Methods Cynomolgus monkeys were randomly assigned to three treatment groups (saline, etanercept, and anti-TNF α Mab) by body weight and each group contained two male and two female monkeys. Abscess formation was induced with subcutaneous inoculation of *Staphylococcus aureus*.

Results Intravenous administration of anti-TNF α Mab on days -7, -3, 1, and 4 delayed the onset and reduced the incidence and the severity of *S. aureus*-induced abscess formation as compared with the saline-treated group at both 10^9 and 10^{10} CFU/ml of bacterial inocula given on day 1. In contrast, no improvement in subcutaneous abscess formation as measured by swelling scores was observed in the receptor construct-treated animals as compared with the saline-treated animals when delivered with the same regimen at the same doses as anti-TNF α Mab. Rather, treatment with receptor construct resulted in a modest increase in the incidence and severity of abscess formation with inocula of 10^9 and 10^{10} CFU/ml.

Conclusion Our results provide the initial evidence that a specific anti-TNF α monoclonal antibody therapy administered at 3 mg/kg provides a beneficial effect against *S. aureus*-induced infection as shown in this non-human primate model of abscess formation.

THU0042 **IN VIVO PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS**

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Background Pro-inflammatory and anti-inflammatory cytokines play important roles in the rheumatoid activity process.

Objectives This study aims to demonstrate the cytokine profiles in rheumatoid arthritis (RA) patients with active and inactive disease process.

Methods Fifty-three Chinese rheumatoid arthritis patients (8M:45F) and 38 healthy individuals (19M:19F) were enrolled into the study. RA patients were classified as having active disease or in remission according to clinical and serological criteria (morning stiffness, joint pain, joint tenderness, joint swelling, ESR, CRP). At venepuncture, the patients and healthy controls did not have any active infective process. All except 3 patients (in remission) were on SAARDs. Sera obtained in the morning were assayed for the following cytokines: TNF α , IFN γ , TGF β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 and for rheumatoid factor (RF) by the ELISA method. HLA-DRB1*04 typing was performed using PCR-RFLP.

Results Forty-two patients had active disease and 11 patients were in remission. Rheumatoid factor was detected in 28 patients and HLA-DR*0405 allele was significantly elevated in RA patients ($p < 0.001$). TNF α , IL-6 and IL-8 were significantly elevated in RA patients when compared to controls ($p < 0.01$, $p < 0.001$, $p < 0.018$, respectively). Patients with RF positivity were noted to have much higher levels of these 3 pro-inflammatory cytokines. There was however no association with HLA-DR*0405. The anti-inflammatory TGF β was noted to be significantly lower in active patients when compared to inactive patients or controls ($p < 0.05$).

Conclusion In conclusion, the serum pro-inflammatory cytokines were significantly elevated in RA patients irrespective of their clinical disease activity while serum TGF β was significantly

depressed in active RA patients and normalises when in remission. The exact role that TGF β plays in RA is yet to be fully elucidated.

THU0043 **THE EXPRESSION OF CYCLOOXYGENASE, LIPOXYGENASE AND NEUROPEPTIDES IN MUSCLE TISSUE OF PATIENTS WITH POLYMYOSITIS AND DERMATOMYOSITIS**

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Background

Objectives To investigate pathogenesis of inflammation in muscles of patients with idiopathic inflammatory myopathies (IIM) – dermatomyositis (DM), polymyositis (PM) by studying expression of cox, lox and neuropeptides, using *in situ* hybridization and immunohistochemistry.

Methods Muscle tissue samples were obtained by needle biopsy from affected and non-affected sites distinguished by MRI-STIR technique. The second biopsy was performed after treatment. For studying cox, lox we used *in situ* hybridization with mRNA probes – antisense cox1, 2 and lox 5. Neuropeptides, such as substance P (SP), calcitonin gene related peptide (CGRP), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY), were evaluated by using immunohistochemistry.

Results 11 patients with DM or PM (9 female, 2 male) were examined. The expression of all investigated molecules (cox1, cox2, lox5, CGRP, SP, VIP, NPY) was detected on all muscle tissue. mRNA expression of cox and lox was increased in affected tissue (detectable expression was on 47 sections of 53–89%) in comparison with non-affected (40 sections of 64 $\pm$ 63%) and tissue after treatment. Expressing cells were muscle cells, inflammatory cells and vessels. Cox1 expression was found mainly on inflammatory cells and vessels, cox2 on muscle cells, inflammatory cells and vessels, lox5 mainly on muscle cells. Expression of neuropeptides was detected mainly on vessels, not on nerve cells. SP and CGRP were expressed in all tissues, VIP and NPY mainly in affected tissue. CGRP was expressed on smaller and thicker vessels in non-affected tissue and on bigger and thinner vessels in affected tissue, SP on smaller and thicker vessels in affected tissue. VIP and NPY staining was weak.

Conclusion Expression of all investigated molecules was observed in all muscle tissue samples from IIM patients. This expression was increased in affected tissues compared with non-affected and tissue after treatment, which indicates the role of cox, lox and neuropeptides in pathogenesis of IIM. Expression of neuropeptides was detected on vessels, not on nerve cells that suggests activation of vascular system in inflamed tissue.

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THU0044 **METHOTREXATE INHIBITS EX-VIVO IFN-GAMMA PRODUCTION IN WHOLE BLOOD OF RHEUMATOID ARTHRITIS PATIENTS**

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Background The mechanism by which methotrexate (MTX), a well known antifolate inhibits inflammation is still unknown. The inflammation in rheumatoid arthritis is mediated by pro-inflammatory cytokines such as TNF α and IFN γ .

Objectives To assess whether cytokine production in blood of RA patients is inhibited after taking MTX.

Methods Blood was obtained from 25 RA patients at T = 0 h and T = 2 h after taking MTX (10 mg or) for the first time. Heparinized Whole Blood Cultures (WBC; dilution 1/4) using anti-CD3 and anti-CD28 as stimulus were performed, with and without addition of folic acid (40 g/ml) to the culture. Because MTX has been reported to induce IL-10 production all experiments were also carried out with and without excess neutralising mAb to IL-10. After incubation for 72 h supernatants were measured by ELISA.

Results IFN γ production by WBC of 25 RA patients before (T = 0 h) and after (T = 2 h) MTX.

Abstract THU0044 Table 1

	IFN γ *ng/ ml	IFN γ , FA added	IFN γ , anti IL 10 added	IFN γ , FA and anti IL 10
T = 0 h	53(12)	39(10) ²	94(17)	78(16)
T = 2 h	19(5) ¹	48(12) ³	27(6) ⁴	93(17)

*Mean (SEM). ¹2h after intake of MTX, IFN γ production is inhibited (p = 0.0001); ²Folic acid by itself inhibits IFN γ production (p < 0.0001); ³Folic acid completely reverses the inhibition by MTX; ⁴In the presence of anti-IL-10 similar results were obtained.

Conclusion MTX inhibits *ex vivo* IFN γ production in RA patients by a folate dependent mechanism. MTX does not act via induction of IL-10. Similar results were found for TNF α and GM-CSF. IL-8 production is not affected by MTX.

THU0045 LOCAL PRODUCTION AND MODULATION OF COMPLEMENT PROTEINS IN RHEUMATOID ARTHRITIS SYNOVIUM

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Background

Objectives Rheumatoid arthritis (RA) is a severe chronic disease characterised by inflammation of synovial tissue, which causes pain and dysfunction of joints and leads to their destruction. In RA, complement has been implicated in inflammation and joint destruction based on studies showing reduced levels of native complement components and increased levels of complement metabolites in plasma, synovial fluid and synovial tissue of RA patients. However, there is limited information on local production and activation of key factors of the complement cascade in RA synovium and their potential modulation by novel anti-cytokine therapies.

Methods Using *in situ* hybridization, immunohistochemistry and Western blot techniques, the presence of complement proteins C3, factor B and Sc5b-9 and of the complement receptors C3aR and C5aR were evaluated in RA and osteoarthritic (OA) synovium. In addition, C3 and factor B levels in synovial fluid were

determined by ELISA. Functional assessment was performed by examination of the effects of adenovirus-based sTNF-R p55 gene transfer into RA synovial fibroblasts in the SCID mouse model.

Results Complement proteins C3 and factor B were synthesised mainly around terminal vasculature in all (6/6) RA synovial specimens whereas complement receptors C3aR and C5aR were expressed in adjacent inflammatory infiltrates. In contrast, only limited local production of these factors could be observed in OA. Different activation of the complement cascade was illustrated by more intensive degradation of factor B in RA as compared to OA synovial fluid (mean: 28 vs. 48 μ g/ml). Overexpression of sTNFRp55 revealed upregulation of C5 but not C3 receptor expression in the transduced RA synovial fibroblasts indicating differential effects of anti-cytokine therapies on complement factors in RA synovium.

Conclusion We conclude that local complement production and activation may play an important role in RA synovium. Moreover, modulation or inhibition of local complement production, e.g. by biologic agents and anti-C5 antibodies, which are currently investigated in clinical trials, could be an attractive therapeutic approach for RA.

THU0046 SERUM SOLUBLE SELECTINS LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND SYSTEMIC SCLEROSIS

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Background Soluble forms of adhesion molecules may be found in the circulation, and increased levels of selectins have been reported in some connective tissue diseases. It was suggested that the measurement of levels of circulating soluble selectins is useful as an indicator of inflammatory disease.

Objectives To determine serum levels of soluble (s) E-, L-, and P-selectin in patients with rheumatoid arthritis (RA) and systemic sclerosis (SSc), and to determine whether levels of these molecules correlate with measures of disease activity and pulmonary involvement.

Methods Serum levels of sE-, L-, and P-selectin were determined by sandwich ELISA in 34 patients with RA, 30 patients with SSc and 15 healthy subjects. The patients with RA were classified according to disease activity, functional status, Larsen score reflecting anatomical joint damage, and the presence of pulmonary involvement. The presence of pulmonary fibrosis and DLCO were determined in patients with SSc.

Results The mean soluble selectin levels are shown in Table 1. sE- and sL-selectin levels were significantly higher in patients with active RA compared to inactive patients (respectively p < 0.05 and p < 0.05). Although sP-selectin level was higher in active RA patients than those in inactive patients, the difference did not reach statistically significant (p = 0.06). Significant correlations were found between sL-selectin and CRP (p < 0.05), and white blood cell count (p < 0.01) and between sP-selectin and CRP (p < 0.01), and erythrocyte sedimentation rate (p < 0.01). There were no correlations between selectin levels and Larsen score. sE-selectin level was increased in RA patients with advanced functional impairment (56.0 $\pm$ 39.8 in stage III-IV vs. 32.7 $\pm$ 13.6 ng/ml in stage I-II, p < 0.05). The presence of pulmonary involvement in patients with RA was not correlated with soluble selectin levels. sE-selectin levels was significantly higher