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 Erythematous (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 is 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 inversely correlated with apoptosis.

Conclusions: 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.

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