Objectives The objective was to assess the distribution and possible associations of HLA-DRB1 alleles in the Russian population with long standing rheumatoid arthritis.

Methods Forty four patients (pts) with RA (2 males, 42 females, mean age 53.3 ± 10.85 yr. and mean disease duration 12.9 ± 7.71 yr. and 135 controls were typed for HLA-DRB1 alleles using PCR based molecular techniques. Radiological outcome was measured by scoring X-rays of the hands and feet using the standard radiographs of Larsen (Larsen score). Patients were stratified by RF status and the results were analysed using the Fisher exact test.

Results The frequency of individuals with DRB1*04 ($p < 0.00001$) was significantly higher, DRB1*05 ($p < 0.04$) and *07 ($p < 0.006$) were lower in the pts in compare with the controls. HLA-DRB1*04 proved to be significantly increased in seropositive pts ($p < 0.038$). RF(+) pts with homozygous status for HLA-DRB1*04 ($p < 0.04$) had significantly higher radiological score ($p < 0.05$).

Conclusion Protracted RA in the Russian population is characterized by high frequency HLA-DRB1*04 and low distribution DRB1*05 and DRB1*07. In RF(+) RA pts there was association between the presence DR4 and radiological severity.

AB0035 PLATELET MICROPARTICLES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background Platelet microparticles (PMP) contain pro-coagulant as well as pro-inflammatory substances like platelet activating factor (PAF) and P-selectin. Rheumatoid arthritis (RA) is associated with increased concentrations of both PAF and P-selectin, but the link with PMP has never been investigated.

Objectives To test the hypothesis that patients with RA have elevated levels of PMPs and that these levels are associated with disease activity.

Methods This was a descriptive study in patients with RA, using neither anticoagulants nor corticosteroids. The patients were divided in two groups, according to disease activity. Criteria for active RA were: ESR at least 28 mmh and/or CRP at least 28 mg/dl plus more than 8 painful and more than 5 swollen joints. Criteria for RA in remission were: ESR < 28 mmh and/or CRP < 28 mg/dl plus no painful and no swollen joints. PMPs were determined with flowcytometry (CD61/annexin-V). The values were compared with those from healthy volunteers, using non-parametric tests (Mann-Whitney).

Results Ten patients with active RA and ten patients with RA in remission were included. There were 5 men and 15 women. The mean age was 61 years, the mean disease duration was 6 years and 75% was rheumatoid factor positive (comparable in both groups). The mean number of PMPs in RA patients was $3,173 \times 106/L$ versus $451 \times 106/L$ in healthy volunteers ($p = 0.005$). See Table 1 for further results:

Abstract AB0035 Table 1

	Active RA	RA in remission	Healthy
DAS	6.8 (5.1–7.5)	2.1 (1.3–2.4)	-
PMPs $\times 10^6/L$	2,104 (120–22,441)	504 (121–5,562)	118 (35–1,881)
Mann-Whitney	$p = 0.01$	$p = 0.028$	

Conclusion This study confirms the hypothesis that patients with RA have higher numbers of PMPs; moreover, the presence of PMPs is associated with disease activity. This observation is most probably the missing link between the studies on the PMP content in general and the raised PAF- and P-selectin levels in sera from patients with RA. Moreover, one may speculate on the possible meaning of these elevated PMPs for the well-known high mortality due to cardiovascular disease in patients with RA. More research may clarify this.

AB0036 POLYMORPHISM OF CHOLESTEROL CRYSTALS IN SYNOVIAL FLUID

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Background The aim of the study was to evaluate the frequency of cholesterol crystals occurrence in synovial fluid (SF) in rheumatic diseases as well as characteristics of crystals.

Objectives We have studied 1000 synovial fluid samples from 854 patients. The most numerous was group of rheumatoid arthritis (RA) patients (573 cases) and osteoarthritis (OA).

Methods Synovial fluids were taken on heparin as anticoagulant. SF sediments were examined under polarised microscope.

Results Cholesterol crystals were demonstrated in 16 samples taken from 7 patients with RA only. Most of the crystals occurred as typical large flat rectangular or romboid plates. They ranged in size from 8 to 100 μm and they showed strong birefringence with negative or positive elongation. Less often, they appeared as long rods or curved needle-shaped crystals.

Conclusion The results showed rare occurrence of cholesterol crystals in synovial fluid samples. Polymorphism of cholesterol crystals under polarised microscope may be a cause of difficulties in synovial crystals differentiation.

OP0010 NEUTRALISATION OF IL-18 ACTIVITY IS A DISEASE-MODIFYING THERAPY IN THE MURINE COLLAGEN-INDUCED ARTHRITIS MODEL

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Background Interleukin-18 (IL-18) is a member of the interleukin-1 cytokine family. *In vivo* IL-18 has been demonstrated as promoting the development of a TH1 response in synergy with IL-12. Patients with rheumatoid arthritis (RA) have in their joints significant levels of IL-18 and IL-12.

Objectives This study was aimed at studying the effect of neutralising endogenous IL-18 *in vivo* in collagen-induced arthritic (CIA) mice, to define the therapeutic potentials of IL-18 blockade for the treatment of patients with RA.

Methods Two distinct IL-18 neutralising strategies were used, i. e. a recombinant human IL-18 binding protein (rIL-18BP) and a polyclonal rabbit anti-mouse IL-18 IgG, to treat CIA mice in a

therapeutic protocol (after disease onset). The effect on disease severity (visual scores) as well as parameters of cartilage and bone destruction were evaluated.

Results Clinical scores were significantly reduced after IL-18 blockade (rhIL-18BP 1 mg/kg, $p < 0.001$, $n = 13$; anti-IL18 IgG, 2 mg, $p < 0.05$, $n = 9$, Mann Whitney test, treated versus placebo groups). Histological examination showed cartilage protection (decrease erosion scores, $p < 0.05$) that was accompanied by significantly reduced levels of serum cartilage oligomeric matrix protein (an indicator of cartilage turnover) and VDIPEN expression (a neoepitope present after digestion by matrix metalloproteinases). X-ray analysis of joints provided evidence of reduced bone erosion.

Conclusion These results clearly demonstrate that neutralising endogenous IL-18 is therapeutically efficacious in the CIA model and support the use of IL-18 neutralisation as a novel cartilage and bone sparing therapy for the treatment of destructive arthritis. Recombinant hIL-18BP could therefore represent a new disease-modifying anti-rheumatic drug that warrants testing in clinical trials in patients with rheumatoid arthritis.

OP0018 EARLY TREATMENT OF RECENT-ONSET RHEUMATOID ARTHRITIS PATIENTS IMPAIRS THE EFFECT OF HLA CLASS II ANTIGENS ON THE PROGRESSION OF JOINT DESTRUCTION

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Background HLA class II antigens influence disease progression as measured by extend of joint destruction in RA. This effect is supposed to be caused by formation of auto reactive T-cells after presentation of (auto) antigens in the context of HLA class II.

Objectives To investigate whether in patients with recent-onset RA early DMARD treatment could prevent the involvement of autoreactive T cells, we analysed the association of HLA class II and joint damage in 110 patients with early RA that were treated according to the pyramid strategy with DMARDs and 98 patients with early RA that were promptly treated with DMARDs at two weeks after the first visit.

Methods DNA isolation, DRB1 typing and subtyping and DQB1 typing were performed. Extend of joint damage was measured by the modified Sharp score of the radiographs of hand and feet.

Results In the early treatment group the increase in the median Sharp score in the SE+ group was 1.0 to 5.0 in contrast to 1.0 to 3.0 in the SE- group ($P > 0.5$). However, in the pyramid group the increase in joint damage was 0.0 to 16.0 in the SE+ group in contrast to 0.0 to 5.0 in the SE- group ($p < 0.005$). On the other hand, in the RF+ group of the early treatment group the increase in joint damage was 1.0 to 6.5 in contrast to 1.0 to 1.0 in the RF- group ($p < 0.005$). In the pyramid group the increase in joint damage was 0.5 to 17.0 in the RF+ in contrast to 0.0 to 3.0 in the RF- group ($p < 0.005$).

Conclusion This study shows that early anti-rheumatic drug treatment abolishes the effect of HLA class II alleles on extend of joint damage in early RA patients.

OP0019 ENDOGENOUS DNA AND ARTHRITIS

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Background Recent studies have shown that DNA, in addition to functioning as hereditary material, is able to activate the immune system. In particular, bacterial DNA displays *in vivo* and *in vitro* immunostimulatory properties. These properties have been attributed to certain unique properties of bacterial DNA including high content of unmethylated cytosines in CpG dinucleotides. Although eukaryotic nuclear DNA is not immunostimulatory, we have found that mitochondrial DNA (mtDNA), like bacterial DNA, can activate inflammation.

Objectives The aims of our studies were to assess the arthritogenicity in mice of endogenous mtDNA sequences and to assess a potential role in human inflammatory disease.

Methods Highly purified mtDNA as well as synthetically produced oligonucleotides were injected intra-articularly into murine knee joints. The development of arthritis was evaluated histopathologically. In addition, we analysed by PCR the synovial fluids (SF) of rheumatoid arthritis (RA) patients for the presence of free mtDNA.

Results Intra-articular injection of mtDNA and fragments of the mitochondrial genome containing unmethylated CpG sequences gave rise to arthritis in mice. This inflammation was mediated by macrophages and their products, such as TNF-alpha. In contrast, T/B lymphocytes or granulocytes did not participate in the onset or progression of the disease. The SF of 40/54 RA patients tested contained extracellular, PCR-amplifiable mtDNA.

Conclusion Our results indicate that endogenous mtDNA is pro-inflammatory and may participate in the induction and/or propagation of arthritis in man.

OP0020 TUMOUR NECROSIS FACTOR ALPHA ACTS DIRECTLY ON OSTEOCLAST FORMATION BY SYNOVIAL MACROPHAGES AND MONOCYTES IN RHEUMATOID ARTHRITIS

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Background Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology that results in progressive destruction of bone and cartilage in affected joints. It has been reported that in mouse bone marrow cell cultures TNF-alpha directly induces osteoclast formation and IL-1alpha stimulates osteoclast activation, and that this osteoclast formation and activation is independent of osteoprotegerin ligand (OPGL) signalling system.^{1,2}

Objectives TNF-alpha and IL-1alpha considered master cytokines in the process of RA but little is known about the direct effects of these cytokines on development of human osteoclasts. This study aims to determine the role of TNF-alpha and IL-1alpha, on synovial macrophage- and monocyte-osteoclast differentiation in RA.

Methods Macrophages and monocytes isolated from RA patients were cultured with TNF-alpha and/or IL-1alpha on glass coverslips and dentine slices in the presence of M-CSF. Anti-TNF-alpha, anti-IL-1alpha and osteoprotegerin (OPG) was added to this cultures. Cultures were assessed for cytochemical and functional evidence of osteoclast differentiation.