between TNF-alpha and ESR ($p < 0,02$), arthritis ($p < 0,01$), capillaritis ($p < 0,01$), lymphadenopathy ($p < 0,001$); the negative correlation was observed with serum albumin ($p < 0,02$) in females. The level of TNF-alpha was the low-positive in 2 pts (4,5%), the moderate-positive in 8 pts (18,1%), the high-positive in 26 pts (59%) in males. There was a positive correlation between TNF-alpha and SLAM index ($p < 0,01$), SLEDAI ($p < 0,06$), antiphospholipid syndrome ($p < 0,01$), IgG-anticardiolipin antibodies ($p < 0,03$) in male group.

Conclusion There are differences between the level, the clinical value of TNF-alpha in male and female cohorts of pts with SLE. The measurement of TNF-alpha maybe a useful tool for monitoring disease activity, in the male cohort especially.

AB0015 SYNOVIAL FLUID ANALYSIS: CORRELATION BETWEEN REAGENT STRIPS ANALYSIS AND WHITE BLOOD CELL COUNT

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Background

Objectives To determine if reagent test strips used to analyse inflammatory synovial fluid (SF) is as performing as white blood cell (WBC) count.

Methods 100 synovial fluid specimens obtained were tested using two techniques:

- reagent strips used to test urine (multistix 8SG Bayerdiagnostics) to check the presence of leucocytes. An inflammatory SF was defined as a strip showing more than a trace for leucocytes. The analysis was performed by an investigator unaware of WBC count and of diagnosis.
- White blood cell count. An inflammatory SF was defined as $WBC \geq 2000 \text{ cells/mm}^3$.

Specificity, sensitivity and like hood ratio (LR) of the reagent strip in diagnosing inflammatory SF were calculated.

Results 100 samples SF were tested. The performance of reagent strip were: sensitivity: 82% (IC: 95%); specificity was 88% (IC: 95%) LR: 5.6.

Conclusion Our study suggest that an evaluation of SF by reagent strips could be of interest to guide the diagnosis.

OP0030 CARTILAGE DEGRADATION BY IL-18 IS PREDOMINANTLY MEDIATED BY IL-1 GENERATION

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Background Interleukin-18 (IL-18) is a member of the IL-1 family of proteins that exerts proinflammatory effects.¹ It was formally known as IGIF and is a pivotal cytokine for the development of Th1 responses.² IL-18 is structurally related to IL-1b both cytokines need ICE or caspase-1 for cleavage of the precursor to release the bioactive molecules. Apart from immune-stimulatory activity, IL-18 induces production of TNF and IL-1 *in vitro*. IL-18 synthesis is found in both articular chondrocytes and osteoblasts and, with respect to cartilage, IL-18 promotes gene expression of nitric oxide synthase, inducible cyclooxygenase, IL-6 and stromelysin.

Objectives The goal of the present study was to investigate whether IL-18 mediates cartilage degradation directly or via induction of other cytokines, such as IL-1 and IFN-g.

Methods Patellae of C57Bl/6, IL-1b deficient, Balb/C and IFN-g deficient mice were used for *in vitro* cartilage degradation experiments with either murine IL-18 or IL-1b. Patellar cartilage was pre-labelled with 35S-sulphate. The explants were cultured for 24 h, 48 h or 72 h in either IGF-1 (0.25 ug/ml), IGF-1/IL-18 (10 and 100 ng/ml) or IGF-1/IL-1b (10 ng/ml). Patellae were washed in saline, fixed in 4% formaldehyde and subsequently decalcified in 5% formic acid. Patellae were punched out of the adjacent tissue, dissolved and the 35S content was measured by liquid scintillation counting. For IL-1 inhibition we added IL-1Ra (10 ug/ml) or an ICE inhibitor (2.5 uM) to the culture system.

Results IL-18 exposure for 24 or 48 h did not induce cartilage degradation, determined as release of prelabelled proteoglycans. Cartilage degradation by IL-18 was only found after a 72 h culture period. In contrast, IL-1b did already induce marked cartilage degradation after 24 h. To investigate whether generation of another destructive cytokine was responsible for the delay of cartilage degradation we added IL-1Ra or ICE inhibitors to the culture system. Blocking of IL-1 resulted in almost complete protection against IL-18 mediated cartilage degradation. Additionally, studies in IL-1b -/- mice showed that IL-1b was pivotal in IL-18-induced cartilage degradation. Since IL-18 is a potent inducer of IFN-g, we investigated whether IFN-g induction by IL-18 was involved. It was revealed that IL-18 mediated cartilage destruction was partly dependent on IFN-g induction.

Conclusion The present study demonstrated that IL-18 induces cartilage destruction *in vitro*. Here we showed that IL-1b generation, due to IL-18 exposure was essential for marked cartilage degradation. In addition, it was shown that induction of IFN-g was also involved in IL-18-induced cartilage degradation. These findings implicated that IL-18 contributed to cartilage destruction by induction of IL-1b and IFN-g. Inhibition of both IL-18 and IL-1b generation by ICE inhibitors might be a protective therapy in RA.

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OP0031 PROLONGED EXPOSURE OF T CELLS TO TNF DOWNREGULATES TCRZ AND EXPRESSION OF THE TCR/CD3 COMPLEX

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Background A role for tumour necrosis factor α (TNF) in the pathogenesis of chronic inflammatory is now firmly established. Paradoxically, TNF also has potent immuno-modulatory effects on CD4⁺ T lymphocytes, since antigen specific proliferative and cytokine responses are suppressed following prolonged exposure to TNF. We studied whether TNF attenuated T cell activation by uncoupling proximal TCR signal transduction pathways using a mouse T cell hybridoma model. In this model, IL-2 production following peptide stimulation was suppressed by up to 90% by chronic TNF exposure, and was restored to control levels within

days of withdrawing TNF signals. Dose-response studies indicated that TNF-treated T cells required TCR engagement with higher peptide concentrations for longer periods of time for commitment to IL-2 production. Subsequent experiments revealed that chronic TNF exposure led to a reversible loss of TCR ζ chain expression, in part through a reduction in gene transcription. Downregulation of TCR ζ impaired TCR/CD3 assembly and expression at the cell surface, and uncoupled membrane proximal tyrosine phosphorylation events, including phosphorylation of the TCR ζ chain itself, CD3 ϵ , ZAP-70 protein tyrosine kinase and LAT. Intracellular Ca²⁺ mobilisation was also suppressed in TNF-treated T cells. Signals transduced through a single chain Fv antibody/FcR γ chain chimeric receptor that recognises native type II collagen, and which utilises TCR ζ for IL-2 gene transcription, were also attenuated. We propose that TNF may contribute to T cell hyporesponsiveness in chronic inflammatory and infectious diseases, such as rheumatoid arthritis, by mechanisms which include downregulation of TCR ζ expression. We speculate that by uncoupling proximal TCR signals TNF could also interrupt mechanisms of peripheral tolerance which are dependent upon intact TCR signal transduction pathways.

Objectives

Methods

Results

Conclusion

OP0032 INVASION OF SYNOVIOCYTES IS INHIBITED BY GENE TRANSFER OF TNF-BP OR IL10 IN AN *IN VITRO* INVASION MODEL

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Background In RA fibroblast like synoviocytes (FLS) degrade and invade into adjacent cartilage. An *in vivo* model is the SCID-mouse/Human cartilage/synoviocyte model (S. Gay). Previous studies indicate that the invasive behaviour of fibroblast like synoviocytes can be tested *in vitro* in a matrigel transwell system. Matrigel, mainly composed of laminin and collagen IV, serves as a model for cartilage.

Objectives The aim of this study was to compare the invasive behaviour of FLS from RA and osteoarthritis (OA) patients and to investigate the effect of adenoviral (Ad) transfer of genes encoding IL-10 and TNF-binding protein (p55) (TNF-BP) on the invasive behaviour of FLS from RA patients.

Methods FLS from 43 RA and 28 OA patients obtained from synovial tissue harvested at joint replacement surgery, were seeded at confluency in serum free medium on top of a matrigel coated transwell filter with 8 μ m pores. Medium with 10% Fetal Calf Serum and 10% Human Serum was used in the lower compartment. Three days post incubation cells were fixated, stained and the invaded synoviocytes on the lower side of the filter were counted. To test the effect of IL10 and TNF-BP, RA synoviocytes were infected overnight with 5, 10, 50 and 100 plaque forming units (pfu)/cel of Ad. IL10, Ad. TNF-BP or Ad. luciferase (negative control) and then tested in the invasion model.

Results Significantly more RA synoviocytes invaded through the matrigel (median = 4035 cells) as compared to OA synoviocytes (median = 1900 cells; $p < 0.001$). IL10 and TNF-BP gene

transfer both resulted in a dose dependent inhibition of invasion with a maximal inhibition of 93.7% (± 10.7) and 86.6% (± 14.5) respectively, while luciferase gene transfer showed a maximal inhibition of 17.6% (± 1.7).

Conclusion In conclusion, the invasive behaviour of FLS can be studied in the matrigel transwell system. This assay discriminates between the invasive behaviour of RA and OA FLS. The invasive behaviour of RA synoviocytes can be strongly inhibited by IL10 indicating that IL10 is able to downregulate the proteins involved in invasive growth. The inhibitive effect of TNF-BP indicates that continuous production of TNF is involved in invasive behaviour of synoviocytes.

OP0033 SYSTEMIC ADENOVIRUS MEDIATED GENE THERAPY USING A CHIMERIC SOLUBLE IL-1 RECEPTOR TYPE II ? IGG PROTEIN REDUCES ACUTE INFLAMMATION IN ANTIGEN-INDUCED ARTHRITIS

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Background The anti-inflammatory effects of IL-1 inhibition are well established but most studies have focused on the endogenous IL-1 receptor antagonist (IL-1Ra) protein. However, IL1ra has a very short plasma half-life and hundred-fold excess over IL-1 is required to obtain a 50% inhibition of IL-1 responses. Another naturally occurring inhibitor, soluble IL-1 receptor type II (sIL-1R2) may offer a higher IL-1 neutralising activity and several studies have emphasised its potential function as a regulatory "decoy" for IL1b.

Objectives To construct soluble IL-1 receptor type II chimeric protein with an IgG heavy chain and to evaluate its therapeutic potential in a murine arthritis model using a gene therapy approach.

Methods The murine IL-1R2 extracellular domain was cloned using a RT-PCR protocol from total cytoplasmic RNA, sequenced and a chimeric expressing gene constructed by the addition of a XbaI fragment encoding the constant and hinge regions of a murine IgG1 heavy chain. The IL-1R2IgG coding insert was subcloned into an E1-deleted adenoviral shuttle expression plasmid under the control of a CMV promoter and the resulting plasmid used to construct a standard first generation E1-deleted recombinant adenovirus (Ad-sIL1R2 Ig) using standard homologous recombination techniques in 293 cells. Stock viral preparations were produced in 293 cells and purified by ultracentrifugation in two CsCl² gradients. Viral titer (pfu/ml) was determined by plaque-assay. An EGFP (enhanced green fluorescent protein) expressing recombinant adenovirus (Ad-EGFP) was prepared using a similar protocol. Expression was confirmed by Western blotting using transient expression on HeLa cells under serum free-conditions.

The therapeutic effects of this approach was tested in the acute murine antigen-induced arthritis model (AIA).

Results Western blotting under reducing and nonreducing conditions confirmed expression and dimerization of the chimeric protein. More importantly, adenovirus-mediated systemic expression of sIL1R2 Ig suppressed acute inflammation *in vivo* in murine AIA. Mice were injected into the tail vein with 10⁹ pfu Ad-sIL1R2Ig or Ad-EGFP three days before induction of arthritis by intraarticular injection of 100 μ g mBSA into the right knee. The left knee was injected with PBS as a control. Joint inflammation