differentiate into Th2 effectors. In contrast, naive T cells are capable of differentiating into Th2 cells with appropriate stimulation suggesting that the bias in Th1 differentiation of most RA patients is acquired during T cell maturation presumably in response to antigenic stimulation.

THU0118 SEQUENTIAL EP1 AND EP4 REGULATION IN A RABBIT MODEL OF ANTIGEN-INDUCED ARTHRITIS

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Background Prostaglandin E2 (PGE2) exerts its actions through the binding of the high affinity receptors EP. There are 4 isoforms of EP receptors with extracellular domains highly conserved, each coupling different intracellular transduction systems. Distribution of EP subtypes in the target tissues is therefore central to the pathways elicited by PGE2.

Objectives The aim of this work was to study the regulation of EP1 and EP4 receptors in the inflamed synovial tissue.

Methods We have studied different time periods in a rabbit model of antigen-induced arthritis: 24, 48 and 72 h (acute phase) and 1, 2 and 3 weeks (chronic phase). Knee synovial fluid (SF) volume and white cell count were measured. Total RNA was extracted from the synovial membranes and expression of EP1 and EP4 receptors was studied by RT-PCR techniques. A fraction of synovial tissue was fixed and embedded in paraffin for morphological studies.

Results At the acute stages of the disease the animals depicted a synovial effusion that peaked at 48 h. The highest white cell infiltration was found at 24 h and was dominated by neutrophils; at 72 h neutrophils and mononuclear cells showed similar concentrations in the SF. At longer time-periods only half of the cases showed a synovial effusion, of smaller proportion. The acute histopathologic lesions consisted of leakage of plasma elements with prominent neutrophil invasion. At 72 h lining hyperplasia developed and there was a switch to mononuclear cell infiltration at the subintima. At further stages, fibrotic changes and increases vascularisation were also found. EP1 receptor expression was up-regulated at every point of study, when compared to controls. EP1 expression increased in a time dependent fashion, peaking at 2 weeks (14-fold vs. control). On the other hand, healthy controls expressed moderate EP4 levels, which were dramatically reduced at the acute stage of the disease (90% reduction at 72 h). At the latest periods studied, there was a normalisation and even slight up-regulation of EP4 expression.

Conclusion Our data may suggest that EP1 receptor mediates some of the PGE2 actions in experimental arthritis. This actions do not only include plasma leakage and activation of early inflammatory mediators, as EP1 up-regulation continues to increase when these typical features of acute arthritis are no longer evident. EP4 down-regulation at the early stages of the disease may suggest that this molecule do not participate in oedema and neutrophil activation induced by PGE2 in this experimental model. Restoring of the balance between PGE2 receptors in arthritis could be of help for the control of the inflammatory reaction.

THU0119 RHEUMATOID ARTHRITIS MYELOID CELLS EXPRESS AN INTRINSIC ABNORMAL CAPACITY FOR ACTIVATION

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Background Evidence suggests that monocyte precursor cells in rheumatoid arthritis (RA) might become partially activated in the bone marrow or the peripheral blood before they enter the rheumatoid synovium. However, whether this is the result of pro-inflammatory cytokines leaking from the inflamed synovium into the circulation or rather a functional abnormality of myeloid cells has not yet been conclusively delineated.

Objectives To test the hypothesis that monocytes in RA might express an intrinsic abnormality for activation, we investigated the function and the phenotype of peripheral blood monocytes from patients with RA and healthy controls *in vitro*.

Methods Monocytes were isolated by negative selection from patients with active, early RA and from patients with long standing RA under effective anti-inflammatory therapy with disease modifying antirheumatic drugs (DMARDs). A cell culture system was employed that permitted the differentiation of monocytes to macrophages. Expression of surface molecules indicative of activation and/or differentiation was assessed by flow cytometry.

Results No differences were detected for the expression of HLA-DR, CD14, CD40, CD68, CD80 and CD86 on freshly isolated monocytes between patients and controls. During differentiation to macrophages, the expression of HLA-DR, CD14 and CD80 was increased in RA monocytes. Interestingly, patients with untreated, active early disease manifested an even higher propensity to upregulate HLA-DR during differentiation compared to patients with long standing disease under effective anti-inflammatory therapy. In contrast, the expression of CD40, CD68 and CD86 was reduced in myeloid cells from RA patients during differentiation. This feature was more pronounced in patients with long standing disease, but was not correlated with clinical activity.

Conclusion The data indicate that monocytes from RA patients might have an intrinsically increased ability for activation during differentiation. This ability might contribute to clinical disease activity and emphasises the systemic nature of the disease. The data further suggest that long standing disease might alter the functional phenotype of circulating monocytes. However, the contribution of continuous treatment with DMARDs to this phenomenon remains to be elucidated.

THU0120 POLYMORPHONUCLEAR NEUTROPHIL NADPH OXIDASE PRIMING AND ACTIVATION IN RHEUMATOID JOINTS: CORRELATION WITH DISEASE ACTIVITY AND SYNOVIAL CONCENTRATIONS OF TNF, IL-8 AND GM-CSF

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Background Superoxide production by polymorphonuclear neutrophil (PMN) participates in the rheumatoid joint lesions and mainly depends on NADPH oxidase activation. Tumour necrosis factor (TNF), interleukin-8 (IL-8) and granulocyte/macrophage colony stimulating factor (GM-CSF) have a priming effect on NADPH oxidase, depending on the phosphorylation of p47phox, a major cytosolic component of NADPH oxidase.

Objectives We sought to determine if the status (primed/activated) of NADPH oxidase of articular PMN was correlated with rheumatic disease activity and TNF, IL-8 and GM-CSF synovial concentrations.

Methods Knee joint fluid aspiration was performed in 17 patients, 11 with RA and 6 with spondyloarthritis (SpA). Usual clinical and biological data were recorded for each patient, allowing a DAS28 scoring. NADPH oxidase activity was analysed with cytochrome c reduction technique. Phosphorylation of p47phox was evaluated with isoelectrofocusing and immunoblotting using a specific antibody. Cytokines concentrations were measured with ELISA kits.

Results NADPH oxidase activity was increased in the majority of RA knee joints, and to a lesser extent in the SpA group. In the RA group, the NADPH oxidase activity was correlated positively with DAS28, with the PMN count in the synovial fluid, with the degree of p47phox phosphorylation, and with the synovial concentrations of TNF and IL-8. Concentrations of GM-CSF remained very low or undetectable in all patients. In the RA group only, the DAS28, the PMN count and the phosphorylation of p47phox were correlated with synovial concentrations of TNF and IL-8.

Conclusion NADPH oxidase activation, through an increased phosphorylation of p47phox, seems to be implicated in joint inflammation during RA. This phenomenon could be related to high level of TNF and IL-8.

THU0121 BCL-XL AND PLASMA CELL SURVIVAL IN RHEUMATOID SYNOVIUM

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Background Bcl-xL is a Bcl-2 homologue that regulates lymphocyte apoptosis. It has been reported that co-culture of B cells with rheumatoid synovial stromal cells induces upregulation of Bcl-xL, consequently inhibiting B cell apoptosis. Impaired apoptosis of auto-antibody producing B cells may contribute to the perpetuation of inflammation in rheumatoid arthritis (RA). The phenotype of synovial T lymphocytes in RA – Bcl-xL^{high}/Bcl-2^{low}, may also be linked to the enhanced survival of T lymphocytes within the joint.

Objectives In this study, we examined the expression of Bcl-xL in rheumatoid synovium using immunohistochemistry.

Methods Sections of synovium were obtained intraoperatively from patients with RA (n = 18). Specimens from patients with osteoarthritis were used as non-inflammatory controls (n = 9). Staining was carried out using a polyclonal rabbit antibody to Bcl-xL. Peripheral blood lymphocytes were used as positive biological controls. Detection of cell death was carried out using the TUNEL assay (terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick end labelling). Identification of plasma cells within the lymphocyte population was performed using a monoclonal antibody to CD138.

Results Bcl-xL was expressed in both RA and OA tissue. Of note, there was a marked infiltrate of Bcl-xL positive cells in 13 of the 18 RA sections with scattered positive cells in a further 2 sections. Only 1 of the 9 OA sections demonstrated a similar degree of plasma cell infiltration and Bcl-xL expression, although a few positive plasma cells were noted in the remaining sections.

Plasma cells infiltrates that were Bcl-xL positive were noted to be TUNEL-negative on consecutive sections.

Conclusion Upregulation of Bcl-xL by plasma cells in rheumatoid synovium may prevent their elimination by apoptosis and may play a role in the pathogenesis in rheumatoid arthritis.

THU0122 ANTIOXIDANT VITAMINS AND LIPID PEROXIDATION IN PATIENTS WITH RHEUMATOID ARTHRITIS: ASSOCIATION WITH INFLAMMATORY MARKERS

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Background Rheumatoid arthritis (RA) is a chronic inflammatory disease that has been associated with an increase of cardiovascular disease (CVD). However, the mechanism underlying the high incidence of CVD is unclear. It has been postulated that the chronic inflammation associated with RA could lead to an accelerated atherogenesis. Lipid peroxidation and low antioxidants levels are associated with increase of CVD and RA. Furthermore, inflammatory markers and cytokines has related with inflammatory responses and pathogenesis in RA and also atherosclerosis.

Objectives To evaluate in RA patients the vitaminic status and their relation with secretory phospholipase A2 (sPLA2) and other inflammatory markers, and also lipid peroxidation parameters.

Methods The study comprises 30 patients with diagnosed RA according ACR 1987 criteria under treatment and 30 matched controls. Inflammatory markers studied were sPLA2, VCAM, ICAM, TNF α , IFN γ , CRP, ESR and fibrinogen. Lipid profile, size distribution of LDL subclasses and the binding affinity of LDL to chondroitin-6-sulfate glycosaminoglycan (GAG) were analysed. The vitamins A and E were determined in plasma by HPLC. The susceptibility of LDL oxidation was evaluated by the kinetics of conjugated dienes formation induced by hemin. Lag phase (LP), maximal rate of diene production (MR), and maximum diene production (MDP) were studied.

Results RA patients had significant higher plasma levels of sPLA2, VCAM, ICAM, TNF α , IFN γ , CRP, ESR and fibrinogen compared with controls. LDL cholesterol in patients with RA was lower (p < 0.05). Plasma RA patients had significantly higher levels of small dense LDL and lower levels of large light LDL than controls (p < 0.01). LDL affinity (Kd) to GAG were significantly higher in RA patients than in controls (p < 0.05). No differences were observed in vitamins and among the different phases of the dienes formation. In RA patients, a negative correlation between vitamins A and E and sPLA2 (p < 0.01), as well as between vitamins and CRP (p < 0.01), was observed.

A positive correlation between Kd LDL and LP (p < 0.05) in RA patients was observed.

Conclusion These results suggest that inflammation influences the vitaminic status in RA patients. This alteration combined with the presence of atherogenic small LDL with high affinity for GAG may contribute synergistically to the higher risk for atherosclerotic CVD reported in RA patients.

THU0123 APOPTOSIS MARKERS IN RHEUMATOID ARTHRITIS

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