CIRCULATING TREGS AND TH17 CELLS PERCANTEGES IN CLASS IV DIFFER FROM OTHER CLASSES OF LUPUS NEPHRITIS

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INTRODUCTION AND AIMS:

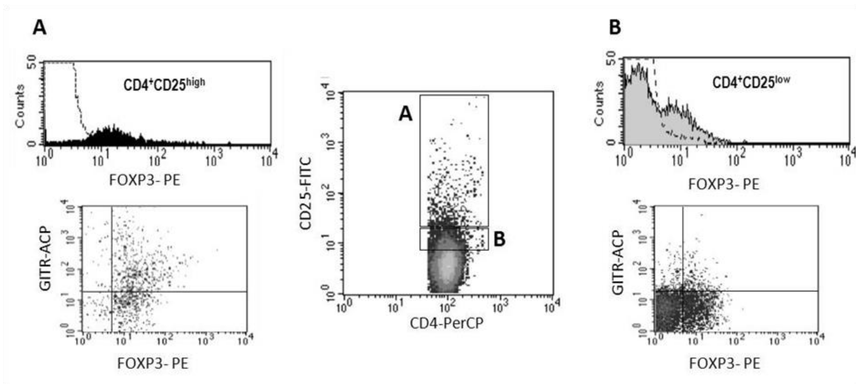
Lupus nephritis (LN) is a severe complication of systemic lupus erythematosus. T lymphocytes with regulatory properties (Tregs) play a role in preventing autoimmunity, are involved in LN pathogenesis and may also determine glomerular lesions in LN. Their potential use as LN biomarkers is investigated.

The aim of our study was to assess the relationship between repeated measurements of Tregs proportions, histopathology classes and five-year clinical outcomes in LN patients with different disease duration and activity.

METHODS:

Forty eight LN patients with different disease duration and activity were enrolled (Tab.1), and then followed-up for 5 years. Their mean age, disease duration and activity (SLEDAI) at baseline was 41.1 years, 9.8 years and 8.3 points, respectively. Their blood was collected twice: at baseline and after 6 months. Populations of Tregs and Th17 cells were analysed by flow cytometry (Fig.1), in relation to clinical parameters and previously established LN classes assessed according to the ISN/RPS 2003 classification.

Fig. 1. Example of the Tregs identification with use of unique marker expression patterns. Flow cytometry identifies $CD25^{high}$ $FOXP3^{+}$ Treg within the $CD4^{+}$ T cell population in peripheral blood. $CD4^{+}CD25^{high}$ T cells (A) express T cell-specific 'pattern' of FOXP3 which is different from $CD4^{+}CD25^{+}$ (B) T cells representing the activated $CD4^{+}$ T cells. Analysis showed that 90% of $FOXP3^{+}$ cells were in the $CD25^{high}$ and 35% in the $CD25^{+}$ populations. It also revealed the distribution of receptor GITR (a negative regulator of Treg cells) in each cell population. The dotted black line represents isotype IgG–PE control for anti-FOXP3 antibody.



RESULTS:

Lymphocytes percentages in class IV were different from classes III, V or a combination of III and V. In the latter classes, the percentages of the Tregs and Th17 cells were significantly lower, whereas in class IV the increase in FOXP3 in the Tregs and Th17 cells over six months period was significantly higher (Tab.2). Changes in glomerular filtration rate and SLEDAI within 5 years did not correlate with single or repeated Tregs measurements.

CONCLUSIONS:

Differences in lymphocyte proportions between class IV and other classes may suggest its distinct pathogenesis and requires further investigations on their role as a potential LN class surrogate biomarker.

REFERENCES:

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Table 1. Demographic and clinical characteristics.

	Baseline visit
Female / male [n]	44 / 4
Age [years] (mean ± SD)	41,1 ± 12,9
Disease duration [years] (mean ± SD)	9,8 ± 8,6
Immunosuppressive therapy [n] (%)	
CCS	20 (41,7)
CCS + AZA	12 (25)
NONE	5 (10,4)
CCS + HCQ	3 (6,3)
CCS + MMF	2 (4,2)
CCS + CSA	2 (4,2)
CCS + AZA + HCQ	1 (2,1)
CCS + AZA + CSA	1 (2,1)
CCS + CYP	1 (2,1)
CCS + MTX + CSA	1 (2,1)
SLEDAI score [pt] (mean ±SD)	8,3 ± 6,2
Renal features [n] (%)	
Urinary casts	9 (18,8)
Leukocyturia	14 (29,2)
Hematuria	14 (29,2)
24-h urine protein [mg] (mean ±SD)	1224 ± 3764
eGFR [ml/min]	87,3 ± 28,1
Class of nephritis (ISN/RPS 2003) [n] (%)	
II	1 (3)
III	6 (18,2)
IV	15 (45,5)
V	2 (6,1)
II + V	1 (3)
III + V	5 (15,2)
IV + V	1 (3)
FSGS	1 (3)
VI	1 (3)
Autoantibody positivity [n] (%)	
Antinuclear antibodies	42 (87,5)
Anti-dsDNA	11 (22,9)
Complement [mg/dl] (mean ±SD)	
C3 (N:90-180)	122,3 ± 13
C4 (N:10-40)	17,2 ± 1,7
ESR [mm/H] (mean ±SD)	36,6 ± 23,1
CRP [mg/l] (mean ±SD) (N:0-9)	2,4 ± 3,0

Legend:
AZA – azathioprine; CCS – corticosteroids; CRP – C-reactive protein; CSA – cyclosporine; CYP – cyclophosphamide; ESR – erythrocyte sedimentation rate; FSGS – focal segmental glomerulonephritis; HCQ – hydroxychloroquine; MTX – metothrexate; SLEDAI – SLE disease activity index;

Table 2. Factors differentiating histopathology LN classes.

Class of LN vs other classes	**Correlating factor**		**p**
III + V	C4	↓	0,0007
	FOXP3% in $CD4^{+}CD25^{+}$	↓	0,009
	FOXP3 MFI in $CD4^{+}CD25^{+}$	↓	0,00003
	FOXP3 MFI in $CD4^{+}$	↓	0,02
	FOXP3 MFI in $CD4^{+}CD25^{+}CD127^{+}$	↓	0,01
III	FOXP3 MFI in $CD4^{+}CD25^{high}$	↓	0,03
	delta FOXP3% in $CD4^{+}CD25^{high}$	↓	0,02
	FOXP3 MFI in $CD4^{+}CD25^{+}CD127^{+}$	↓	0,01
IV	FOXP3% in $CD4^{+}CD25^{high}$	↓	0,03
	delta FOXP3% in $CD4^{+}CD25^{high}$	↑	0,02
	FOXP3% in $CD4^{+}CD25^{+}CD127^{+}$	↓	0,03
	delta FOXP3% in $CD4^{+}CD25^{+}CD127^{+}$	↑	0,003
V	FOXP3 MFI in $CD4^{+}CD25^{+}$	↓	0,0006

Legend:
C4 - complement concentration; delta - difference between two measurements (0 and +6 months); FOXP3 - forkhead box protein 3; MFI – mean fluorescence intensity;

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