patient and physician global disease activity assessment (GDA) in a Likert scale (0–4), ESR and CRP. Those patients that fulfilled 5 or more of the next criteria were considered to have an active disease: pain score > 25 mm; TJC > 5; SJC > 3; patient GDA > 1; physician GDA > 1; ESR > 25 mm and RCP > 0.8 mg/dl. DAS28 score was also calculated. Student t test and Pearson correlation test were applied for statistical analysis.

Results IL-15 and TNF- α serum levels from RA patients were significantly higher than that from healthy donors (314.6 ± 72.3 vs. 12.9 ± 3.1 pg/ml, $p = 0.0001$; and 233.1 ± 97.9 vs. 74.2 ± 30 pg/ml, $p = 0.06$, respectively). The percentage of CD69 positive PBL in RA patients was also higher than in controls (12.6 ± 1.2 vs. 9.7 ± 0.8 ; $p = 0.03$). The percentage of CD69⁺ PBL correlated with DAS score ($r = 0.382$; $p < 0.05$). In addition, MS and TNF- α levels also correlated ($r = 0.49$; $p = 0.015$). Mean serum concentrations of IL15 and TNF- α in patients with active disease were higher than these cytokine levels in patients considered inactive (274 vs. 112.4 pg/ml and 337.6 vs. 211 pg/ml, respectively), although this difference did not reached statistical significance likely due to the low number of patients with inactive disease ($n = 6$).

Conclusion Our data showed a correlation between CD69 expression on PBL and clinical parameters, suggesting a pathogenic role for this molecule in RA. Further studies are needed to evaluate the clinical significance of elevated levels of IL15 in RA patients.

THU0087 INCREASED GLUCOCORTICOID BETA MRNA IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF RHEUMATOID ARTHRITIS PATIENTS

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Background Rheumatoid Arthritis is an autoimmune inflammatory disease. A glucocorticoid resistance has been postulated as a factor involved in the pathogenesis of this disease.¹ The mechanisms involved in this GC-resistance has not been elucidated. Glucocorticoids' effects are mediated through an intracellular receptor, the glucocorticoid receptor (GCR). There are two isoforms of the GCR termed alpha and beta. The Beta isoform has recently been demonstrated to act as a dominant negative inhibitor of the classic glucocorticoid receptor alpha.² As a potential *in vivo* inhibitor of GCR alpha activity, the expression of GCR beta isoform may be an important factor regulating target cell responsiveness to glucocorticoids.

Objectives This study evaluated if there is an increase in GCR beta mRNA in peripheral blood mononuclear cells (PBMNC) from active newly diagnosed rheumatoid arthritis (RA) patients as compared to PBMNC from normal controls.

Methods PBMNC were obtained from 9 active RA patients and 9 normal controls, matched by age and sex. A patient was defined as having an active disease if 3 of the following criteria were fulfilled: Erythrocyte sedimentation rate > 30 mm/hour, morning stiffness > 1 h, > 6 tender joints, > 3 swollen joints. The GCR alpha and beta were evaluated by semiquantitative RT-PCR. The RT-PCR products for both isoforms of glucocorticoid receptor were electrophoretically fractionated on 2% agarose gels stained with ethidium bromide. The intensity of ethidium bromide fluorescence was measured densitometrically and expressed as optical density units (ODU). The presence of statistically significant

differences in ODU between groups was evaluated by the Wilcoxon test for matched pairs.

Results RA patients presented a significant increase in the expression of GCR beta mRNA (median 571, range 208–968 ODU vs median 317, range 83–563 ODU in controls; $P = 0.007$) with no change in GCR alpha mRNA compared to controls.

Conclusion RA patients present an increased expression of the inhibitory beta isoform of the GCR in PBMNC. Glucocorticoids have well known anti-inflammatory properties, therefore an increased proportion of the inhibitory isoform of the glucocorticoid receptor could lead to a glucocorticoid-resistance status, which could be a pathogenetic factor in RA by favouring the persistence of inflammatory processes.

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THU0088 PREVENTION OF A NOVEL AND NEWLY DISCOVERED ANTI-ARTHRITIC COMPOUND SX-5452 ON DEVELOPMENT OF COLLAGEN-INDUCED ARTHRITIS IN DBA/1 MICE AND AUTOIMMUNE RESPONSES IN MRL/LPR MICE

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Background In trying to elucidate the value of new drug regimens in rheumatoid arthritis (RA), it is of critical importance to use animal models which share as many features as possible with the human disease. Collagen 0 induced arthritis (CIA) is characterised by development of both cellular and humoral immune responses directed against heterologous and autologous CII. Newly discovered diphenylpyrimidine amino acetamide derivatives pretreatment has been shown *in vitro* to reduce anti-CII antibody formation. Against this background, we wanted to investigate the clinical effects of SX-5452 on CIA and MRL/lpr.

Objectives To investigate the effectiveness of SX-5452 in preventing the development of arthritis/autoimmune diseases and to compare the potency/mechanisms with Leflunomide, a novel anti-rheumatic drug.

Methods Male DBA/1 mice and female MRL/lpr/lpr mice were used. The degrees of swelling, redness were evaluated macroscopically and intensity of inflammation was evaluated by histologically. At the same time the antibody formation was evaluated *in vivo* and *in vitro*.

Results Oral administration of SX-5452 (0.03 mg/kg/day–10 mg/kg/day) from the day of immunisation with CII strongly and dose-dependently suppressed the development of arthritis. SX-5452 at the dose of 10 mg/kg prevented completely the development of arthritis in both arthritic score and histological changes. Furthermore administration of SX-5452 at the dose of 10 mg/kg one a week also suppressed almost completely the development of CIA. It also showed inhibitory effect on the production of anti-CII antibody in CIA mice as well as anti-DNA antibody in MRL/lpr mice. These effects were at least three-fold more potent than those of leflunomide. MAPK-pathway and tyrosine phosphorylation are raised as the possible molecular mechanisms.

Conclusion The development of CIA and autoimmune responses in MRL/lpr mice were markedly suppressed by SX-5452 via inhibition of anti-CII/anti-DNA antibodies formation. Since SX-5452 exerts no overt toxicity after repeated administration in mice, it is an interesting candidate for therapeutic use in rheumatoid arthritis.

THU0089 INHIBITION OF RHEUMATOID ARTHRITIS SYNOVIOCYTES GROWTH BY TAURINE CHLORAMINE

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Background Hyperplasia of synovial membrane, a characteristic feature of rheumatoid arthritis (RA), is proposed to result from either the enhanced proliferation of fibroblast-like synoviocytes (FLS) or the resistance of these cells to apoptotic death. Therapeutic approaches to inhibit an excessive growth of RA FLS are not satisfactory. We have previously shown that taurine chloramine (Tau-Cl), a chlorinated derivative of a dominant free amino acid taurine (Tau), formed by neutrophils during respiratory burst, inhibits FGF-triggered proliferation of RA FLS (Arthritis Rheum 1999;42:2552).

Objectives The aim of present study was to investigate the mechanism of Tau-Cl inhibition of RA FLS growth.

Methods FLS, isolated from the synovial membrane of RA patients, were stimulated *in vitro* for 18–72 h with 10 ng/ml of either rhPDGF or rhTNF- α . Tau or Tau-Cl were added (at 100–500 μ M concentration) together with the stimuli. Cell proliferation was determined by incorporation of 3H-TdR. To evaluate the cytotoxicity of tested compounds, and to estimate the recovery of the cells from the cultures, the lactate dehydrogenase (LDH) activity was measured in culture supernatants and in cell lysates, respectively. The expression of proteins regulating the cell-cycle progression or apoptosis, was estimated by Western blotting.

Results The rate of spontaneous proliferation of FLS ranged from 176 to 4639 cpm. Both, PDGF and TNF- α raised it significantly by 6 and 2 times, respectively. Tau was neither cytotoxic nor affected the recovery/proliferation of RA FLS. In contrast, Tau-Cl inhibited both the proliferation and the cell recovery in a dose-dependent manner. Independent of the type of stimulus, both responses were reduced by 70% in the presence of 250 μ M, and were almost completely blocked (by 90%) in the presence of 500 μ M concentration of Tau-Cl. At the same concentrations (250 and 500 μ M) Tau-Cl inhibited PDGF- and TNF- α - triggered expression of PCNA (proliferating cell nuclear antigen) but has no effect on the expression of either the Cdk-4 (the cyclin-dependent kinase-4), Cdk-inhibitors (p16, p21, p27) or the anti-apoptotic, Bcl-2, protein. At the late time points (48–72 hrs) only the higher (500 μ M) concentration of Tau-Cl exerted cytotoxic (50%) effect.

Conclusion We report that Tau-Cl, a physiologic factor originated from activated neutrophils, blocks proliferation of RA FLS by affecting the common event critical for the cell cycle progression: the expression of PCNA protein, known to act as a cofactor for DNA polymerase δ . Thus, Tau-Cl might be promising therapeutic agent for RA. Whether Tau-Cl triggers apoptosis of RA FLS is under investigation.

THU0090 TAP1 AND TAP2 GENE POLYMORPHISM IN RHEUMATOID ARTHRITIS IN EASTERN FRENCH POPULATION

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Background The Transporter associated with antigen processing (TAP) gene products are involved in the processing of endogenous peptides that bind to human leukocyte antigen (HLA) class I molecules and polymorphism within these genes could alter the immune responses, the phenomenon relevant to the development of autoimmune diseases.

Objectives In this study, we examined the polymorphism of TAP1 and TAP2 genes in patients with rheumatoid arthritis (RA).

Methods TAP1 and TAP2 typing was performed in 138 white RA patients and 100 healthy controls, all of them originating from Eastern France. TAP1 polymorphic residues at position 333 and 637 and amino acid variants 379, 565, 651 and 665 in TAP2 gene were determined using amplification refractory mutation system (ARMS)-PCR. This method enabled us to determine 4 TAP1 alleles (TAP1A to TAP1D) and 8 TAP2 alleles (TAP2A to TAP2H). All patients and controls had been HLA DRB1* genotyped.

Results The polymorphic residues TAP1/333 and TAP1/637 did not show any difference in their distribution between patients and controls.

Similar findings were observed for TAP2/379 and TAP2/665. However, we found an increased frequency of Thr homozygosity and heterozygosity at position 565 in TAP2 gene (RA vs Controls: 25.3% vs 14%; p: 0.032; OR: 2.09; CI: 1.01–4.38). Similarly, the prevalence of subjects who were homozygote and heterozygote for Cys/651 was increased in the RA group (RA vs Controls: 22.4% vs 11%; p: 0.02). The frequency of TAP2F was higher in RA patients (24.5%) as compared with controls (11.3%), without significance after correction (p: 0.029; p corr: 0.17).

Finally, we found no linkage disequilibrium between DRB1* associated -alleles and amino acid substitution Thr/665 whereas Cys/651 was not independent of DRB1*04, a strongly RA-linked allele.

Only a few patients expressing Thr at position 565 in TAP2 gene were associated with severe clinical manifestations.

Conclusion We evidenced in this study an association between a particular amino acid residue, namely Thr/665 in TAP2 gene, and RA.

This association was found to be weak and did not seem to be a predictor for the severity of the disease.

THU0091 IL-8 PRODUCTION INDUCED BY PROTEIN I/II, A MODULIN FROM ORAL STREPTOCOCCI, IN SYNOVIOCYTES FROM RHEUMATOID ARTHRITIS (RA) PATIENTS INVOLVES THE MAPKS AND FAK SIGNALLING PATHWAYS AND NF-KB

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