current and future pharmacological interventions of the prostaglandin pathway. The strong suppressive effect on SP in vagus projected areas reveals the importance of CAP in complex brain networks.

A2.8 ENHANCED NEUTROPHIL EXTRACELLULAR TRAP FORMATION IN RHEUMATOID ARTHRITIS PATIENTS IS CORRELATED WITH HIGH LEVELS OF RHEUMATOID FACTOR (RF)

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Background/Objectives Neutrophils are the most abundant cell type identified in joints from patients with rheumatoid arthritis (RA), with a key role in inflammation and cartilage damage. Activated neutrophils form extracellular traps (NETs) with potent pro-inflammatory and immunostimulatory activity. Consequently, we sought to assess the role of NET release (NETosis) in RA pathogenesis and whether RA specific autoantibodies (rheumatoid factor [RF]) are correlated to this phenomenon.

Materials/Methods Peripheral blood neutrophils were isolated from active RA patients (n = 6) (Disease activity score, DAS28 > 5.1) and healthy control subjects (n = 7). NET formation from neutrophils, both spontaneous and following incubation with RA serum (n = 7) or synovial fluid (n = 7), was assessed by immunofluorescence microscopy, using co-staining with myeloperoxidase and 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI). The percentage of NET releasing cells was determined by examining 200 cells per sample in a double blind fashion. Extracellular DNA content was quantified by fluorescence spectrometry (picogreen) and NET fold increase was calculated based on the extracellular DNA content produced by healthy unstimulated neutrophils.

Results Freshly isolated neutrophils from the peripheral blood of RA patients underwent spontaneous NETosis at higher rates compared to healthy controls ($12 \pm 2.1\%$ versus $3.2 \pm 0.9\%$, $p < 0.05$). Notably, neutrophils isolated from RA synovial fluid exhibited even higher rates of NETosis. Incubation of healthy neutrophils with RA serum or synovial fluid induced NET release compared to the effect of normal serum ($16 \pm 2.5\%$ and $9 \pm 1.5\%$, versus $3.2 \pm 0.7\%$ $p < 0.005$). Moreover, quantification of the extracellular DNA content revealed that neutrophils from RF positive RA patients (n = 6) exhibited significantly increased spontaneous NET formation compared to RF negative patients (n = 8) (542 ± 115.2 ng/ml versus 199 ± 52.84 ng/ml, $p = 0.008$). Treatment of healthy neutrophils with either serum or synovial fluid derived from RF positive RA patients increased NETs compared to cells treated with normal serum (1.45 ± 0.19 and 4.85 ± 1.5 fold increase, $p < 0.05$). Inhibition studies are in progress to address the role of inflammatory cytokines (TNF- α , IL-6, IL-1b) and immune complexes in RA NETosis. Preliminary data show that NET induction in healthy neutrophils upon treatment with RA serum/synovial fluid may be mediated through Fc γ receptors.

Conclusions We found that neutrophils from RA patients have enhanced NET formation, driven by soluble factors found in RA sera and synovial fluid, and this is associated with presence of RF. Further studies will address whether NETs are involved in the initiation of adaptive immune responses in humans and in mouse model of arthritis, and whether suppression of NETosis may ameliorate arthritis in RA mouse models.

A2.9 HIGH SERUM-CHOLESTEROL LEVELS BY EITHER LOW DENSITY LIPOPROTEIN RECEPTOR DEFICIENCY OR A CHOLESTEROL-RICH DIET RESULT IN SYNOVIAL ACTIVATION AND OSTEOPHYTE FORMATION DURING EXPERIMENTAL OSTEOARTHRITIS

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Background and Objectives Atherosclerotic studies show that scavenger receptors on macrophages are capable of taking up oxidised low density lipoprotein (oxLDL), resulting in increased inflammatory properties of the macrophage. Accumulated LDL can be oxidised in an inflammatory milieu such as OA, possibly resulting in oxLDL uptake of synovial macrophages. We investigated whether increased LDL levels lead to more severe OA pathology in experimental induced OA.

Materials and Methods LDL receptor deficient (LDLR^{-/-}) mice and their wild type (WT) controls received either a cholesterol-rich or control diet for 120 days. Experimental OA was induced by intra-articular injection of collagenase. 36 days after OA induction, mice were sacrificed and total knee joints and serum were collected. Bone marrow derived cells were differentiated into type two macrophages and pre-incubated with oxLDL for 24 hours and stimulated with S100A8. RNA was analysed for gene expression. Data are depicted as mean $\pm$ SEM.

Results WT mice receiving a normal diet developed moderate cartilage destruction (6.1 ± 1.5), synovial thickening (1.4 ± 0.2) and osteophyte formation ($32.4 \mu\text{m}^2 \pm 14.6$). Serum LDL levels were significantly higher in LDLR^{-/-} mice compared to WT mice (7.33 mmol/L ± 0.46 and 0.54 mmol/L ± 0.04 respectively; $p < 0.05$), which was additionally increased by a cholesterol-rich diet (38.73 mmol/L ± 3.11 ; $p < 0.0001$). Despite differences in serum LDL levels, no significant differences between the four groups were found regarding synovial thickening and cartilage destruction. Expression of S100A8 by the synovial lining, however, was increased after receiving a cholesterol-rich diet, suggesting synovial activation. Furthermore, a cholesterol-rich diet increased ApoB accumulation in synovial lining macrophages of LDLR^{-/-} mice. Interestingly, at the tibial plateau, LDLR^{-/-} mice showed almost a fourfold increase of osteophyte formation compared to WT mice ($206.3 \mu\text{m}^2 \pm 36.3$; $p < 0.05$). When receiving a cholesterol-rich diet, osteophyte formation at the lateral side of the tibial plateau in LDLR^{-/-} mice further increased from $107.0 \mu\text{m}^2 \pm 49.3$ to $309.4 \mu\text{m}^2 \pm 41.7$ ($p < 0.05$). In vitro stimulation of oxLDL-laden macrophages with S100A8 showed a significant decrease of IL-10 expression and an increase of BMP6 expression compared to macrophages that were not pre-incubated with oxLDL.

Conclusions Increased serum cholesterol levels by either LDL receptor deficiency or a cholesterol-rich diet increase oxLDL uptake by synovial lining macrophages and synovial activation. In accordance with in vitro data, this synovial activation by oxLDL leads to an inflammatory milieu with increased S100A8 levels, resulting in increased osteophyte formation.

A2.10 INCREASED IMMUNOLOGIC EXPOSURE TO NECROTIC CELL REMNANTS IN PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME OWING TO DEFECTIVE DNASE-I ACTIVITY AND THE PRESENCE OF OPSONIZING IGG AUTOANTIBODIES IN SERUM

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Background and Objectives Our recent experiments have suggested that similarly to SLE, patients with primary Sjögren's syndrome (SS) manifest significantly increased phagocytosis of necrotic cell debris (secondary necrotic cell material, SNEC). This phenomenon has been attributed to serological aberrations of these patients, as indicated by the capacity of patients' sera to promote the uptake of SNEC by healthy phagocytes. In this study, we comparatively investigated the role of serum DNase-I activity and IgG immunoglobulins from SS, SLE and RA patients in the promotion of SNEC-phagocytosis by healthy monocytes.

Materials and Methods The activity of DNase-I was assessed by single radial enzyme-diffusion assay (SRED) in the serum of patients with SS (n = 60), SLE (n = 22) and RA (n = 14) and healthy donors (HBD, n = 52). Total IgG immunoglobulins were isolated by negative selection from the serum of patients and controls using Melon Gel Resin columns. SNEC were prepared by heat-induced necrosis of normal lymphocytes and labelling with propidium iodide. The influence of serum components on SNEC-phagocytosis was assessed by flow cytometry in admixture experiments using normal phagocytes and SNEC pre-incubated with whole sera or purified serum IgG from patients or HBD.

Results Serum DNase-I activity in patients with SS and SLE was found significantly reduced compared to HBD and RA patients ($p < 0.0001$) and correlated inversely with the ability of these sera to promote SNEC-phagocytosis by healthy monocytes ($p = 0.0003$). The capacity of HBD sera to promote SNEC-phagocytosis by normal monocytes was significantly increased (by 90%) following the addition of the DNase-I-specific inhibitor G-actin (800 µg/ml), supporting the important physiological role of DNA degradation by serum DNase-I in the prevention of SNEC-phagocytosis. SNEC opsonised with IgG isolated from autoimmune patients or from HBD were found to be similarly ingested by normal monocytes. However, in the presence of normal serum, the opsonisation of SNEC with IgG isolated from SS or SLE sera was found to induce significantly increased SNEC-phagocytosis, compared to that observed with SNEC opsonised with IgG isolated from HBD sera ($p = 0.001$).

Conclusions Our results indicate that, in a manner similar to SLE, SS patients are characterised by deficient serum DNase-I activity. Such reduced serum capacity for degradation of nucleic acids, in conjunction with the opsonisation of SNEC by serum autoantibodies appears to lead to increased exposure of the immune system of these patients to necrotic cell debris, to enhanced SNEC-phagocytosis and consequently to the inflammatory responses that characterise the disorder.

A2.11 INVOLVEMENT OF THE NUCLEIC ACID RECOGNISING TOLL-LIKE RECEPTORS TLR7 AND TLR9 IN THE PATHOGENESIS OF EROSIVE ARTHRITIS

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Background There is substantial evidence that abundant release and/or insufficient removal of endogenous nucleic acids can trigger autoimmune reactions via activation of nucleic acid recognising Toll-like receptors (TLR) such as TLR7 and TLR9, which may lead to the development of SLE and other systemic autoimmune diseases. However, in RA the involvement of these TLRs is less well understood. Interestingly, in rats with pristane-induced arthritis (PIA) disease can be transferred by T cells together with antigen-presenting-cells pre-activated with TLR7 or TLR9 agonists (Hoffmann *et al*, J Autoimmunity 2011; 36: 288–300).

Objective We aimed to study the role of TLR7 and TLR9 in the pathogenesis of inflammatory erosive arthritis by antagonising them in PIA and the KRN serum transfer model.

Methods Arthritis was induced in rats with the mineral oil pristane, and in C57Bl/6 mice by injection of KRN serum. Immunoregulatory oligodeoxynucleotide (ODN) sequences (IRS) antagonising TLR7 or TLR9 were applied either subcutaneously (PIA) or intra-peritoneally (KRN). A non-inhibitory ODN was used as control and PBS served as placebo. Arthritis was scored using established scoring systems, inflammation and bone erosion were quantitatively analysed by histology. Serum and cell culture cytokine levels were measured by ELISA.

Results While the TLR7 inhibitor and the control ODN showed no effect on arthritis development and severity, the TLR9 antagonist reduced arthritis severity significantly in PIA. Bone erosion was almost completely abolished, whereas it was moderately aggravated in animals treated with the TLR7 inhibitor. Furthermore, IL-6 serum levels were significantly reduced in animals treated with the TLR9 antagonist. However, these beneficial effects were only observed when the inhibitor was applied before disease onset. Moreover, neither inhibitor affected arthritis onset and severity in the serum transfer model, which is independent of the adaptive immune system.

Summary and Conclusions Inhibition of TLR9 significantly reduced inflammation and bone erosion in PIA but not in the KRN serum transfer model that reflects the late effector phase of erosive arthritis. Therefore, these results suggest important involvement of the DNA (CpG) recognising TLR9 in the initiation of autoimmune arthritis whereas nucleic acid binding TLRs do not seem to play a major role in the later phases of the disease. Antagonizing TLR9 in human RA may only act beneficial in the earliest phase of the disease.

A2.12 PHENOTYPE CHANGES OF BLOOD EOSINOPHILS REFLECT ACTIVITY AND SEVERITY IN SYSTEMIC SCLEROSIS

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Background and Objectives We investigated whether surface marker expression analysis of blood eosinophils may reflect disease activity in systemic sclerosis (SSc), since a role for eosinophils in the pathogenesis of SSc may be suggested from the observation of increased counts in bronchoalveolar lavage.

Materials and Methods By flow cytometer analysis, eosinophils in whole blood were identified in 32 consecutive untreated patients using surface marker CD16 and CD9. Surface expression of markers CD11b, CD44, CD48, CD54, CD81 and HLA-DR was measured. Data were related to clinical measurements of the disease activity.

Results An increased blood eosinophil population with low surface expression of CD9 was identified in patients with early SSc, i.e. a disease duration of <2 years, compared to patients with longer disease duration ($P = 0.003$) and controls ($P = 0.029$). CD81 expression was lower in SSc patients compared to healthy individuals ($P = 0.003$). In patients with early SSc, CD81 levels correlated inversely to degree of skin involvement ($r = -0.67$; $P = 0.009$). CD48 levels were increased in early SSc and were associated with an increased concentration of alveolar nitric oxide in these patients ($r = 0.84$; $P < 0.001$). CD16 expression on blood eosinophils was also higher in patients with early disease and was associated with loss of nailfold capillaries ($r = -0.78$; $P < 0.001$).