SNPs and to measure the distribution of IL-10 SNPs in RA versus controls.

Methods DNA of high and low IL10 producers were sequenced. Subsequently, the association between LPS-induced IL-10 production and previously described panel was analysed.

Results The following SNPs were identified:

-3575 A to G, -2849 A to G, -2763 A to C and -1330 A to G. Previously we have identified 4 ancestral IL-10 haplotypes. The current SNPs are: IL10.1 R3-AAAA-(IL10G)-GCC, IL10.2 R2-TGCG-(IL10G)-ACC, IL10.3 R2-AGAA-(IL10G)-GCC, and IL10.4 R2-TGCC-(IL10G)-ATA. To investigate whether these SNPs were functional we analysed the LPS-induced IL-10 production of 161 healthy donors with a specific genotype: -3575: AA (n = 38) 1896 ng/ml, AT (n = 76) 3232 ng/ml, TT (n = 47) 3195 ng/ml. -2849: AA (n = 21) 2115 ng/ml, AG (n = 75) 2950 ng/ml, GG (n = 65) 3111 ng/ml (MW-test both $p < 0.05$). Next, the analysis was repeated in a different group of donors: 135 partners of patients with SLE/MS: -3575: AA (n = 29) 4190 ng/ml, AT (n = 71) 4521 ng/ml, TT (n = 35) 4401 ng/ml (MW-test $P = 0.6$). -2849: AA (n = 26) 3845 ng/ml, AG (n = 41) 4577 ng/ml, GG (n = 68) 4543 ng/ml (MW-test $P = 0.04$, for G carrier versus non G: $P = 0.02$). Next, the distribution of -2849 SNP was compared in RA patients compared to controls. Control-Panels were 1) partners of MS-SLE patients and 2) organ donors. RA-patients were: 1) incident RA cases, 2) outpatient consecutive RA and 3) RA patients from our early arthritis cohort. Control 1) AA 27, AG 42, GG 71; 2) AA 16, AG 64, GG 88; RA patients 1) AA 3, AG 38, GG 51; 2) AA 24, AG 141, GG 152; 3) AA 16, AG 74, GG 91. Total chi-square: $P = 0.0014$.

Conclusion We have previously found that the allele IL-10R3 microsatellite was less frequent in three ethnic groups of RA patients (Afro-americans $p < 0.01$, English $p < 0.008$ and Scottish $p < 0.02$). The SNP that defines the haplotype on which R3 is located is also less prevalent in three groups of dutch RA compared to two groups of dutch controls. These data suggest that a high innate IL-10 production is a risk factor for RA. This may be due to the B-cell stimulating properties of IL-10.

THU0027 EFFECTS OF LEFLUNOMIDE ON CYTOKINE PRODUCTION IN CULTURED RHEUMATOID SYNOVIAL CELLS

P Vergne, B Liagre, P Bertin, C Bonnet, M Rigaud, JL Beneytout. *Department of Rheumatology, University of Medicine, Limoges, France*

10.1136/annrheumdis-2001.824

Background Leflunomide (A 771726) has been shown recently to be effective in rheumatoid arthritis (RA). It inhibits cell proliferation in activated lymphocytes. Its faster onset of action could be due to both antiinflammatory and immunoregulatory properties.

Objectives The present study evaluated the effects of the drug on cytokine production in cultured human rheumatoid synovial cells.

Methods Adherent synovial cells were obtained by collagenase digestion of synovium, isolated from RA patients undergoing surgery. Between passages 4 and 6, cultured synovial cells were incubated with 6 different concentrations of leflunomide (10,20,40,60,80 and 100 μ M) and dexamethasone for 24 h. Lipopolysaccharides (LPS) were or were not added for the last 6 h of incubation. Supernatants were assayed for IL-1 receptor antagonist (IL-1 RA), soluble type I receptor of tumour necrosis

factor (sTNF RI) and interleukin-6 (IL-6) productions by ELISA. Statistical analysis of differences was carried out by analysis of variance (ANOVA).

Results IL-1 RA and IL-6 production of LPS-stimulated synovial cells were respectively 2.4- and 1.7-fold higher than that of unstimulated cells. LPS did not enhance the production of sTNF RI. Leflunomide increased significantly ($p < 0,001$) the production of IL-1 RA in a dose dependent manner with a plateau effect beyond 80 μ M. Leflunomide decreased significantly ($p < 0,001$) the IL-6 production by 70 to 75% in a dose dependent manner. On the other hand, leflunomide did not have a significant effect on sTNF RI production. Dexamethasone decreased all the cytokine productions.

Conclusion Leflunomide could have an immunomodulatory action in the cytokine network involved in the pathogenesis of RA with a decrease production of the proinflammatory cytokine IL-6 and increase synthesis of the antiinflammatory cytokine IL-1 RA.

This work has been supported by Aventis Pharma laboratory.

THU0028 DIFFERENT VARIANTS OF TNF-ALPHA MRNA TRANSCRIPTS ARE EXPRESSED IN RATS WITH COLLAGEN-INDUCED ARTHRITIS

¹MF Seidel*, ²MP Junier, H Vetter^{3*}. ¹Experimental Rheumatology, Rheumaklinik, Bad Bellingen, Germany; ²Neuroplasticité Et Thérapeutique, INSERM Unite 421, Creteil Cedex, France; ³Medizinische Poliklinik, Universität Bonn, Bonn, Germany

10.1136/annrheumdis-2001.825

Background Tumour necrosis factor alpha (TNF- α) is found in both, autoimmune and normal conditions and may therefore not mediate inflammation by overexpression alone. Upregulation of polymorph TNF- α peptides caused by single base pair (bp) exchanges in mRNA transcripts might modify inflammatory events in arthritis.

Objectives We wished to examine variants of TNF- α transcripts expressed in collagen-induced arthritis (CIA) as a classical model of autoimmune diseases.

Methods Severe osteodestructive CIA was induced in male Dark Agouti rats, the nature of arthritis was confirmed by radiology. Total articular RNA from diseased animals was used for cDNA synthesis. PCR was performed for the 705 bp TNF- α propeptide and the amplified bands were cloned in E. coli. Two clones were sequenced and the results compared to the NIH gene bank. To confirm polymorph expressions ribonuclease protection assays (RPA) were performed with RNA from normal spleen tissue and joints from healthy and arthritic animals.

Results Radiology showed severe osteodestruction in CIA animals. Two clones with a complete open reading frame for the 705 bp TNF- α propeptide transcript were obtained by RT-PCR. Single base pair mismatches were found at position 365 and 568 leading to amino acid exchanges of proline versus leucine and lysine versus glutamic acid, respectively. Based on the NIH database four previously published rat TNF- α sequences showed up to twelve single base pair mismatches. In RPA analyses we found protected bands at 705 bp size in all conditions. In addition, smaller bands indicated the presence of transcripts with several single base pair mismatches. Some fragments were not observed in healthy control joints.

Conclusion The two novel TNF- α transcripts described here are expressed in arthritic and control animals displaying a nonspecific polymorphism. The additional presence of smaller bands in the RPA suggest the coexpression of similar transcripts which

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

¹F Nishisaka, ²K Fukuda, ³S Soenn, ¹M Saito, ¹M Oh, ¹T Matsushita, ¹H Kikuchi.
¹Orthopaedic Surgery, Sakai Hospital, Kinki University, School of Medicine, Sakai;
²Orthopaedic Surgery, Kinki University, School of Medicine, Osakasayama; ³Orthopaedic Surgery, Nara Hospital, Kinki University, School of Medicine, Ikoma, Japan

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.

REFERENCES

- 1 Yeh LA, Augustine AJ, Lee P, Riviere LR, Sheldon A. Interleukin-4, an inhibitor of cartilage breakdown in bovine articular cartilage explants. *J Rheumatol.* 1995;**22**(9):1740–6
- 2 Fukuda K, Kumano F, Takayama M, Saito M, Otani K, Tanaka S. Zonal differences in nitric oxide synthesis by bovine chondrocytes exposed to interleukin-1. *Inflamm Res.* 1995;**44**(10):434–7

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

VV Yurovsky. Department of Medicine, University of Maryland – Baltimore, Baltimore, USA

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

V Pittoni, M Bombardieri, A Spadaro, A Ossandon, FR Spinelli, E Taccari, V Riccieri, T Rinaldi, C Alessandri, G Valesini. *Terapia Medica, Unità Di Reumatologia, Università "La Sapienza", Rome, Italy*

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