TOLERANCE AND REJECTION ASSOCIATED TRANSCRIPTS IN LOW-RISK KIDNEY TRANSPLANT RECIPIENTS: PROSPECTIVE STUDY

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Introduction and aim

Numerous B and T cells associated gene transcripts were identified in operationally tolerant and in transplant recipients suffering from rejection but little is known about their long-term expression in low risk kidney transplant recipients under immunosuppression.

The aim of this study was to compare the molecular profile of 28 selected B and T cell tolerance/rejection related transcripts in peripheral blood and grafts of kidney transplant recipients with/without rejection episode in 1 year follow-up.

Methods

In this prospective single center study, 48 consecutive low-risk (PRA<20%), first kidney recipients treated with TAC/MMF based immunosuppression were enrolled. The expressions of 28 genes, previously found to be associated with tolerance or rejection1-5, and lymphocytes subsets, were analyzed in peripheral blood using RT-qPCR and FACS at POD 0, 7, 14, 90 and 365. Intragraft expression of these genes was analyzed during rejection.

Results

Table 1. Patient´s demographics and clinical characteristics.

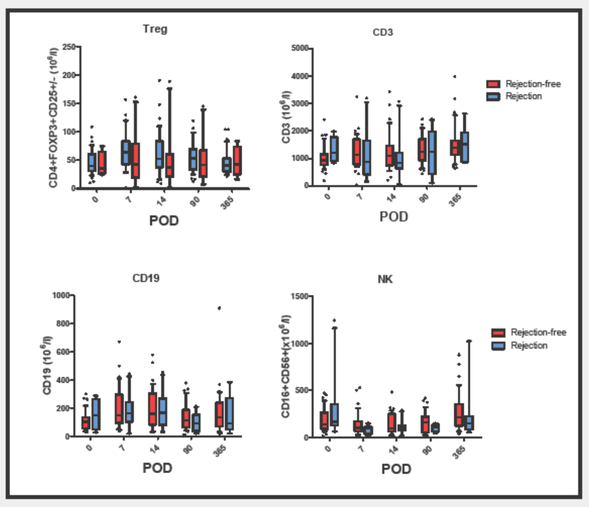
	Rejection-free (n=38)	Acute TCMR (n=10)	p
Donor´s characteristic			
*Age, years	46 [16; 67]	50.5 [18; 63]	0.819
Gender (male/female), n	28/10	6/4	0.397
Expanded criteria donors (0/1/2)	20/9/9	6/2/2	0.917
Recipient´s characteristics			
*Age, years	55.8 [36.6; 76.5]	53.7 [27.7; 64.2]	0.269
Gender (male/female)	29/9	8/2	0.805
*PreTx BMI	27.7 [20.6; 34.3]	28.7 [19.8; 33.9]	0.501
*HLA mismatch	2.0 [0; 5])	3.5 [1; 5]	0.228
*PRA, %	2 [0; 16])	0 [0; 8]	0.892
*Dialysis time, days	698 [156; 1812]	1189 [209; 2747]	0.648
*Cold ischemia, hod	16.9 [9.5; 25.6]	17.2 [12.9; 23.3]	0.909
Hemodialysis/Peritoneal dialysis, n	35/4	8/1	0.940
No induction/Basiliximab induction	9/29	5/5	0.103
Maintenance immunosuppression			
MMF/TAC or CyA	13	1	
MMF/TAC or CyA/steroids for 10 days	7	2	
MMF/TAC or CyA/steroids	18	7	0.411
***Serum creatinine (µmol/l)**			
At 7 POD	234.1 [82; 798.7]	427.2 [110.6; 744.5]	0.381
At 14 POD	170.6 [64.1; 838.6]	244.7 [105.4; 838.6]	0.304
At 90 POD	124 [77.7; 289.2]	150.8 [92.4; 313.3]	0.096
At 365 POD	118.9 [72.3; 311.5]	169.9 [79.4; 338.6]	0.033
GFR (ml/s), mean (SD)			
At 3 months	0.78 [0.34; 1.47]	0.76 [0.40; 1.21]	0.825
At 1year	0.87 [0.21; 1.6]	0.69 [0.34; 1.1]	0.102

*Data are presented as medians [min; max]

● Flow cytometry analysis

Longitudinal analysis of the lymphocytes subsets in the peripheral blood (Tregs as CD4+CD25+FoxP3+ and CD4+CD25+CD127−, NK cells as CD16+CD56+, T lymphocytes as CD3+ and B lymphocytes as CD45+CD19+CD3-) has not shown a statistical significant difference in their counts (Fig.1).

Figure 1. Lymphocytes subsets in kidney transplant recipients with/without rejection during 1 year follow-up.



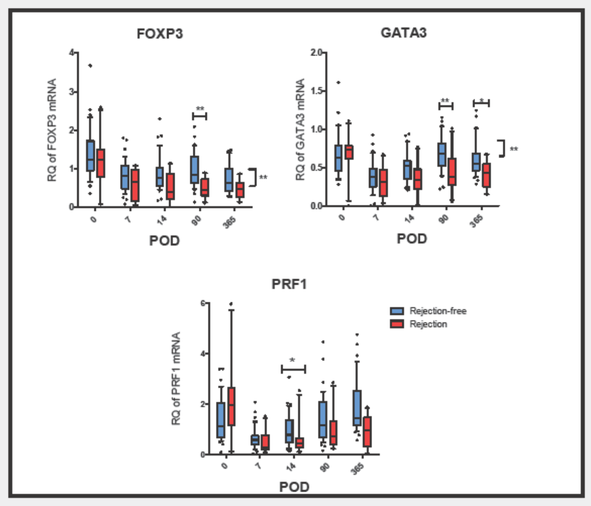
The lymphocytes subsets (Tregs as CD4+CD25+FoxP3+ and CD4+CD25+CD127−, NK cells as CD16+CD56+, T lymphocytes as CD3+ and B lymphocytes as CD45+CD19+CD3-) in rejection-free kidney recipients (n=38) and patients with rejections (n=10) during 1 year follow-up. Data are given as medians with 10-90 percentile.

● RT-qPCR analysis

○ Longitudinal analysis of blood samples

- ✔ Rejection free patients (n=38) compared to patients with acute T-cell mediated rejection TCMR (n=10) experienced significantly higher expression of T cell transcripts FOXP3 and GATA3 during the whole follow-up (GLM repeated measures test) and PRF1 at 14 POD (Kruskal-Wallis test) (Fig. 2).

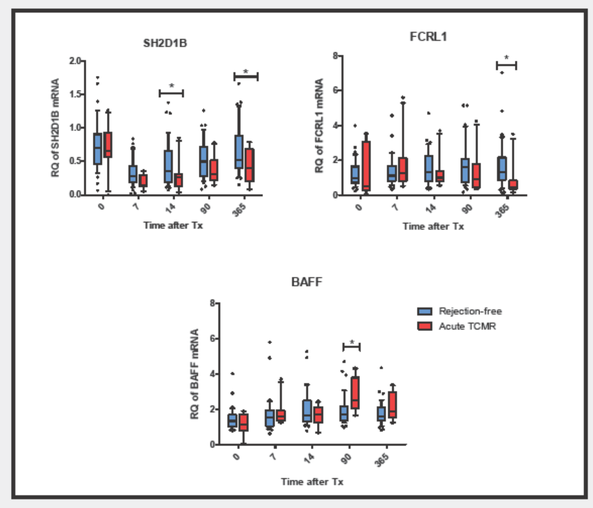
Figure 2. The expression of T-cell related genes in recipients with/without rejection during 1 year follow-up.



*The expression of T cell-related genes FOXP3 (Treg transcription factor), GATA3 (Th2 transcription factor) and PRF1 (perforin) in peripheral blood of rejection-free kidney recipients (n=38) and patients with rejections (n=10) during 1 year follow-up. Data are given as medians with 10-90 percentile. Kruskal-Walis test. GLM repeated measured test. *p<0.05, **p<0.01.*

- ✔ Patients with rejections had significantly lower expression of B-cell transcripts SH2D1B at 14 and 365 POD and FCRL1 at 365 POD and lower expression of BAFF at 90 POD in the peripheral blood (Fig.3).

Figure 3. The expression of B-cell related genes in recipients with/without rejection during 1 year follow-up.

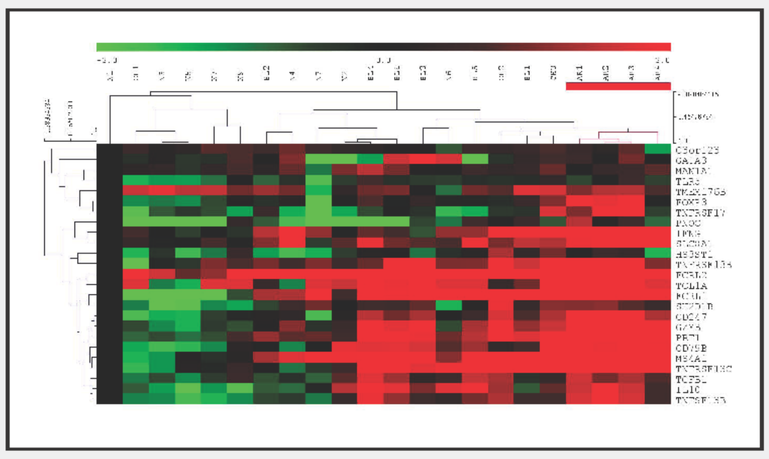


*The expression of B cell-related genes SH2D1B (SH2-domain adapter), BAFF (B-cell activating factor), FCRL1 (Fc receptor-like playing role in B cell activation) in peripheral blood of rejection-free kidney recipients (n=38) and patients with rejections (n=10) during 1 year follow-up. Data are given as medians with 10-90 percentile. Kruskal-Wallis test. *p<0.05, **p<0.01.*

○ Intragraft gene expression

In kidney graft tissue, the expressions of T-cell (GZMB, PRF1, CD247, FOXP3, IFNγ) and B-cell transcripts related to rejection and tolerance (FCRL1, FCRL2, BAFF, MS4A1, SLC8A1, CD79B, BAFF-R, TACI, TCL1A, BCMA, SH2D1B) were significantly higher (p<0.05) during acute TCMR (n=4) compared to biopsies with chronic TCMR (n=3), borderline changes (n=6) or normal morphology (n=9) (Fig. 4).

Figure 4. Hierarchical clustering of 28 T- and B- cell related genes in 22 kidney graft biopsies (N, normal histopathological finding; BL, borderline changes; CH, chronic rejection; AR, acute T cell mediated rejection).



Conclusion

Higher peripheral gene expression of both tolerance and rejection markers during the follow-up in rejection free patients while higher intragraft transcripts during acute rejection suggest the effect of immunosuppression load on analyzed variables.

References

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