STEROID FREE IMMUNOSUPPRESSION IS ASSOCIATED WITH ENHANCED TH1 TRANSCRIPTS

Petra Hrubá[1], Irena Brabcová[1], Eva Krepsová[1], Alena Sekerková[3], Janka Slatinská[2], Ilja Stříž[3] and Ondřej Viklický[1,2]

[1]Transplant Laboratory, Center for Experimental Medicine; [2]Department of Nephrology and Transplant Center;

[3]Department of Clinical and Transplant Immunology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Introduction and aim

Steroid withdrawal from immunosuppressive regimen offer several metabolic advantages, however, it might be associated with higher rejection incidence. The aim of this prospective study was to evaluate transcripts associated with immune response in steroid- free and steroids-based regimens in kidney transplantation.

Patients and Methods

In this prospective study 33 low-risk (PRA<20%), first deceased kidney transplant recipients received basiliximab induction and maintenance immunosuppression consisting of TAC/MMF. In steroid free-regimen group only perioperative bolus was used (n=14), and in the second group after initial bolus tapered steroids were discontinued at 10 day (n=9). Control group received initial steroid bolus and low dose steroid therapy for the whole follow-up (n=10).

The expressions of 28 genes, associated with alloimmune response/tolerance[1-5], were measured in the peripheral blood by RT-qPCR at POD 0, 7, 14, 90 and 365 and lymphocyte subpopulations were monitored by flow-cytometry.

Results

Subject characteristic

Main pre-transplant donor ´s and recipient ´s characteristics did not differ significantly among groups, with the exception of cold ischemia time (Table 1). Kidney graft function was not significantly influenced by steroid avoidance regimens (Table 2) in our small cohort of patients.

Table 1. Patient ´s demographics

	No steroids (n=14)	Steroids 10 d only (n=9)	Controls (n=10)	p
Donor´s characteristic				
*Age, years	44 [20; 65]	46 [18; 63]	56 [28; 67]	0.231
Gender (male/female), n	10/4	6/3	8/2	0.800
Expanded criteria donors (0/1/2)	8/3/3	6/2/1	2/4/4	0.280
Recipient´s characteristics				
*Age, years	46.7 [36.6; 64.2]	53.2 [40.9; 68.2]	61.9 [42.4; 69.1]	0.057
Gender (male/female)	12/2	7/2	7/3	0.869
*PreTx BMI	27.5 [23.8; 36.7]	28.8 [21.1; 33.9]	27.9[20.7; 33.9]	0.926
*HLA mismatch	2.5 [1; 5])	2 [0; 5]	3.5 [2; 5]	0.142
*PRA, %	2 [0; 16])	0 [0; 8]	6 [0; 26]	0.098
*Dialysis time, days	795.5 [156; 2746]	760.5 [209; 1225]	572 [161; 1257]	0.579
*Cold ischemia, hod	17.1 [14.7; 22.9]	16.1 [13.5; 24]	19.0 [15.9; 25.6]	0.043
Hemodialysis/Peritoneal dialysis, n	13/1	8/1	8/2	0.632

*Data are presented as medians [min; max]

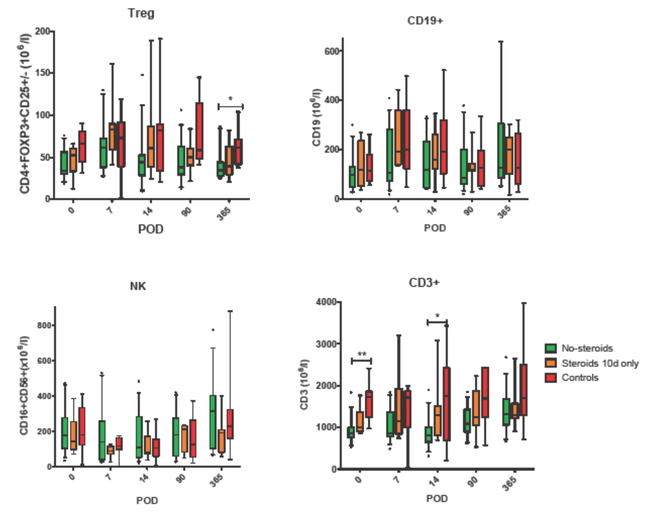
Table 2. Clinical outcome of patients in 1 year follow-up.

	No steroids (n=14)	Steroids 10 d only (n=9)	Controls (n=10)	p
Serum creatinine (µmol/l), median [min; max]				
At 3 months	143.7 [86.6; 289.2]	122.7 [79.4; 235.7]	136.1 [90.6; 168.2]	0.988
At 1year	142.3 [89.3; 231.6]	122.9 [84.9; 181.6]	129.9[99.7; 266.1]	0.656
GFR (ml/s), mean (SD)				
At 3 months	0.80 (0.28)	0.83 (0.27)	0.79 (018)	0.912
At 1year	0.75 (0.30)	0.93 (0.37)	0.77 (0.17)	0.420
Bioptically verified rejections or infections				
Borderline changes, %	28.5% (4)	44.4% (4)	30% (3)	0.707
Acute TCMR, % (n)	7.1% (1)	11.1% (1)	20% (2)	0.632
Chronic TCMR, % (n)	0	11.1% (1)	0	0.253
Graft kidney failure	0	0	0	
Pyelonephritis, % (n)	7.1% (1)	0	0	0.497
BKV, % (n)	0% (0)	11.1% (1)	30% (3)	0.085

Flow cytometry analysis

Longitudinal analysis of the lymphocytes subsets has shown **a trend toward lower number of both Tregs and CD19+B cells in steroid avoidance group**. However, with the exception of Tregs number at 365 POD, no difference was statistically significant (Fig.1).

Figure 1 The lymphocytes subsets in steroids-free and steroids-based regimens.



*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 kidney recipients receiving standard immunosuppression with steroids (controls) or in patients with no-steroids or with steroids withdrawal 10 days after transplantation (Steroids 10d only). Data are given as medians with 10-90 percentile. Kruskal-Wallis test. *p<0.05*

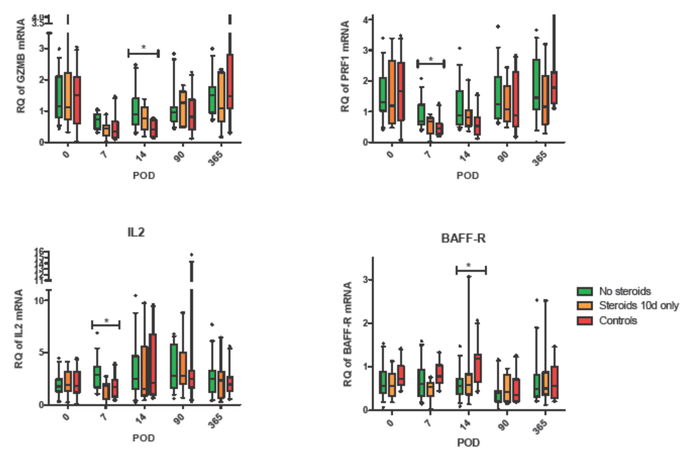
RT-qPCR analysis

Steroid free immunosuppression is associated with higher inflammatory response

Both steroid free regimens were associated with higher cytotoxic T lymphocytes and NK cells derived GZMB (granzyme B) expression at POD14 and PRF1 (perforin) at POD7. The higher proinflammatory cytokine IL-2 expression at POD7 was detected only in no-steroids group (Fig.2).

Steroids increased the expression of BAFF-R (receptor of B-cell activating factor) at POD7 and POD14.

Figure 2 The longitudinal expression of selected genes in steroids-free and steroids-based regimens.



*The expression of T cell cytotoxic effector molecules GZMB and PRF1, pro-inflammatory cytokine IL-2 and receptor of B-cell activating factor (BAFF-R) in peripheral blood of kidney transplant recipients in no-steroids group, in steroids 10d only and in controls, receiving standard immunosuppression with steroids. Data are given as medians with 10-90 percentile. Kruskal-Wallis test. *p<0.05*

Conclusion

Steroid free immunosuppression early after kidney transplantation is associated with enhanced expression of Th1 associated transcripts in peripheral blood that suggest higher susceptibility for early acute rejection in those patients. This observation needs further clinical validation.

References

1. *Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B et al. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 2001;344(13):947-954.*
2. *Sawitzki B, Gerlach U, Yamaguchi H, Vogt K, Haase S, Volk D et al. Pre-Transplant Analysis of Whole Blood Toag-1 Gene Expression and CD4+CD45RO-CD62L-TEMRA Frequencies Identifies High Risk Patients. Am J Transplant 2010;10:1-2.*
3. *Muthukumar T, Dadhania D, Ding RC, Snopkowski C, Naqvi R, Lee JB et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. New Engl J Med 2005;353(22):2342-2351.*
4. *Sagoo P, Perucha E, Sawitzki B, Tomiuk S, Stephens DA, Miqueu P et al. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. Journal of Clinical Investigation 2010;120(6):1848-1861.*
5. *Newell KA, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M et al. Identification of a B cell signature associated with renal transplant tolerance in humans. J Clin Invest 2010;120(6):1836-1847.*

Supported by GACR No. P301/11/1568 and MZO 00023001.