THU0034 HIGHER IL-10 CONCENTRATIONS IN THE SYNOVIAL FLUID FROM RA PATIENTS WHEN COMPARED WITH SPONDYLOARTHROPATHY-DERIVED FLUIDS

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Background Several investigations of synovial fluid (SF) and membrane derived T cells show a predominant Th1 response with high IFN-gamma production from rheumatoid arthritis (RA), and a predominant Th2 response with high IL-4 and IL-10 production in T cells from spondyloarthritis (SpA) joints.

Objectives To investigate the concentrations of IFN-gamma, TNF-alpha, IL-4, IL-10, the Th1 propagating cytokines IL-12 and IL-18, and the monokine IL-1 in sera and synovial fluids from RA and SpA patients.

Methods Patients were selected according to the ACR classification criteria for RA (n = 13), and the ESSG classification criteria for SpA (n = 14). Cytokine concentrations were measured using commercial ELISA kits. Results are expressed as mean $\pm$ standard deviation, significance was calculated using the Mann-Whitney U-test for unpaired groups. Sera from 8 healthy donors served as controls.

Results IFN-gamma concentrations were significantly lower ($4,3 \pm 2,6$ pg/ml), but IL-18 ($78,8 \pm 29,3$ pg/ml), TNF-alpha ($105,4 \pm 40,9$ pg/ml), and IL-10 ($100,1 \pm 69,7$ pg/ml) concentrations were significantly higher in RA than in SpA-SF (IFN-gamma $12,9 \pm 10,4$ pg/ml, $p = 0,009$, IL-18 $55,1 \pm 44,1$ pg/ml, $p = 0,038$, TNF-alpha $74,1 \pm 85,0$ pg/ml, $p = 0,044$, IL-10 $14,1 \pm 19,9$, $p < 0,0001$). IL-4 and IL-12 concentrations were rather low in RA- and SpA-SF. Serum concentrations of all investigated cytokines in RA and SpA differed – if at all – only marginally from the situation in healthy donors.

Conclusion Synovial fluid concentrations of the Th1 and Th2 cytokines in RA and SpA do not generally reflect the state of Th1/Th2 balance at the single cell level. There is strong evidence for substantially higher amounts of IL-10 in RA than SpA synovium, reflecting strong antiinflammatory processes are present in RA synovium. The observed Th1/Th2 balances in both investigated diseases are not explained by the corresponding IL-12 and IL-18 SF concentrations.

THU0035 CYTOKINE PRODUCTION BY CD3⁺ T CELL IN SYNOVIAL FLUID FROM PATIENTS AFFECTED BY PSORIATIC ARTHRITIS AND RHEUMATOID ARTHRITIS

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Background Psoriatic arthritis (PsA) is an inflammatory arthropathy associated with psoriasis, characterised by synovitis which often leads to destruction of cartilage and bone.

The mechanism of joint damage is unclear, but it's likely that cytokines play critical roles. In PsA cytokines have been previously measured by enzyme-linked immunoassays; however, these molecules are not very stable in inflammatory fluids and are detectable only when produced in large amount.

Objectives The aim of our study was to analyse the cytokines production in synovial fluids of patients affected by psoriatic arthritis.

Methods We analysed cytokines production *ex vivo* by single cell analysis in flow cytometry in synovial fluids of 9 patients affected by PsA. As control group we used synovial fluids from 8 patients affected by Rheumatoid Arthritis (RA). Synovial fluid leukocytes isolated on Ficoll gradients were stimulated with phorbol-myristate-acetate and ionomycin, treated with brefeldin, permeabilized and stained with anti-CD3, anti-IL4 and anti-IFNg.

Results In 9 patients affected by PsA, IFNg-producing cells were the vast majority (43%, 46%, 75%, 55%, 51%, 42%, 38%, 32%, 20% of CD3⁺ cells). A similar predominance was observed in 8 patients with rheumatoid arthritis (64%, 36%, 35%, 75%, 38%, 29%, 46%, 26%).

Conclusion IFNg is the typical cytokine produced by TH1 T cells, thus the results of our study suggest that TH1 T cell selectively home to synovial tissue in PsA, as already shown in RA.

A better understanding of cytokine production in psoriatic arthritis synovium in a larger number of patients may help to clarify the pathogenesis of the disease and lead to the development of more specific and effective therapeutic agents.

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THU0036 THE EFFECT OF INFLIXIMAB ON PLASMA LIPOPROTEINS

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Background We investigated the effect of Infliximab, a mouse-human chimaeric monoclonal antibody that binds and inhibits the activity of TNF α , on Lipid Metabolism in patients with Rheumatoid arthritis (RA) and Psoriatic arthritis (PsA).

Objectives

Methods Fifteen persons hospitalised in our division of rheumatology were studied. Over a period of eight months 5 male and 10 female patients with a mean age of 56,7 years (age range: 30–81) were included in this study. The subjects included 7 patients with rheumatoid arthritis (RA) according to the ARA criteria (12) and 8 patients with Psoriatic arthritis (PsA). Eight patients had been treated with methotrexat (MTX), 4 with other DMARDs than MTX, 2 patients only received NSAIDs and 5 corticosteroids. Treatment resistant patients with active disease (fulfilling inclusions criteria) received infusions of 3 mg/kg infliximab (at week 0, 2, 6 14, 22 and 30). Two of the patients received 4 mg/kg infliximab after 14 weeks. Lipoprotein (a), Cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were measured by commercially available kits from Boehringer Mannheim (Mannheim, Germany).

Results Infliximab was well tolerated; no toxic effects were observed, no major abnormalities in hematologic findings were noted during or after the study. There was no significant difference either in total cholesterol before and after treatment (209 ± 25 vs. 25 ± 36 mg/dl) or in LDL-cholesterol (1311 ± 24 vs. 1188 ± 4 mg/dl). Lp (a) levels also did not show a change during treatment (median: 1,1 vs. 1,4 mg/dl). However there was a significant rise in triglyceride (TG) levels during treatment with Remicade (1122 ± 48 vs. 1333 ± 5 mg/dl, $p < 0.01$). HDL levels on the other hand were significantly lowered (566 ± 12 vs.

50 ± 1 mg (dl, $p < 0.006$) by the treatment. The changes of the main parameters are summarised in Table 1.

Abstract THU0036 Table 1

	Before Remicade	After Remicade	Significant p-value
TC	209 ± 25	205 ± 36	
LDL-Chol	131 ± 24	118 ± 43	
HDL-Chol	56 ± 12	50 ± 13	$p < 0.006$
TG	112 ± 48	133 ± 53	$p < 0.01$
Lpa	1.1	1.4	

Values of the lipid parameters in our 15 patients.

Conclusion This study shows that elevated TG and decreased HDL levels occur in patients with RA and PsA after Infliximab therapy. We suggest that these changes in lipid metabolism may be a factor responsible for an increased development of vascular pathology.

THU0037 CLASS SPECIFIC RHEUMATOID FACTORS AND INTERLEUKIN-13 IN RHEUMATOID ARTHRITIS

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Background In rheumatoid arthritis (RA) the production of rheumatoid factor (RF) by B-lymphocytes could depend on the action of different cytokines.

Objectives Aim of this study was to detect interleukin 13 (IL-13), a potent modulator of B-cell functions¹, in sera of RA patients in order to investigate the relationship of this cytokine, with different class specific RFs.

Methods We studied 36 consecutive patients with RA, classified according to Arnett criteria and 20 healthy subjects matched for age and sex. In all patients, we evaluated the main clinical and laboratory parameters including the determination of IgM-RF, IgA-RF, IgG-RF and IL-13 by immunoenzymatic methods.

Results We found detectable levels of IL-13 (>1.56 pg/ml) in 86.1% of RA patients and in 40% of controls ($p < 0.0006$). The IL-13 serum levels were significantly ($p < 0.00001$) higher in patients with RA (mean/lower and upper 95% CI = 165.4/99.3–231.6 pg/ml) respect to the controls (mean/lower and upper 95% CI = 3.9/0.8–7.0 pg/ml). The serum levels of IL-13 correlated with those of IgM-RF ($r = 0.591$; $p < 0.0001$) and IgA-RF ($r = 0.505$; $p < 0.002$), while we didn't find any relationship with IgG-RF, IgM, IgA and IgG levels. IgA-RF levels correlated with those of IgM-RF ($r = 0.548$; $p < 0.0005$) and IgG-RF ($r = 0.543$; $p < 0.0006$), while no relationship between IgM-RF and IgG-RF ($r = 0.222$; n.s.) was found. The comparison of patients with or without the different class specific RFs revealed that patients with IgM-RF had significantly ($p < 0.018$) higher levels of IL-13 respect to the patients without (mean/lower and upper 95% CI = 202.4/125.8–278.9 vs 12.6/2.2–22.9 pg/ml), while this finding was not observed for IgA-RF (mean/lower and upper 95% CI = 191.1/114.3–267.9 vs 59.3/58.6–177.2 pg/ml) and IgG-RF (mean/lower and upper 95% CI = 239.4/48.6–430.2 vs 147.6/74.5–220.7 pg/ml). We didn't find any relationship of serum IL-13 levels with clinical and other laboratory signs of disease activity.

Conclusion In conclusion our results show that IL-13 is involved in pathogenetic mechanism of RA, suggesting that IL-13 source could derive from an excessive joint or intravascular production by immune system. A relevant action of IL-13 seems to be related with a selective isotype production of IgM-RF.

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THU0038 CYTOKINE AND GROWTH FACTOR REGULATION OF RA AND OA SYNOVIAL MACROPHAGE DIFFERENTIATION INTO OSTEOCLASTS AND DENDRITIC CELLS

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Background Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease in which an unknown autoantigen is presented to T cells. In RA synovial tissue, fully differentiated dendritic cells (DC) are found in close association with T cells. DC are thought to be the major antigen presenting cells in RA. It has previously been shown that human monocytes differentiate into DC when stimulated with GM-CSF and IL-4 with terminal differentiation promoted by TNF α . We have shown that circulating monocytes and synovial macrophages are stimulated to differentiate into functional osteoclasts in the presence of RANKL, M-CSF and dexamethasone (Dex).

Objectives We aimed to determine whether synovial macrophages preferentially differentiate into DC or functional osteoclasts in the presence of specific combinations of cytokines/growth factors which are known to be involved in the pathogenesis of RA and other inflammatory bone conditions.

Methods Synovial macrophages were isolated from the knee and hip of RA and OA patients. The cells were seeded onto dentine slices and coverslips and cultured for 1, 7 and 14 days in the presence/absence of different combinations of the following factors: RANKL (30 ng/ml), Dex (10–8M), MCSF (0–50 ng/ml), GM-CSF (0–50 ng/ml), IL-4 (10 ng/ml), TNF α (10 ng/ml). Macrophage-osteoclast differentiation was quantified by counting the number of multinucleated, tartrate-resistant acid phosphatase (TRAP) cells present, and by measuring their ability to carry out lacunar resorption on dentine slices. Mature DC were identified by the expression of the marker CD83.

Results In the presence of RANKL, M-CSF and Dex, RA and OA synovial macrophages were maximally stimulated to form osteoclasts; a few cells in these cultures were CD83 positive. The majority were TRAP positive and capable of forming resorption pits on dentine. GM-CSF, IL-4 and TNF α stimulated maximum expression of the mature DC marker CD83 in the cultures; these cultures were TRAP negative and incapable of resorbing dentine. Synovial macrophages cultured in the presence of RANKL and GM-CSF alone resulted in the formation of large multinucleated cells which expressed the marker CD83; a few of these large multinucleated cells were also TRAP positive. Cell cultures treated with GM-CSF were capable of significantly less dentine resorption than cultures treated with RANKL and M-CSF. The addition of RANKL did not modify CD83 expression in any of the above cytokine/growth factor combinations.