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

AP Cairns, AD Crockard, AL Bell. *Queen's University Department of Rheumatology, Musgrave Park Hospital, Belfast, UK*

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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

¹MS Kurowska, ¹E Kontry, ¹I Janicka, ¹W Rudnicka, ²J Kowalczewski, ¹W Maslinski. ¹Department of Pathophysiology and Immunology, ²Clinic of Orthopaedy, Institute of Rheumatology, Warsaw, Poland

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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

AM Ortiz, R Garcia-Vicuña, E Tomero, A Laffon, I González-Alvaro. *Rheumatology, Hospital Universitario de La Princesa, Madrid, Spain*

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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),

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

¹A Goecke, ²M Alamo, ²M Cuchacovich, ¹C Sepulveda, ²H Gatica. ¹Physiology Program ICBM Faculty of Medicine; ²Rheumatology Service, Hospital Clinico University of Chile, Santiago, Chile

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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

¹MN Itoh, ¹M Tanaka, ¹S Mishina, ²T Murata, ³K Ohno. ¹Pharmacology II/Discovery Research Laboratories; ²R&D Planning; ³Chemistry I, Dainippon Pharmaceutical Co., Ltd., Suita, Japan

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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.