may in part correspond to previously reported sequences. The absence of some fragments in healthy control joints is indirect evidence for differentially upregulated TNF- α peptide variants in arthritis.

THU0029 EFFECT OF IL-4 ON IL-1 INDUCED INTRACELLULAR Ca^{2+} CHANGES AND INHIBITION OF PG SYNTHESIS IN ARTICULAR CHONDROCYTES

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10.1136/annrheumdis-2001.826

Background Studies suggest that interleukin-1 (IL-1) is the key mediator of arthritis and we have investigated the effect of IL-1 on cartilage degradation. We demonstrated that IL-1 inhibited proteoglycan (PG) synthesis via enhanced nitric oxide (NO) synthesis by chondrocytes. We also demonstrated that IL-1 increased intracellular Ca^{2+} ion concentration ($[\text{Ca}^{2+}]_i$) of chondrocytes. On the other hand, interleukin-4 (IL-4) is a T-cell derived 20 kDa glycoprotein and antagonise the cytokine mediated cartilage degradation.

Objectives We examined the effect of IL-4 on IL-1-enhanced NO synthesis in parallel with intracellular Ca levels and PG synthesis.

Methods Bovine articular chondrocytes were obtained. PG synthesis was measured with ^{35}S sulfate incorporation. NO levels were measured by use of an NO chemiluminescence analyzer. Ca^{2+} imaging was carried out with Fura2-AM loaded chondrocyte using the digital fluorescence-imaging system.

Results In the presence of IL-4, IL-1-enhanced NO release was completely abolished. IL-4 functions mainly as anti-inflammatory, as well as IL-10 and IL-13. To our knowledge, this is the first report demonstrating an inhibitory effect of IL-4 in NO release stimulated by IL-1. Alterations in $[\text{Ca}^{2+}]_i$ provide a ubiquitous cell signalling system that mediates a variety of cellular process. IL-1 evoked an increase in the levels of $[\text{Ca}^{2+}]_i$, a response characterised by a steep onset and followed by a slower decline to preexisting values. IL-4 also abolished the IL-1-induced $[\text{Ca}^{2+}]_i$ increase in the chondrocytes. We examined the effect of IL-4 on PG synthesis. Although IL-4 did not affect PG synthesis, IL-1-inhibited PG synthesis was reversed with the addition of IL-4. It is possible that IL-4 reversed the IL-1-inhibited PG synthesis through the inhibition of NO synthesis and $[\text{Ca}^{2+}]_i$ increase.

Conclusion IL-4 plays a chondroprotecting effect antagonising with IL-1-inhibited PG synthesis.

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THU0030 INCREASED COLLAGEN PRODUCTION BY HUMAN LUNG FIBROBLASTS IN THE RESPONSE TO TUMOUR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND

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10.1136/annrheumdis-2001.827

Background Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), also called Apo-2 ligand, is a member of the TNF family that has been reported to induce apoptosis in a variety of transformed cell lines, as well as in normal human hepatocytes *in vitro*. Among the family members, TRAIL displays highest homology to CD95 ligand, receptor of which may not only mediate apoptosis of T cells, but also mediate the proliferation of normal human fibroblasts. Considering structural and functional similarities between TRAIL and CD95 ligand, we examined the effects of soluble TRAIL on normal human lung fibroblasts. Collagen a2(I) mRNA expression in fibroblasts was measured by RT-PCR, with ribosomal protein S9 as an internal standard. Normalised collagen mRNA expression was increased in fibroblasts stimulated with TRAIL for 1 or 7 days, with peak response (> 5 -fold increase) at 10 ng/ml TRAIL. The increased expression of collagen a2(I) gene was confirmed by cDNA microarray that also revealed 72 other genes with expression level increased and 108 genes with expression level decreased ≥ 2.2 -fold in comparison with quiescent fibroblasts. There was little, if any, effect of TRAIL on fibroblast proliferation. In addition, the expression of TRAIL was found in CD8^{+} T cell clones that had undergone oligoclonal expansion in the lungs of patients with systemic sclerosis (scleroderma) and were able to stimulate collagen production in lung fibroblasts *in vitro*. These data suggest that TRAIL can enhance collagen production by fibroblasts that are resistant to TRAIL-induced apoptosis.

Objectives

Methods

Results

Conclusion

THU0031 HIGH SYNOVIAL FLUID AND SERUM IL-18 LEVELS IN PATIENTS WITH RHEUMATOID AND PSORIATIC ARTHRITIS

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10.1136/annrheumdis-2001.828

Background IL-18 is a recently identified cytokine with a broad array of proinflammatory actions. IL-18 is expressed at sites of chronic inflammation in human autoimmune diseases and is thought to play a pathogenetic role especially in those immune-mediated diseases with a predominant Th1 cytokine profile.

Objectives The aim of this study was to measure IL-18 levels in sera and synovial fluids (SF) of patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA) and osteoarthritis (OA).

Methods Eighty-one patients were studied, of which thirty-three with RA (11M, 22F; mean age 54.7 ± 12.8 yrs), twenty-nine

with PsA (23M, 6F; mean age 48.1 ± 14.2 yrs) and nineteen with OA (9M, 10F; mean age 63.6 ± 8.8 yrs). After centrifugation sera and SF were stored at -20°C and a sandwich enzyme-linked IL-18 immunoassay was performed. Statistical analysis was carried out using non parametric methods.

Results IL-18 levels were significantly higher in SF than in sera of either RA (mean value $\pm$ SD = $6,758 \pm 3,702$ pg/ml vs $575 \pm 1,118$ pg/ml, $p = <1 \times 10^{-6}$), PsA ($4,234 \pm 2,834$ pg/ml vs 556 ± 634 pg/ml, $p = <1 \times 10^{-5}$) and OA patients ($1,054 \pm 1,215$ pg/ml vs 283 ± 542 pg/ml, $p = 0.002$). Serum IL-18 levels were significantly higher in RA ($p < 0.05$) and in PsA ($p < 0.01$) than in OA, while no significant difference between RA and PsA serum levels was found. SF IL-18 levels were significantly more increased in RA than in PsA ($p < 0.01$) and OA ($p < 1 \times 10^{-8}$). SF IL-18 levels in PsA were also higher than in OA ($p < 0.0002$).

Conclusion This study indicates that IL-18 levels are augmented in immune-mediated inflammatory arthropathies such as RA and PsA as against OA where degenerative mechanisms are generally associated with mild inflammation. The difference found is more striking if SF rather than sera were considered. The increased concentration of IL-18 found in SF as against sera in the three groups of patients suggests a pivotal role of this cytokine in local mechanisms of inflammation.

THU0032 THE ASSOCIATION OF SERUM MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITOR LEVELS WITH SCLERODERMA DISEASE SEVERITY

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10.1136/annrheumdis-2001.829

Background Matrix metalloproteinase 3 (MMP-3) is reported to play an important role in the pathogenesis of Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA). Studies have also investigated the association of different tissue inhibitors of MMPs (TIMPs) with fibrosis in scleroderma (SSc).

Objectives To evaluate the correlation of serum MMP-1,3 and TIMP-1 with severity and disease specific markers of SSc and RA.

Methods Serum MMP-1,3 and TIMP-1 were measured in 42 SSc patients (age range 28–68 y, mean 47 y) and compared to measurement in 29 RA and healthy age/sex individuals. Elevated values of MMPs and TIMP-1 were defined as those greater than 2 S. D above normal mean. All SSc and RA patients were scored for disease severity.

Results Serum MMP-1 was significantly elevated in 8/42 (19%) SSc patients ($p = 0.01$) but only in 2/29 (7%) RA patients ($p = 0.2$). Whereas MMP-3 levels were elevated in 10/29 (34%) RA patients ($p = 0.002$), it was elevated in only 5/42 (12%) SSc patients (0.03). TIMP-1 was found elevated in 17/42 (40%) SSc patients ($p = 0.001$) and in only 4/29 RA patients (a strong trend towards significance $p = 0.052$). We found a significant association between the elevation of both MMPs and TIMP-1 levels, with the severity of SSc. Those who had an increase of more than one MMP and/or TIMP, demonstrated life-threatening major organ involvement such as severe lung fibrosis, diffuse GI, or kidney involvement and severe cardiac failure. Contrary to that in SSc, the severity of RA was associated with MMP-3 only.

Conclusion We confirm previous observations that MMPs and TIMPs may play an important role in various rheumatic diseases. Whereas serum increase of MMP-3 correlated with RA severity, SSc severity was characterised mainly by the increase of both MMP-1 and TIMP-1. This suggests that the MMPs and TIMPs involved in SSc are different than those playing a role in RA. These factors may play a synergistic role in the severity of systemic sclerosis.

THU0033 THE CHEMOKINE MIP-1ALPHA IN RHEUMATOID ARTHRITIS – POSSIBLE SIGNIFICANCE ON INFLAMMATION AND ANAEMIA

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10.1136/annrheumdis-2001.830

Background Chemokines play a key role in modulating leukocyte functions at sites of inflammation. In rheumatoid arthritis (RA) an enhancement of the chemokine macrophage inflammatory protein-1alpha (MIP-1a) was observed and may be indicative for inflammatory processes.¹ In addition to its proinflammatory activities several lines of evidence indicate that MIP-1a modulates the proliferation of hematopoietic progenitor cells.²

Anaemia is a common feature of active rheumatoid arthritis. Very little information on the significance of MIP-1a on the pathogenesis of anaemia in RA is available.

Objectives Basing on theoretical, haematological and immunological data, the chemokine MIP-1a was to be determined about its importance in RA concerning the activity of inflammation and the development of anaemia.

Methods 84 patients (18 male, 66 female) with rheumatoid arthritis (ACR criteria) were included to detect inflammatory processes and anaemia. White and red blood cell count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum iron and the rheumatoid factor were assessed by routine laboratory methods. MIP-1a, serum amyloid A (SAA) and transferrin receptor (TfR) were measured by solid phase enzyme immunoassay; erythropoietin (EPO) and neopterin by radioimmunoassay techniques.

Results High MIP-1a levels were accompanied by increased parameters of inflammation (SAA, CRP, ESR). Patients with RA and anaemia showed significantly higher MIP-1a levels than those without anaemia ($p < 0.03$). However, high MIP-1a levels together with decreased serum iron were not associated with increased EPO levels. It might be of importance that patients with high MIP-1a levels in parallel with anaemia and activation of inflammation had a twofold frequency of disability pension compared to RA patients without anaemia.

Conclusion The enhanced expression of MIP-1a in RA is indicative of the inflammatory activation. Moreover besides the regulation of inflammatory processes this chemokine may influence the pathogenesis of anaemia in RA.

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