PCR-RFLP methodology was used for -1149 G/T SNP detection. PCR: The 137 base pairs (bp) region of the PRL extrapituitary promoter was amplified by employing the following primers: forward 5'GGAGTGCAAGATAAAGG and reverse 5'-CTCCTCGAGGTGAATTATTCTCGTT. RFLP: Apol restriction endonuclease was used. The genotypes we identified were: TT homozygote characterized by 120bp+17bp, GG homozygote by 85bp+35bp+17 bp and GT heterozygote by 120bp+85bp+35bp+17bp DNA fragments. Results were evaluated by χ² test with Bonferroni correction.

Results: In the SLE group there was no difference in genotype and allele frequencies compared to healthy individuals. With respect to specific organ manifestation of SLE we detected an association between G allele and arthritis (Pc=0.0086;OR 2.56,CI 1.43-4.59). According to age when SLE was diagnosed we observed an inverse correlation between G allele and arthritis (Pc=0.0086;OR 2.56,CI 1.43-4.59). According to age when SLE was diagnosed we observed an inverse correlation between G allele and arthritis (Pc=0.0086;OR 2.56,CI 1.43-4.59).

Conclusions: The presence of G allele and GG genotype of the PRL extrapituitary promoter -1149 G/T SNP is associated with certain clinical features of SLE namely arthritis and age of SLE onset.

AB10
Systemic Lupus Erythematosus: the involvement of PI3K/Akt/mTOR pathway in cellular cycle and in early apoptosis
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Objective: PI3K/Akt/mTOR signaling pathway plays a critical role in many cellular functions. Until now there are no data regarding this signaling pathway and p27kip1 status in peripheral blood mononuclear cells (PBMCs) of patients with Systemic Lupus Erythematosus (SLE). Therefore, we proposed to analyze in SLE PBMCs the activity of Akt, the expression level of their substrate p27kip1 and the relationship with cell cycle progression as well as the contribution of mTOR to the increased apoptosis of SLE PBMCs.

Methods: Akt and mTOR activities were evaluated by measuring their phosphorylation level, using immunoblotting. The expression level of p27kip1 in PBMCs and lymphocytes was analyzed by immunoblotting and FACS. Quantification of apoptosis and the cell cycle distribution were performed using FACS.

Results: The phosphorylation level of Akt at Thr308 was statistically significant higher while the expression level of p27kip1 was two times lower in SLE than in HC PBMCs. The percentage of lymphocytes accumulated in S and G2/M cell cycle phases are significantly increased in SLE than in HC. Statistical analysis demonstrated that p27kip1 expression level correlated with the percentages of lymphocytes in cell cycle phases. Moreover, in SLE lymphocytes, p27kip1 expression level was indirectly correlated with apoptosis. The phosphorylation level of mTOR was similar in SLE and HC PBMCs, probably due to the therapy. In SLE lymphocytes, where an increased apoptosis was found, mTOR activity was inverse correlated with apoptosis.

Conclusions: Increased activity of Akt, observed in SLE lymphocytes, explains the reduced expression of its substrate p27kip1. This defect seems to be involved in SLE lymphocytes passage by G1/S cell cycle checkpoint. Therefore, SLE lymphocytes accumulate in S and G2/M cell cycle phases towards apoptosis or proliferation. The inverse correlation of mTOR activity and apoptosis rather suggested an anti-apoptotic role of this kinase.

AB11
Abnormalities at the level of E3 ubiquitin ligases in peripheral blood T cells from Systemic Lupus Erythematosus patients

Objective: It is now unanimously accepted that autoimmunity could be defined as a breakdown of mechanisms responsible for self-tolerance. Among peripheral tolerance mechanisms, T cells anergy plays an important role. Previously, in patients with Systemic Lupus Erythematosus (SLE) it was demonstrated that peripheral T cells are resistant to anergy induction. Since some of E3 ubiquitin ligases (Cbl-b, GRAIL) are involved in T cells tolerance we initiated a study in order to establish the role of these ligases in SLE T cells anergy.

Methods: Our study included fifty eight SLE patients with active disease, forty nine with inactive disease and fifty healthy controls (HC). The patients were selected and characterized in Bucharest hospitals according to the American Rheumatism Association criteria. Cbl-b and GRAIL protein expression levels in peripheral blood mononuclear cells (PBMCs)/T cells were determined by western blotting using Cbl-b and GRAIL specific antibodies. The expression level of cbl-b mRNA in PBMCs/T cells was evaluated by RT-PCR.

Cbl-b expression and distribution in T cells as well as its co-localization with lipid rafts was analyzed by confocal microscopy.

Results: Both Cbl-b and GRAIL protein expression levels were significantly reduced in SLE, especially in patients with active disease, than in HC PBMCs. Also, a reduced level of Cbl-b mRNA was identified in active and inactive SLE T cells by comparison with HC. Confocal microscopy analysis confirmed these results showing that in SLE T cells Cbl-b has a reduced and diffuse expression and did not co-localize with preformed large lipid raft domains.

Conclusions: Our results suggested that abnormal expression of some E3 ligases could be involved in enhanced response of SLE T cells even if these are stimulated under anergic conditions.

AB12
Eicosanoid production in the peripheral blood mononuclear cells (PBMC) from patients with systemic sclerosis

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