atanasova, G. Fuchs-Moll, et al., Adaptive and innate immune responses in a rat orthotopic lung transplant model of chronic lung allograft dysfunction, Transpl. Int. 28 (1) (2015 Jan) 95–107.
[9] F. Issa, R.J. Robb, K.J. Wood, The where and when of T cell regulation in transplantation, Trends Immunol. 34 (3) (2013 Mar) 107–113.
[10] W. Li, A.C. Bribriesco, R.G. Nava, et al., Lung transplant acceptance is facilitated by early events in the graft and is associated with lymphoid neogenesis, Mucosal Immunol. 5 (5) (2012 Sep) 544–554.
[11] W. Sommer, A.K. Knöfel, N. Madrahimov, et al., Allogeneic CD4 + CD25high T cells regulate obliterative bronchiolitis of heterotopic bronchus allografts in both

porcinized and humanized mouse models, Transplantation. 99 (3) (2015 Mar) 482–491, https://doi.org/10.1097/TP.0000000000000063.
[12] Q. Shi, Y. Niu, H. Cao, et al., CD28 superagonist antibody treatment attenuated obliterative bronchiolitis in rat Allo-orthotopic tracheal transplantation by preferentially expanding Foxp3-expressing regulatory T cells, Transplant. Proc. 44 (4) (2012 May) 1060–1066.
[13] D. Piloni, M. Morosini, S. Magni, et al., Analysis of long term CD4+CD25highCD127- T-reg cells kinetics in peripheral blood of lung transplant recipients, BMC Pulm Med. 17 (1) (2017 Jul 18) 102.
[14] J. Salman, F. Ius, A.K. Knoefel, et al., Association of Higher CD4+ CD25high CD127low, FoxP3+, and IL-2+ T cell frequencies early after lung transplantation with less chronic lung allograft dysfunction at two years, Am. J. Transplant. 17 (6) (2017 Jun) 1637–1648.
[15] J.M. Berthelot, C. Jamin, K. Amrouche, et al., Regulatory B cells play a key role in immune system balance, Joint Bone Spine 80 (2013) 18–22.
[16] E.I. Dijke, J.L. Platt, P. Blair, et al., B cells in transplantation, J. Heart Lung Transplant. 35 (2016) 704–710.
[17] P.A. Blair, L.Y. Noreña, F. Flores-Borja, et al., CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients, Immunity 32 (2010) 129.
[18] C. Mauri, M. Menon, The expanding family of regulatory B cells, Int. Immunol. 27 (10) (2015 Oct) 479–486.
[19] F. Flores-Borja, A. Bosma, D. Ng, et al., CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation, Sci. Transl. Med. 5 (2013) 173ra23.
[20] A.S. Chong, R. Sciammas, Match making the B-cell signature of tolerance to regulatory Bcells, Am. J. Transplant. 11 (2011) 2555–2560.
[21] H.M. Silva, M.C. Takenaka, P.M. Moraes-Vieira, et al., Preserving the B-cell compartment favors operational tolerance in human renal transplantation, Mol. Med. 18 (2012) 733–743.
[22] I. Latorre, A. Esteve-Sole, D. Redondo, et al., Calcineurin and mTOR inhibitors have opposing effects on regulatory T cells while reducing regulatory B cell populations in kidney transplant recipients, Transpl. Immunol. 35 (2016 Mar) 1–6.
[23] F.E. Lund, T.D. Randall, Effector and regulatory B cells: modulators of CD4(+) T cell immunity, Nat. Rev. Immunol. 10 (2010) 236–247.
[24] Y. Zhao, J.R. Gillen, A.K. Meher, et al., Rapamycin prevents bronchiolitis obliterans through increasing infiltration of regulatory B cells in a murine tracheal transplantation model, J. Thorac. Cardiovasc. Surg. 151 (2016) 487–496.
[25] F. Meloni, N. Solari, S. Miserere, et al., Chemokine redundancy in BOS pathogenesis. A possible role also for the CC chemokines: MIP3-beta, MIP3-alpha, MDC and their specific receptors, Transpl. Immunol. 18 (2008) 275–280.
[26] M. Estenne, M.I. Hertz, Bronchiolitis obliterans after human lung transplantation, Am. J. Respir. Crit. Care Med. 166 (2002) 440–444.
[27] M. Sato, T.K. Waddell, U. Wagnetz, et al., Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction, J. Heart Lung Transplant. 30 (2011) 735–742.
[28] G.M. Verleden, R. Vos, B. Vanaudenaerde, et al., Current views on chronic rejection after lung transplantation, Transpl. Int. (10) (2015 Apr).
[29] C. Del Fante, L. Scudeller, T. Oggionni, et al., Long-term off-line extracorporeal Photochemotherapy in patients with chronic lung allograft rejection not responsive to conventional treatment: a 10-year single-Centre analysis, Respiration. 90 (2) (2015) 118–128.
[30] D. Lilleri, G. Gerna, F. Bruno, et al., Systemic and local human cytomegalovirus-specific T-cell response in lung transplant recipients, New Microbiol. 36 (2013) 267–277.
[31] C. Mauri, A. Bosma, Immune regulatory function of B cells, Annu. Rev. Immunol. 30 (2012) 221.
[32] A. Nouël, Q. Simon, C. Jamin, J.O. Pers, S. Hillion, Regulatory B cells: an exciting target for future therapeutics in transplantation, Front. Immunol. 5 (2014) 11.
[33] A. Khoder, A. Sarvaria, A. Alsuliman, et al., Regulatory B cells are enriched within the IgM memory and transitional subsets in healthy donors but are deficient in chronic GVHD, Blood 124 (2014) 2034.
[34] E.C. Rosser, K. Oleinika, S. Tonon, R. Doyle, A. Bosma, N.A. Carter, K.A. Harris, S.A. Jones, N. Klein, C. Mauri, Regulatory B cells are induced by gut microbiota-driven interleukin-1β and interleukin-6 production, Nat. Med. 20 (11) (2014 Nov) 1334–1339, https://doi.org/10.1038/nm.3680 Epub 2014 Oct 19. PubMed PMID: (5326801).