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Relationship between leptin and regulatory T cells in systemic lupus erythematosus: preliminary results

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Abstract. – OBJECTIVE: Crescent literature data demonstrated a role of adipokines in immune responses, particularly leptin is involved in wide spectrum of pro-inflammatory functions. Several evidences suggested that leptin is able to inhibit T regulatory cells proliferation and function *in vitro* models. In the present study, we investigate the relationship between leptin and circulating T regulatory cells (Tregs) in patients affected by systemic lupus erythematosus (SLE).

PATIENTS AND METHODS: 13 SLE patients and 11 healthy controls were enrolled. Metabolic syndrome and cardiovascular parameters were evaluated. Serum leptin levels were detected by commercial ELISA kit and circulating regulatory T cells were determined by FACS analysis as CD4+CD25highFOP3+ lymphocytes.

RESULTS: Metabolic syndrome, defined by ATPIII criteria, was more prevalent in SLE compared to controls (38.4% *vs.* 0%, $p = 0.04$), as well as arterial hypertension (38.4% *vs.* 0%, $p = 0.04$). We did not find significant differences in mean leptin levels among SLE and controls (13.13±1.51 ng/ml *vs.* 9.48±8.67 ng/ml, $p = 0.6$). Mean Tregs percentage of total CD4 were 1.27±0.9 in SLE *vs.* 2.8±1.2 in healthy controls ($p = 0.001$). We found a negative correlation between leptin levels and Tregs percentage of total CD4 in SLE patients ($r = 0.4$, $p = 0.01$).

CONCLUSIONS: Our results suggest a role of leptin in the regulation of circulating T regulatory cells amount in human SLE.

Key words:
Leptin, Tregs, SLE.

Introduction

In recent years the concept of adipose tissue has completely changed. The white adipose tissue (WAT) was always considered place of energy storage. In fact, the WAT carries out many biological functions and secretes a large number of molecules, including the adipokines[1-4]. The adipokines are involved not only in the control of energy metabolism and in the modulation of appetite, but also in the functions of the gastrointestinal, reproductive, cardiovascular and nervous systems. Crescent literature data demonstrated a role of adipokines in immune responses[5-8]. Leptin is the first discovered adipokine, produced by ob gene, and belongs to the long-chain helical cytokine family, such as interleukin-2 (IL-2) and IL-12. Leptin exerts pro-inflammatory actions both in innate and adaptive immunity[5-12]. Hyperexpression of leptin in Systemic Lupus Erythematosus (SLE) is widely described, in relation to immune dysfunction, cardiovascular risk and insulin-resistance[13-15].

Growing evidences suggest the involvement of regulatory T cells (Tregs) in the pathogenesis of SLE. Tregs were originally described by Sakaguchi et al as CD4+ T-cells that constitutively express the receptor of a chain of IL-2 (CD25)[16]. Actually, the most relevant class of Tregs are CD4+ with the highest expression of CD25 and low expression of CD127 (the α chain of the IL-7 receptor)[17-19]. The hallmark of Tregs is Foxp3, a transcription factor with a central role for the development and function of Tregs[17-19]. In lupus-prone mice, the treatment with antibodies anti-CD25 induces the development of nephirits, whereas the transfer of CD25+ T cells suppresses antibodies production and reduces the prevalence or slows the progression of renal disease[20-22]. Several reports describe a functional impairment of Tregs derived from lupus-prone mice[20-22]. In human SLE, the findings about Tregs number and function are controversial. However, compelling evidences suggest a substantial loss of suppressive capacity of Tregs from SLE patients[20-23].

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Starting from the observation of a reduction of Tregs concentration in visceral adipose tissue with obesity progression in mice models[24], several researchers have begun to investigate the relationship between metabolism and Tregs. De Rosa et al[25] demonstrated that leptin is able to reduce the proliferative potential of Tregs upon T cell receptor stimulation and that Tregs express high levels of leptin receptor. In the same experimental model, the administration of an anti-leptin antibody reversed the anergic status of Tregs in vitro. Moreover, a greater numbers of Tregs were reported in leptin-deficient mice than in wild-type[25,26]. These findings suggest an interplay between adipocytes and Tregs that could be mediated by leptin.

In this study we investigated the relationship between leptin with Tregs in a SLE cohort.

Patients and methods

Study Population

13 patients affected by SLE according to SLICC classification criteria[27] and 11 healthy controls were enrolled in University Campus Bio-Medico Outpatients Clinic, Rome. Patients and controls were female. Local Ethics Committee approved the study and informed consent was obtained from all subject enrolled.

Clinical Evaluation and Laboratory Assessment

Clinical history in SLE patients and controls concerned fertility or menopausal status, history of diabetes mellitus, dyslipidemia or hypertension, familiar or personal cardiovascular diseases (CVD) history. For patients and controls Waist/Hip ratio, body mass index, resting blood pressure were recorded. In SLE patients disease feature, disease and medication history were assessed. Exclusion criteria were subjects younger than 18 years, pregnancy, estro-progestinic assumption, nephropathy (defined as MDRD <50 ml/min/1.73 m^2, nephritic or nephrotic syndrome), increased serum liver enzymes (>2xN) and history of cancer. None of the SLE patients was in treatment with more than 12.5 mg/day of prednisone or equivalent during the 6 months prior of the study.

Metabolic Syndrome was defined according to ATPIII criteria[28].

Blood specimens were collected after an overnight fast and stored at -80°C until assayed.

Leptin

Serum leptin levels were determined by commercial ELISA kit (Leptin sandwich ELISA, DRG Instruments GmbH, Germany)[29].

T regulatory lymphocytes

PBMC derived from SLE patients and healthy controls were incubated with surface markers FITC Mouse Anti-Human CD4 (clone RPA-T4, BD Pharmingen, Becton, Dickinson and Company, Buccinasco, MI, Italy) and APC Mouse Anti-Human CD25 (clone M-A251, BD Pharmingen, Becton, Dickinson and Company, Buccinasco, MI, Italy). Stained cells were fixed and permeabilized with BD Human FoxP3 Buffer Set (BD Pharmingen, Becton, Dickinson and Company, Buccinasco, MI, Italy), according to manufacturer instruction. Then, cells were incubated with PE Mouse anti-Human FoxP3 (clone 259D/C7, BD Pharmingen, Becton, Dickinson and Company, Buccinasco, MI, Italy) or PE Mouse IgG1, κ Isotype Control (MOPC-21, BD Pharmingen, Becton, Dickinson and Company, Buccinasco, MI, Italy). Cells were analyzed by BD FACS Canto II system. T CD4+, CD25high, FoxP3+ cells were expressed as percentage of total CD4 lymphocytes.

Statistical Analysis

Data were analyzed by software for statistical analysis (Prism 5.0, Inc., San Diego, CA, USA). Comparisons of continuous variables among groups were performed by Student's *t*-test. The categorical variables were analysed by Fisher F test. Correlations were calculated by Pearson's test. Two-sided p values <0.05 were considered statistically significant.

Results

Demographic and clinical features of SLE and controls were reported in Table I. None of the patients presented anti-phospholipids, anti-beta2glicoproteinI and Lupus anticoagulant positivity.

Metabolic and Cardiovascular Parameters

In table 2 we described metabolic and cardiovascular parameters. In SLE patients we found a higher prevalence of arterial hypertension compared to controls (38.4% *vs.* 0%, $p = 0.04$). Moreover, in 5 of 13 SLE patients metabolic syndrome was diagnosed compared with controls (38.4% *vs.* 0%, $p = 0.04$).

Table I. Demographics of study cohort.

		SLE	Healthy Subjects	p
	Number, N	13	11	
	Age, years, mean ± SD	45.3 ± 10.8	46.2 ± 8.3	NS
	Sex, M/F	0/13	0/11	NS
Disease features	Kidney disease*, N (%)	0 (0)	0 (0)	NS
	Disease duration, years, mean ± SD	7.6 ± 3.0		NS
	SLEDAI-2K, mean ± SD	5.7 ± 2.5		
	ESR (mm/h), range 0-20, mean ± SD	18.6 ± 5.2	10.4 ± 8.2	0.007
	CRP (mg/dl), range 0-5, mean ± SD	5.3 ± 3.0	1.2 ± 2.4	0.001
	SLE + anti-phospholipid syndrome, N (%)	0 (0)		
Serologic features	ANA ≥ 1:160, N (%)	13 (100)	0 (0)	
	Anti-dsDNA positive, N (%)	6 (46.1)	0 (0)	
	Anti-Sm positive, N (%)	5 (38.4)	0 (0)	
	C3 below inferior limit, N (%)	5 (38.4)	0 (0)	
	C4 below inferior limit, N (%)	4 (30.7)	0 (0)	
	Anti-phospholipid antibodies IgG positive, N (%)	0 (0)	0 (0)	
	Anti-phospholipid antibodies IgM positive, N (%)	0 (0)	0 (0)	
	Anti-beta2glicoprotein I antibodies positive, N (%)	0 (0)	0 (0)	
	Lupus anticoagulant positive, N (%)	0 (0)	0 (0)	
SLE therapy	Mean daily prednisone dosage, mg, mean ± SD	7.8 ± 4.3	0	
	Oral prednisone therapy, N (%)	12 (92.3)	0 (0)	
	Antimalarial, N (%)	13 (100)	0 (0)	
	Azathioprine, N (%)	7 (53.8)	0 (0)	
	Methotrexate, N (%)	4 (30.7)	0 (0)	
	Other immunosuppressant, N (%)	0 (0)	0 (0)	

*Kidney disease: clinical renal disease (nephrosic syndrome, nephritic syndrome, refractory arterial hypertension), isolated urinary abnormalities, WHO class III, IV, V or VI SLE glomerulonephritis, serum creatinine > 1.2 mg/dl.
Table legend: M, male; F, female; SLEDAI 2K, SLE disease activity index 2000; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ANA, antinuclear antibodies.

Table II. Metabolic and cardiovascular parameters.

	SLE	Healthy Subjects	p
Number, N	13	11	
Current smokers, N (%)	5 (38.4)	4 (36.4)	NS
Arterial hypertension, N (%)	5 (38.4)	0 (0)	0.04
Type 2 diabetes mellitus, N (%)	0 (0)	0 (0)	NS
CVD history, N (%) 1 (7.6)	0 (0)	NS	
CVD familiar history, N (%)	5 (38.4)	4 (36.4)	NS
Obesity (BMI ≥30), N (%)			
1 (7.6)	0 (0)	NS	
BMI, mean ± SD 25.6 ± 3.1	22.6 ± 2.3	0.01	
Weight, mean ± SD 68.2 ± 11.3	58.6 ± 5.9	0.02	
Waist/hip ratio, mean ± SD	0.9 ± 0.1	0.8 ± 0.1	NS
Systolic blood pressure, mmHg, mean ± SD	128.9 ± 14.5	112.2 ± 14.3	0.01
Diastolic blood pressure, mmHg, mean ± SD	81.7 ± 9.3	79.2 ± 4.6	NS
Total cholesterol, mg/dl, mean ± SD	187.8 ± 18.5	178.2 ± 20.2	NS
HDL cholesterol, mg/dl, mean ± SD	61.1 ± 13.4	62.0 ± 12.1	NS
Triglycerides, mg/dl, mean ± SD	127.4 ± 57	78.2 ± 35	0.02
HOMA-IR, mean ± SD	2.1 ± 1.1	1.7 ± 0.7	NS
Metabolic syndrome, N (%)	5 (38.4)	0 (0)	0.04

Table legend: CVD, cardiovascular disease; HDL, high density lipoprotein.

Leptin levels

We did not find significant differences in mean Leptin levels among SLE and controls (13.13±1.51 ng/ml *vs.* 9.48±8.67 ng/ml, $p = 0.6$).

T regulatory cells levels

Mean Tregs percentage of total CD4 were 1.27±0.9 in SLE *vs.* 2.8±1.2 in healthy controls ($p = 0.001$).

Relationship between Leptin and Tregs

We found a negative correlation between leptin levels and Tregs percentage of total CD4 in SLE patients ($r = 0.4$, $p = 0.01$) (Figure 1).

Discussion

The adipose tissue, in particular the white adipose tissue, plays an important role in the human organism. The WAT is not only involved in energy metabolism and thermoregulation, but participates in a complex manner to the biological processes[1-4]. The WAT is capable of producing multiple mediators, the adipokines, which are implicated in the function of many organs. In recent years, evidences of immunological functions of adipose tissue have grown, cause of the ability of the WAT to secrete mediators active on the immune system[5-8,14].

The metabolic syndrome is a condition in which the accumulation of adipose tissue is associated with the alteration of cardio-vascular and metabolic parameters. Several studies show that the metabolic syndrome is not merely the sum of individual cardiovascular risk factors, but it is an independent cardio-vascular risk factor[14,30,31].

As already shown, in this study we found an increased prevalence of metabolic syndrome in SLE[13-15].

This phenomenon is certainly multifactorial, however a number of SLE-related factors were associated with metabolic syndrome development. These include the low complement levels, nephritis, disease activity and damage accrual over time. In previous works we showed that leptin is involved in the relationship between disease activity, metabolic syndrome and accelerated atherosclerosis in SLE[14,30]. Leptin is the first adipokine identified and has a molecular structure with four alpha-helixes that gives analogy with proinflammatory cytokines. Immunologic functions of leptin are complex. Leptin is active on innate immunity such as acquired immunity[5-8,14]. The stimulatory action of leptin on T cells has been demonstrated. In particular, leptin promotes the activation of T cells, and the production of IL-2 and interferon gamma (INF-gamma)[14].

Several data suggest an inhibitory capacity of leptin on Tregs. Matarese et al. demonstrated that Tregs express leptin receptor and are able to produce leptin[25,26]. *In vitro*, leptin reduced the proliferative potential of Tregs upon TCR cell receptor stimulation and administration of an anti-leptin antibody restored Tregs proliferation. Moreover, leptin-deficient mice presented higher levels of Tregs compared with wild-type ones[25,26].

In our study, we did not find a significant difference between patients and healthy controls in serum leptin levels, but we found in SLE patients a decreased number of T regulatory cells that negatively correlated with leptin. This is a cross-sectional study conducted on a small cohort of patients, lacking of an *in vitro* analysis of Tregs inhibitory function on T helper cells.

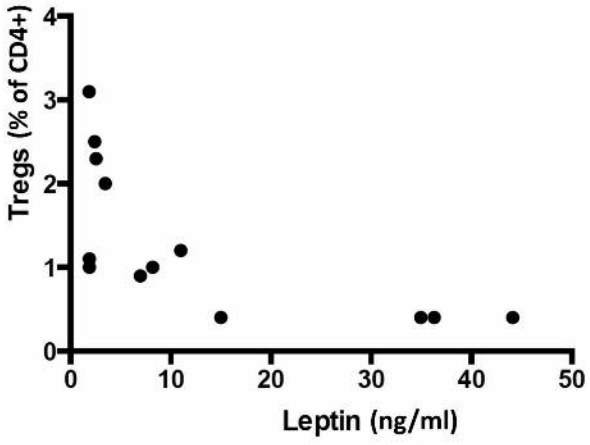

Figure 1. Negative correlation between leptin levels and circulating T regulatory cells in SLE patients (Tregs are expressed as percentage of total CD4+ lymphocytes).

Conclusions

Our data suggest an involvement of leptin in Tregs regulation. However, considering our data we can speculate that in human SLE leptin may not play a pivotal role in modulating Tregs circulating levels, in contrast to *in vitro* evidences. Further studies are required to clarify the capacity of leptin to modulate Tregs function in different subgroups of SLE patients.

Conflict of Interests
The Authors declare that they have no conflict of interests.

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