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

this joint could be a useful site for rapid quantitative screening for skeletal destruction.

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THU0084 THE CD14⁺/CD16⁺ MONOCYTE SUBSET IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Background Most human peripheral blood monocytes strongly express surface CD14, and do not express CD16 (CD14⁺/CD16⁻). A smaller group of monocytes express lower levels of CD14 and also express CD16 (CD14⁺/CD16⁺). This subgroup has different phagocytosing and antigen-presenting capabilities, and is expanded in a number of disease states.¹ Monocyte-derived macrophages and dendritic cells have important roles in the pathogenesis of both rheumatoid arthritis and SLE.

Objectives To determine the percentage of circulating CD14⁺/CD16⁺ monocytes in rheumatoid arthritis and systemic lupus erythematosus (SLE) and relate this to measures of disease activity and treatment.

Methods Peripheral blood was sampled from 31 SLE patients, 19 rheumatoid arthritis patients and 19 healthy controls. The percentage of CD14⁺/CD16⁺ monocytes was determined by immunofluorescence labelling and dual colour flow cytometry.

Results The percentage of CD14⁺/CD16⁺ monocytes was significantly reduced in rheumatoid arthritis (median = 4.90%) compared to normal subjects (median = 7.30%, $p = 0.014$), and in rheumatoid arthritis compared to SLE patients (median = 9.40%, $p = 0.009$). There was a trend towards a higher percentage of CD14⁺/CD16⁺ monocytes in SLE compared to normal subjects, but this did not reach statistical significance. A negative correlation of percentage CD14⁺/CD16⁺ monocytes with serum C-reactive protein level was observed ($r = -0.461$, $p = 0.001$). Drug treatment (including corticosteroid dose) did not correlate with the percentage of CD14⁺/CD16⁺ monocytes in rheumatoid arthritis or SLE.

Conclusion The percentage of CD14⁺/CD16⁺ monocytes in rheumatoid arthritis is lower than in normal subjects and in SLE, and this may contribute to the pathogenesis of rheumatoid arthritis.

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THU0085 IL-15 ENHANCES EXPRESSION OF MRNA ENCODING ANTI-APOPTOTIC PROTEIN BCL-XL AND TRIGGERS PROLIFERATION OF RHEUMATOID ARTHRITIS FIBROBLAST LIKE SYNOVIOCYTES

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Background The hallmarks of rheumatoid arthritis (RA) are leukocytic infiltration of the synovium and expansiveness of fibroblast like synoviocytes (FLS). The abnormal proliferation of FLS

as well as their resistance for apoptosis is thought to be mediated, at least in part, by the overproduction of proinflammatory cytokines and growth factors. IL-15, a T-cell growth factor, has been suggested to play an important role in the pathogenesis of RA. In the present study we tested the hypothesis that IL-15 contributes to FLS expansiveness.

Objectives The aim of this study was to: (i) examine whether RA-FLS express all three receptor chains, i.e. IL-15Ra, IL-2Rb and IL-2Rg required for functional IL-15 receptor complex; (ii) investigate the effect of IL-15 on RA-FLS proliferation; and (iii) analyse IL-15 triggered expression of mRNA encoding anti-apoptotic: Bcl-xL and Bcl-2, and proapoptotic Bcl-xS proteins in these cells.

Methods Synoviocytes isolated from synovial membrane of RA patients (cultured for 2–7 passages) or PBMC from healthy donors were stimulated with TNF- α (10 ng/ml), IL-1b (1ng/ml) or IL-15 (25 ng/ml) in medium supplemented with 5% or 0.1% FCS. After 4 h stimulation, levels of mRNA encoding IL-15Ra, IL-2Rb, IL-2Rg, Bcl-2, Bcl-xS and Bcl-xL were analysed by RT-PCR. The expression of surface IL-15Ra was tested using flow cytometry after 24–48 h stimulation. Proliferation of the FLS was determined based on the incorporation of 3H-thymidine after 72 h pretreatment with 0.1% FCS then 24 h stimulation with IL-15 in medium with 0.1% FCS.

Results Human FLS express mRNA encoding IL-15Ra isoforms and IL-2Rb and γ chains. Interestingly, TNF- α or IL-1 b trigger significant elevation of mRNA encoding IL-15Ra isoforms (3 times), but not IL-2Rb or γ chains. Flow cytometric analysis confirmed the enhancement of IL-15Ra protein expression. The IL-15 receptor complex is functional: IL-15 increased (by ~ 68%) proliferation of FLS in medium with low (0.1%) FCS. Moreover, in these conditions, IL-15 triggers significant elevation of mRNA encoding anti-apoptotic protein Bcl-xL, while the pro-apoptotic Bcl-xS remained unchanged. However, unlike in PBMC, IL-15 fails to rise the level of anti-apoptotic bcl-2 mRNA in FLS.

Conclusion Our studies indicate that IL-15, via enhancement of anti-apoptotic Bcl-xL and increased proliferation is, at least in part, responsible for expansive behaviour of FLS.

THU0086 SERUM IL-15 LEVELS AND PERIPHERAL BLOOD LYMPHOCYTE (PBL) CD69 EXPRESSION IN RHEUMATOID ARTHRITIS (RA) PATIENTS

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Background We have recently described that IL-15 induces higher CD69 expression on lymphocytes compared to several cytokines described in rheumatoid synovium. A growing body of evidence supports that these two molecules can play an important role in the perpetuation of synovitis in RA.

Objectives To determine IL-15 serum levels and the expression of CD69 on PBL from RA patients and to assess their possible correlation with several clinical and biological parameters.

Methods IL-15 and TNF- α serum levels were measured by ELISA (R&D Systems) and CD69 expression on PBL was assessed by flow cytometry, both in RA patients ($n = 25$) and healthy donors ($n = 15$). Each RA patient was evaluated at the time of sample collection for the following variables: morning stiffness (MS), pain visual analogic scale (0–100 mm), number of tender (TJC) and swollen (SJC) joints (Fuchs 28 joints count),