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 spondyloarthropathy (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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10.1136/annrheumdis-2001.998

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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10.1136/annrheumdis-2001.999

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 sinergistically to the higher risk for atherosclerotic CVD reported in RA patients.

THU0123 APOPTOSIS MARKERS IN RHEUMATOID ARTHRITIS

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10.1136/annrheumdis-2001.1000

Background Disregulated apoptosis of T lymphocytes was postulated as one of the potential pathomechanisms of rheumatoid arthritis. In peripheral blood of RA patients atypical clones of T cells were identified, overexpressing Bcl-2 protein and apoptosis resistant. Experimental treatment inducing apoptosis of joint infiltrating T cells and synovial fibroblasts resulted in disease remission in animal models.

Objectives The objective of the study was to examine the expression of apoptosis regulatory proteins (p53, Bcl-2, Bax and Fas) in peripheral blood and synovial fluid lymphocytes as well as sFas concentrations in patients with RA and to compare it with expression in other arthritides. We analyzed the correlation with disease clinical characteristics and treatment. The other aim was to determine the relation of those markers expression in blood and synovial fluid lymphocytes from the same patients.

Methods Study covered 77 RA patients, 18 OA patients 10 patients with other arthritides and healthy control group. Immunocytochemical analysis was performed with indirect immunoperoxidase test. sFas concentration was measured with ELISA test.

Results In RA patients with advanced joint destruction and active disease high overexpression of Bcl-2 was found (over 80% of cells) which correlated to ESR ($r = 0.63$, $p = 0.027$). Bcl-2 expression was significantly higher in RA patients with limited disease than in pts with extra articular disease ($p = 0.02$). No significant difference in other apoptosis markers expression among examined groups was found. We did not observe its relation to disease duration, RF seropositivity, clinical stage or treatment. Serum sFas concentration was significantly higher in extra articular RA ($p = 0.009$).

Conclusion The above results may indirectly suggest the role of lymphocyte apoptosis defect in RA pathogenesis, which demands further investigation.

THU0124 AUTOLOGOUS FIBROBLASTS AS VECTORS FOR ANTI-INFLAMMATORY GENE THERAPY IN MURINE COLLAGEN-INDUCED ARTHRITIS

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10.1136/annrheumdis-2001.1001

Background No treatments effective in the long term are available for rheumatoid arthritis.

Objectives Recent advances in gene therapy and cell therapy have prompted us to design for collagen-induced arthritis (CIA), a new treatment strategy involving injection of autologous fibroblasts transfected with the IL-4 gene using a nonviral method.

Methods A line of immortalised fibroblasts from DBA/1 mice (DBA/1/0 cells) were transfected with a plasmid expressing IL-4 cDNA (DBA/1/IL-4 cells). Xenogeneic fibroblasts from Chinese hamster ovary (CHO) transfected with a plasmid expressing murine IL-4 cDNA were studied also. CIA was caused in DBA/1 mice by injecting bovine type II collagen (CIIB). The cells were injected into the subcutaneous tissue of the back 10 and 25 days after immunisation of the mice by 3. 106 DBA/1/0 or DBA/1/IL-4 or CHO/IL-4 cells.

Results Injection of DBA/1/IL-4 cells was associated with a significant and lasting improvement in the clinical and histological evidence of joint inflammation and destruction as compared with DBA/1/0 cells and CHO/IL-4 cells. The mice injected with DBA/1/IL-4 cells showed decreases in the production of IgG2a

antibody to CIIB and in the proliferation of CIIB-specific nodal T cells.

Conclusion Taken in aggregate, these findings indicate that grafting autologous cells transfected with a gene encoding an anti-inflammatory cytokine is effective in CIA and may attenuate the cytokine imbalance seen in this disease.

Rheumatoid Arthritis – Clinical aspects

AB0037 COMPLICATED RA COURSE WITH ONSET IN ELDERLY AGE

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10.1136/annrheumdis-2001.1002

Background

Objectives

Methods Disease course in 173 RA pts with onset in age older than 50 was analysed. It was demonstrated that in 101 pts (M:F = 12:89, median onset 58.6 y/o, median disease duration up to complication – 6.9 yrs, median age of last examination 67.5, median observation period 7.5) the following complications developed: aseptic bone necrosis, secondary amyloidosis, expressed osteoporosis with osteoporotic bone fractures and compression of vertebral bodies.

Results Systemic manifestations (rheumatoid nodes, internal organs lesions, symptoms of serositis) were determined in 36% of pts, rheumatoid factor in 92%. Vasculopathies including livedo reticularis, capillaritis and trophic ulcers – in 20% of pts.

Aseptic bone necrosis was found in 40 pts (median observation period 10.2 yrs). Their localization: femoral head and/or tectum of cotyloid cavity in 20 pts, subchondral bones of knee joint – in 14, less frequently in wrist, humerus and elbow joints. Repeated aseptic necroses developed in 31 pts.

31 pts had secondary amyloidosis, median disease duration up to diagnosis – 7.6 yrs. This group of pts demonstrated highest inflammatory activity according to clinical and laboratory data. Observation period of pts with already developed amyloidosis was from 2 to 10.5 yrs. 14 pts out of them died during the period of observation.

Developed osteoporosis and compression of vertebrae were observed in 35 pts (median age of fractures 63.8). Radiodensitometry in all pts demonstrated reduced mineral bone density more than 2.5 CD. Localization of skeletal bones fractures (in 24 pts) was as follows: femoral head in 35% of cases, ribs – in 20%, humerus – in 15%, less frequently radius, pelvic bones, feet. Multiple fractures were observed in 40% of cases.

Compressions of vertebral bodies were found in 19 pts, combinations of osteoporotic fractures and vertebral bodies compression – in 9 pts.

Conclusion Thus, follow-up of elderly pts enabled us to select the group of pts with complicated disease course aggravated the prognosis. Reducing of high inflammatory activity of the disease by active therapy will be positive for the disease course, decrease the risk of complications associated with amyloidosis, with diseases activity, aseptic bone necrosis, osteoporosis.

Preventive measures are recommended to decrease osteoporotic complications from the very beginning of the disease.