activation, have been described in RA patients. It has been observed that the expression of the signaling chain subunit of the TCR/CD3 complex, the TCR γ chain, is downregulated in T lymphocytes of RA patients. Chronic TNF-α treatment appears to reproduce many of the TCR signaling defects observed in T cells from RA patients, suppress a broad range of T cell responses and attenuates intracellular Ca²⁺ mobilization. Several studies carried out on patients with rheumatoid arthritis have documented increased endogenous NO synthesis, but its contribution to T cell dysregulation is not known. We investigated the possible role of NO in T cell dysfunction in RA.

Our present data indicate that T cells from RA patients produce > 2.5 times more NO than control healthy donor T cells (p < 0.001). Although NO is an important physiological mediator of mitochondrial biogenesis, mitochondrial mass is similar in RA and control T cells (p = 0.65), whilst increased NO production is associated with increased cytoplasmic Ca²⁺ concentrations in RA T cells (p < 0.001). We observed that T cell NO production decreased in most RA patients following anti-TNF treatment. TNF treatment of Jurkat cells (10-50ng/ml) induces dose dependent NO production (p < 0.001). Furthermore, chronic NO treatment, like TNF, downregulates TCR γ expression. Experiments also indicate that NO seems to regulate TNF induced apoptosis. These data suggest that TNF induced NO production in T lymphocytes is a key modulator of T cell responses, and its overproduction may contribute to perturbations of immune homeostasis in RA.

**AB04**

Function of human invariant NKT cells is regulated by the distinct binding kinetics of their TCRs to the CD1d/glycolipid complexes

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**Background:** Human invariant NKT cells (iNKT) are a unique subset of T cells that co-express an invariant V 24 TCR and receptors from the NK lineage. iNKT cells recognize exogenous and yet unknown endogenous glycolipids presented by a non-classical Ag-presenting complex. Differential responses induced by CD1d/OCH tetramer staining which we named as “high”, “intermediate” and “low”. A homogenous iNKT staining pattern was always observed when CD1d α-GalCer tetramers were used. Ex vivo sorted iNKT clones also showed different staining intensity with CD1d/OCH tetramers in contrast to CD1d α-GalCer tetramers. CD1d/OCH tetramer high iNKT clones proliferated and secreted Th2 cytokines when incubated with CD1d transfectants even in the absence of the antigen. These iNKT clones were highly cytotoxic at low E/T ratios (specific lysis of 80-90% at E/T ratio 3:1). The soluble iNKT TCR from one CD1d/OCH tetramer high iNKT clone bound to CD1d monomers loaded with α-GalCer, OCH and β-GalCer with binding affinity of 0.3 μM, 1.8 μM and 1.9 μM respectively. TCR from CD1d/OCH tetramer low iNKT clone demonstrated low binding affinity to α-GalCer and OCH loaded CD1d complexes - 7.8 μM and 28.5 μM respectively, while no binding was observed to CD1d/β-GalCer complexes.

**Conclusion:** Heterogeneity of human iNKT cell pool was identified with CD1d tetramers loaded with a partial agonist OCH. CD1d/OCH tetramer high iNKT cells and their CD1d/OCH tetramer low counterparts differ with regard to cytokine secretion and cytotoxic activity. The difference in CD1d/OCH tetramer binding is not caused by different TCR expression levels, but by significantly different binding affinities of “HIGH” and “LOW” TCRs for CD1d/lipid complex. These results suggest that the differences in the CDR3γ chain of iNKT cells determine antigen recognition and functional competence of human iNKT cells in vivo.

**AB05**

Deciphering of PI3K/Akt/mTOR pathway in peripheral T lymphocytes of Systemic Lupus Erythematosus patients

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PI3K/Akt/mTOR signaling pathway is involved in cellular activation as well as in apoptosis and reverse of anergy. It is known that all of these cellular processes are disturbed in peripheral T lymphocytes of Systemic Lupus Erythematosus (SLE) patients, an autoimmune disease with an incomplete elucidated ethyology. To the best of our knowledge, there is no literature data regarding PI3K/Akt/mTOR pathway in T lymphocytes from SLE patients; although the results obtained on MRL/lpr mice indirectly suggested a hyperactivation of mTOR kinase. One of the important mTOR substrates is p70S6 kinase, an enzyme involved in protein translation. Based on these data we proposed to study PI3K/Akt/mTOR signaling pathway in SLE T lymphocytes signaling in comparison with healthy donor T lymphocytes. At the beginning the expression of mTOR and p70S6 kinases were analyzed by immunoblotting. Since the phosphorylation level of mTOR and p70S6K can suggest their activation state, the phosphorylation level of these kinases was analyzed by the same method. Preliminary results suggested that the expression level of p70S6 kinase was significantly increased in SLE T lymphocytes than in healthy donor T lymphocytes. As regard the activation state of these kinases experimental data suggested an enhanced phosphorylation of p70S6K without significant phosphorylation of mTOR in SLE T lymphocytes when compared with healthy donor T lymphocytes.