Results Plasma concentrations of TGFb1 were significantly elevated in RA patients compared to OA patients (7.31 ± 1.10 ng/ml vs. 4.11 ± 0.35 ng/ml, mean $\pm$ SEM, $p < 0.05$), the other two isoforms did not show differences. Concentrations of TGFbs isolated from bone were strongly associated with each other with a predominance of TGFb1 >TGFb2 >TGFb3.

Although the concentrations of TGFb1 in bone were slightly elevated in the RA group compared to the OA group (0.49 ± 0.07 vs. 0.38 ± 0.04 ng/mg bone powder) no significant differences were found. No association was found for TGFb levels in peripheral blood and in the corresponding bone extracts. The highest concentrations of TGFb1 from bone were found in patients with reduced bone mineral density. A strong negative correlation was found between bone TGFb1 levels and T-score values ($r = -0.479$, $p = 0.002$).

Conclusion The finding of elevated TGFb1 concentrations in bone samples from patients with osteoporosis is in contrast to earlier findings, which suggest a protective effect of TGFb against matrix degradation. In this study the highest concentrations of extractable TGFb1 were found in RA patients with high bone loss. Thus it may be possible that more proinflammatory features of TGFb are important in bone metabolism, such as the stimulation of IL-6 as described for primary rat osteoblasts. The quantification of total TGFb in the bone matrix may not sufficiently describe the situation *in vivo*. Post translational processes like activation of the latent TGFb and its availability determine the biological effects of this regulatory cytokine.

THU0072 DISTRIBUTION OF SELECTIVE MATRIXINS, TIMP-1, CYTOKINES

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Background The most abundant joint disease is osteoarthritis. Recently it was classified as cartilagenous, synovial or osseous but the unique forms may be combined. The main feature of the disease is imbalance between synthetic and degradative processes within joint tissues. Executive part in degradative processes play matrix metalloproteinases, so called matrixins. *In situ*, they are induced by many stimuli, namely by cytokines and by interaction of different adhesive molecules with their ligands. Having been secreted into the extracellular space, they are activated by serine proteinases, or by other matrixins and inhibited by tissue inhibitors of matrix metalloproteinases, TIMPs.

Objectives

Methods In the sera of OA patients we found matrixins MMP-9, MMP-2 and MMP-3 and TIMP-1 together with cytokines IL-8 and TNF/alpha and adhesive molecules ICAM-1 and VCAM-1. To distinguish between the forms of osteoarthritis, we search for these molecules in all joint tissues that is in cartilage, synovium, granulation tissue, within subchondral bone, synovial fluid and blood serum. The joint tissues and fluids we obtain from surgery during total hip or knee replacement.

Results The joint tissues were derived from 22 patients. We obtained 10 samples of knee joints and 12 samples of hip joints. The patients were 17 female and 6 male, 67.5 ± 9.6 years old, age ranges 48–86 years. The metalloproteinase spectrum in the body fluids and extracts of joint tissues were evaluated by zymography. The concentration of metalloproteinases and TIMP-1 were assessed by ELISA (Biotrak, Amersham Pharmacia

Biotech), the concentration of adhesive immunoglobulins by Bender ELISA (MedSystems Diagnostics GmbH), and the concentration of IL-8 and TNF/alpha were determined on Immulite (DPC, USA).

We found that the most abundant matrixine within cartilage and synovium is either gelatinase A (MMP-2) or stromelysin-1 (MMP-3). On the other hand, within granulation tissue, blood sera and synovial fluid, the prevailing matrixine is gelatinase B or stromelysin-1. In cartilage, synovial fluid and blood serum TIMP-1 is abundant over sum of matrixins, whereas in synovium and granulation tissue the sum of matrixins is abundant over TIMP-1. There is about 2 to 10 times greater concentration of IL-8 in the cartilage than in other joint compartments.

We found significant correlation between levels of sICAM-1 and sVCAM-1, between TNF/alpha and MMP-3, TIMP-1, sICAM-1, sVCAM-1, and IL-8, and between IL-8 and sICAM-1 in all solid joint tissues. In the synovial fluid and blood serum the correlation was less pronounced.

Conclusion There is a great degradative potential within all compartments of osteoarthritic joint due to presence of matrixins, namely that of stromelysin-1. Within cartilage and synovial fluid the proteolytic potential is equilibrated by TIMP-1 whereas within synovium and granulation tissue is not. Synovium and granulation tissue are highly probably the propellers of degradative process in osteoarthritis.

Rheumatoid Arthritis – Aetiology and pathogenesis/Animal models

AB0022 LACTOFERRIN – A POTENT ANTI-INFLAMMATORY AGENT FOR TREATMENT OF ADJUVANT ARTHRITIS

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Background Lactoferrin is a multifunctional immunoregulatory protein, stored in specific granules of neutrophil granulocytes, from which it is released following cell activation. While the biochemical characterisation of lactoferrin as an iron-binding protein has been well described, its physiological role in inflammation remains undefined. Although lactoferrin was proven to exert certain antiinflammatory effects, there is a paucity of data concerning the possibility of administrating lactoferrin as an anti-inflammatory agent in rheumatoid arthritis.

Objectives To evaluate the anti-inflammatory properties of lactoferrin in treatment of adjuvant arthritis ? an adequate model of rheumatoid arthritis.

Methods Adjuvant arthritis was induced in Lewis rats by subplantar inoculation of a complete Freund's adjuvant. Human milk-derived lactoferrin was administered intravenously in the acute phase (8th day after adjuvant inoculation), and in the chronic phase of adjuvant arthritis (29–31 days).

Results The development of adjuvant arthritis in rats was characterised by hind paws swelling and hyperthermia, weight loss, increased white blood cell count and erythrocyte sedimentation rate, an increased level of serum elastase. In approximately 50% of rats a generalisation of inflammation with involvement of intact joints was observed. Treatment with low doses of lactoferrin (3–18 mg/kg) in the acute phase of adjuvant arthritis did not change the course of the disease, while higher doses of lactoferrin (30–60 mg/kg) caused a certain suppression of the inflammatory process, although it was not stable and lasted for only 2

weeks. The serum level of elastase remained unchanged. The administration of lactoferrin in the chronic phase of adjuvant arthritis resulted in a steady suppression of the inflammatory process (decreased hind paws swelling and local temperature, white blood cell count and erythrocyte sedimentation rate, an increase in weight gaining and a decrease in serum elastase level), which lasted until the end of experiment (day 50). This effect was achieved with lower doses of the drug (9 mg/kg), though its strength was dose-dependent. The maximal suppression of adjuvant arthritis was obtained with the repeated injections of lactoferrin (5 injections every other day with total dose of 60 mg/kg) in the chronic phase of adjuvant arthritis (days 23–31). In these rats the serum concentration of elastase was reduced down to normal level and the rate of generalisation was less than 8%.

Conclusion Lactoferrin is a potent anti-inflammatory agent effective in treatment of adjuvant arthritis in rats. The maximal suppression of the inflammatory process is obtained in the chronic phase of adjuvant arthritis.

AB0023 **IN VITRO PERIPHERAL BLOOD MONONUCLEAR CELLS AUTOACTIVITY AGAINST AUTOLOGOUS EPIDERMAL CELLS AND THE PRESENCE OF ANTI-SKIN AUTOANTIBODIES IN PATIENTS WITH RHEUMATOID ARTHRITIS**

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Background We discuss the ability of peripheral blood mononuclear cells (PBMN) of patients with rheumatoid arthritis (RA) or juvenile idiopathic arthritis to damage autologous skin or lyse autologous keratinocytes if co-cultured *in vitro*.

We discuss as well the presence of anti-skin anti-intercellular (ASA-IC), anti-skin anti-basement membrane (ASA-BM) and anti-keratin (AKA) antibodies of IgG class in patients' sera without clinical features of vesicular bullous diseases.

Objectives

Methods A modified *in vitro* skin explant assay originally developed to predict acute graft versus host disease (GvHD) in allogeneic stem cell transplants was used in order to see if GvHR-like histopathological changes occur when patients' PMNC would be co-cultured with autologous skin. ⁵¹Cr release cytotoxic assay was used to prove if observed skin damage was really of an autoimmune origin. An indirect immunofluorescence test on rat, monkey and guinea pig oesophagus substrates was used for the detection and quantification of ASA-IC, ASA-BM and AKA antibodies.

Results We found out that PBMN of 29 out of 34 patients with RA or JIA (85,3%) were able to induce histopathological changes of grade II or above in autologous skin explants. Similarly, we observed that PBMN lysed autologous keratinocytes, but not autologous lymphoblasts when co-cultured *in vitro*.

No skin damage or keratinocytes lysis was observed in healthy controls. Overall ASA-IC were detected in 50 out of 57 studied patients' sera samples (87,7%, $p = 0,0003$). No ASA-BM

positivity was observed in a studied cohort. AKA were detectable in 33 out of 60 patients (55%, $p = 0,0001$).

Conclusion We suggest that PBMN of patients with RA or JIA might recognise similar autoantigen (s) expressed on epidermal cells that give rise an autoimmune response in synovium. However we observed a high incidence of ASA-IC antibodies in patients with RA and JIA we suppose that subclinical pemphigus occurring in this group might be exacerbated with different stimulus including pemphigus inducing drugs.

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AB0024 **ACTIVITIES OF PURINE METABOLISM ENZYMES AND ANTIOXIDANT SYSTEM IN RHEUMATOID ARTHRITIS PATIENTS**

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Background The results of Antioxidant system researches of blood at Rheumatoid arthritis are inconsistent and not enough attention was given to Purine metabolism questions.

Objectives The improvement of Rheumatoid arthritis diagnostics with use of the enzymatic tests.

Methods 71 RA patients and 30 healthy controls were under observation. Adenosin deaminase (ADA), AMP-desaminase (AMPDA), Guanin desaminase (Guanase), Xanthine oxidase (XO), Xanthine dehydrogenase (XDG) activities were determined in blood serum; Glutathione peroxidase (GP), Glutathione reductase (GR), Superoxide dismutase (SOD) activities ? in plasma (pl.) and erythrocytes (er.). ADA, XDG, SOD isoenzymes were determined also. The enzymatic indices were defined in the process of treatment thrice and were analysed depending on clinical specificity of the disease.

Results Increased ADA, Guanase, GRpl., GPer., XO, XDG, SOD activities, decreased AMPDA, GRer., GPpl. activities, increase ADA-2, XDG-2, SOD-1 isoenzymes were observed in RA patients (I degree of activity) in comparison with healthy controls. The increase of the pathological process activity was accompanied with the increase of Guanase, GRpl., XO, SODpl. activities, ADA-2, XDG-2, SOD-1 isoenzymes and the decrease of ADA, XDG, GRer. activities. RA patients with system defects have more increased AMPDA, Guanase, XO activities, ADA-2, XDG-2 isoenzymes, but less ADA, XDG activities in comparison with patients, which had arthritis form of RA. The system defects were characterised with the decrease of SOD, GPer. activities and the increase of GPpl. activity; patients with the arthritis form of RA had contrary changes. We observed more increase AMPDA, Guanase, XO, ADA-2 activities and less ADA activity in fast progressing RA in comparison with slow progressing.

Conclusion The enzymatic indices essentially depended on clinical specificity of the disease and can be used as additional diagnostic criterions in RA.