observed in the both treated groups as compared to the control, but was more expressed using combined therapy (ESR lower by 47,8% and 56,47% and the count of leukocytes by 30,8% and 36,2%). Toxic effect of drugs on liver was not observed. S+P suppressed hepatic stroma inflammatory infiltration with lymphocytes ($p < 0,05$). Pathomorphological examination showed marked positive effect of both kinds of treatment on synovia, soft periarticular tissues and cartilage, but it was more expressed under the combined treatment.

Conclusion The obtained results allow to conclude that S alone and its combination with P had a pronounced antiarthritic effect, showed no toxicity and were well tolerated by animals. These results are in agreement with our previous results, where treatment with S or P separately also exerted antiarthritic effect. Pathomorphological examination at the end of the study demonstrated reduced joint swelling in both treated groups, but the combined therapy with S+P was more effective.

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THU0079 DENDRITIC CELL (DC) SUBSETS IN RHEUMATOID ARTHRITIS (RA) ANALYSED BY 4 COLOUR FLOW CYTOMETRY

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Background Distinct myeloid (MDC) and lymphoid (LDC) DC subsets have been described in humans which are thought to regulate the nature and magnitude of the immune response.¹ MDC have been associated with a T helper 1 (TH1) mediated response while LDC have been associated with a TH2 mediated response.² Inappropriate regulation of DC function may result in diseases such as RA.

Objectives The composition, co-stimulator molecule expression (CD80, CD86, CD40, 41BB-L) and activation (CD83, CD69) state of these two DC subsets was compared between autologous peripheral blood (PB), synovial fluid (SF) and synovial tissue (ST) from patients with RA undergoing joint replacement surgery. DC were defined as lineage negative (CD3-, CD14-, CD16-, CD19- and CD20-) HLA-DR positive cells. MDC were defined as CD11c+ and LDC as CD11c- cells.

Methods Whole blood lysis was used to isolate peripheral blood white cells and size exclusion used to distinguish peripheral blood mononuclear cells (PBMC). Centrifugation was used to isolate cells from freshly drawn SF. ST cells were isolated by mechanical extraction of coarsely minced tissue. Fluorochrome labelled monoclonal antibodies and flow cytometry were used to identify and quantitate cell populations and cell surface proteins of interest. All procedures were carried out either on ice or with cells at 4°C.

Results In the 5 RA patients peripheral blood DC constituted 0.47% (mean, range 0.11–1.28) of PBMC. The ratio of MDC to LDC was 1:3.8. Both MDC and LDC expressed weak/low levels of activation and co-stimulator molecules. DC constituted 2.06% (mean, range 0.78–3.76) of SF mononuclear cells with a ratio of MDC to LDC of 9:1. MDC but not LDC expressed moderate levels of both activation and co-stimulator molecules. ST DCs

constituted 8.41% (mean, range 0.7–22.7) with MDCs making up 100% of DCs in the 5 samples analysed. These MDC expressed high levels of both activation and co-stimulator molecules. In normal healthy adults ($n = 50$) DC constituted 1.07% (mean, median 0.96, 95% confidence limits 0.43–2.5) of PBMC with a MDC to LDC ratio of 1:1.³

Conclusion These preliminary results suggest that MDC play an important role in the pathogenesis of joint disease in RA and support the view that RA is predominantly a TH1 mediated disease.

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Background Lipid profiles in patients with rheumatoid arthritis.

Objectives To investigate lipid profiles in patients with active and inactive rheumatoid arthritis (RA) and to assess the relationship of the inflammatory condition of RA with lipid profiles.

Methods Seventy-two patients with RA and 25 age and sex matched healthy controls were studied. Patients with RA had not been treated with corticosteroid drugs prior to the study. Forty patients had active disease, and 32 had inactive disease. Plasma lipoproteins were fractionated by gradient ultracentrifugation into subfractions, and their chemical composition and mass were determined. Total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol apolipoprotein AI (apo AI)/apolipoprotein B (apo B), lipoprotein (a) [Lp (a)], lipoprotein-associated platelet-activating factor acetylhydrolase (PAF-AH) activity and C-reactive protein (CRP) were measured in both groups.

Results Patients with active RA had significantly lower plasma total cholesterol and high-density lipoprotein (HDL) cholesterol (42.9 vs. 56.1 mg/dl) levels as compared with controls, due to the decrease in the mass of both the HDL2 and HDL3 subfractions. Patients with active RA also had higher plasma triglyceride levels, mainly due to the higher triglyceride content of the very low-density lipoprotein plus the intermediate-density lipoprotein subfraction. The plasma PAF-AH activity in patients with active RA was lower than that in controls, mainly due to the decrease in PAF-AH activity associated with the intermediate and dense low-density lipoprotein subclasses. The levels of apo AI and HDL-cholesterol were significantly lower in patients than in controls (121.4 vs. 147.4 mg/dl). The level of Lp (a) was significantly higher in patients than in controls (29.2 vs. 19.2 mg/dl). The ratios of apo B/apo AI, total cholesterol/HDL-cholesterol, and LDL-cholesterol/HDL-cholesterol were significantly higher in patients than in controls (0.89 vs. 0.61, 4.6 vs. 3.2, 2.7 vs. 1.7, respectively). CRP showed a significant correlation with apo AI ($r = -0.47$, $p < 0.05$).

Conclusion This study suggests that patients with active RA have altered lipoprotein and apolipoprotein patterns that may possibly expose them to higher risk of atherosclerosis. The inflammatory condition of RA may affect the metabolism of HDL-cholesterol and apo AI. Our study to show that patients with active RA exhibit low levels of HDL2 and HDL3 and are deficient in

plasma PAF-AH activity. These alterations suggest that active RA is associated with partial loss of the antiinflammatory activity of plasma Apo B- and Apo A-I-containing lipoproteins.

THU0081 AMELIORATION OF COLLAGEN INDUCED ARTHRITIS BY THROMBIN INHIBITION

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Background The deposition of fibrin observed in arthritic joints has been proved to have a deleterious role.¹

Objectives We wish to explore the effect of the thrombin inhibitor PEG-hirudin in the pathogenesis of the murine collagen-induced arthritis (CIA).

Methods CIA was induced in male DBA/1J mice by intradermic injection of native chicken type II collagen. PEG-hirudin (injected subcutaneously at 1 mg/kg or 0.1 mg/kg per day) was given during 16 days, starting 20 days after the first immunisation (preventive treatment) or at the onset of clinical signs of arthritis (curative treatment).

Results All the mice treated with PEG-hirudin had a significantly prolonged thrombin time compared to control mice. PEG-hirudin, administered in a preventive way, led to significantly reduced incidence and severity of CIA during most of the treatment period, as assessed by clinical scoring. Accordingly, histological features showed a significant diminution of synovial hyperplasia in PEG-hirudin-treated mice compared to untreated mice. There was also a significant down-modulation of the synovial proinflammatory IL-1b and IL-12p35 cytokine mRNAs in treated mice. Intra-articular fibrin, evaluated by immunohistochemistry, was significantly reduced in treated mice compared to control mice and was positively correlated with both clinical and histological scorings. Most importantly, once arthritis was established, there was also a curative effect of PEG-hirudin.

Conclusion PEG-hirudin prevents the onset of CIA in a dose-dependent manner and has also a curative effect on established arthritis, suggesting that thrombin inhibition may offer a new therapeutic approach in arthritis.

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THU0082 INDUCTION OF ADJUVANT ARTHRITIS IN THE MALE LEWIS RAT: LIPOIDAL AMINE EXHIBITS AN "ALL-OR-NOTHING" THRESHOLD RESPONSE DISTINCT FROM THE MYCOBACTERIAL LINEAR DOSE RESPONSE

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Background Marked polyarthritis can be induced in male Lewis rats by injection of either heat-killed *Mycobacterium tuberculosis* (H37Ra, Difco) in paraffin oil (AdA) or lipoidal amine in complete Freund's adjuvant (LA/CFA).

Objectives The present study examined whether or not the disease severity in these two models is related to the dose of the inciting agent.

Methods Adjuvant arthritis was induced in male Lewis rats (190 to 240 g) by administration of *M. tuberculosis* (AdA; 1.25, 2.5, 5, 10, 20 or 40 mg/ml) or lipoidal amine (LA/CFA; 4, 6.25,

12.5, 25, 50, 100, 200, or 400 mg/ml). The clinical evaluation included measurements of body weight and hind paw volume (by water plethysmography) on day 0 and then daily between days 8 to 16. At necropsy on day 16, inflammation (by histopathology) and bone integrity (bone mineral density [BMD] by DEXA; histopathology) were assessed in the tibiotarsal (hock) and femorotibial (knee) joints.

Results Induction of AdA followed a typical dose-response curve for most parameters, including body weight, loss of BMD (hock and knee), inflammation (paw volume and histopathology), and bone erosion (histopathology). In contrast, LA/CFA exhibited an "all-or-nothing" response with a threshold for activity of 50 mg/ml. The severity of arthritis (measured both clinically and morphologically) was comparable at day 16 for AdA engendered by > 1.25 mg/ml of mycobacteria and LA/CFA induced by > 50 mg/ml. These data indicate that AdA and LA/CFA exhibit different dose-response patterns.

Conclusion The practical implication of this finding is that AdA will be the better adjuvant arthritis model for investigating the biology and treatment of inflammatory joint disease over a range of lesion severities.

THU0083 BONE MINERAL DENSITY MEASUREMENTS ARE SUITABLE FOR RAPID QUANTIFICATION OF SKELETAL DESTRUCTION IN RATS WITH ADJUVANT ARTHRITIS

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Background Reduced bone mineral density [BMD] is one of the major features of arthritis in both humans and animals. In a previous study, we showed that BMD was significantly decreased in both the femorotibial (knee) and tibiotarsal (ankle) joints of male Lewis rats with mycobacteria-induced adjuvant arthritis (AdA) even though clinical inflammation was most pronounced in the ankle.¹

Objectives The present experiment measured BMD in the ankles and knees of male Lewis rats with AdA to (1) define which region provides the greatest sensitivity as a rapid screen for anti-arthritis efficacy and to (2) determine the progression of chronic bone loss at these sites.

Methods AdA was induced on day 0 (body weight of rats, ~100 g) by subcutaneous injection of heat-killed mycobacteria in oil; the day of AdA onset (as defined by paw swelling) was day 9. BMD was assessed in knees and ankles on days 12, 13, 14, 15, 16, 17, 18, 25 or 35 (6 normal and 6 arthritic rats/day) using dual energy x-ray absorptiometry (DEXA; Model 4500A, Hologic, Waltham, MA).

Results In control rats, mean BMD for both sites increased by 20% between days 0 to 12, remained constant between days 12 to 18, and then increased again by 10% (ankle) or 25% (knee) by day 35. In AdA rats, hind paw volume relative to baseline values doubled between days 9 to 11 and then increased by another 1.5- to 2-fold to plateau on day 15. Mean knee BMD in AdA animals was 85% of control levels on day 12, fell to 75% by day 15, and ended at 55% on day 35. In contrast, mean BMD in ankles of AdA rats was comparable to control values through day 14, was 85% of controls on days 15 to 18, and decreased to 60% on days 25 and 35.

Conclusion Our data indicate that BMD loss occurs earlier and to a greater degree in the knee of rats with AdA, suggesting that