current and future pharmacological interventions of the prostaglan-
din 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 (pico green) 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 ± 2.1% versus 3.2 ± 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 ± 2.5% and 9 ± 1.5%, versus 3.2 ± 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) [54 ± 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-a, 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 Fcγ 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 initia-
tion 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 OSTEOCYTE FORMATION DURING 
EXPERIMENTAL OSTEOARTHITIS

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Background and Objectives Atherosclerotic studies show that scavenger receptors on macrophages are capable of taking up ox-
dized low density lipoprotein (oxLDL), resulting in increased inflam-
matory 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 experi-
mental 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 ± 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 μm2 ± 14.6). Serum LDL levels were significantly higher in LDLr−/− mice compared to WT mice (7.35 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 μm2 ± 36.3; p < 0.05). When receiving a cholesterol-rich diet, osteophyte forma-
tion at the lateral side of the tibial plateau in LDLr−/− mice further increased from 107.0 μm2 ± 49.3 to 309.4 μm2 ± 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 accord-
ance 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 sug-
gested that similarly to SLE, patients with primary Sjögren's syn-
drome (SS) manifest significantly increased phagocytosis of necrotic
cell debris (secondary necrotic cell material, SNEC). This pheno-
momen 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 com-
paratively 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 nega-
tive 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 phago-
cytes 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 = 0.0008).
The capacity of HBD sera to promote SNEC-phagocytosis by nor-
mal monocytes was significantly increased (by 90%) following the
addition of the DNAse-I-specific inhibitor G-actin (800 µg/ml), sup-
porting 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 autoanti-
bodies appears to lead to increased exposure of the immune system
to these patients to necrotic cell debris, to enhanced SNEC-
phagocytosis and consequently to the inflammatory responses that
characterise the disorder.

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. Immuno-
regulatory oligodeoxynucleotide (ODN) sequences (IR5) antagonis-
ting TLR7 or TLR9 were applied either subcutaneously (PIA) or
intra-peritoneally (KRN). A non-inhibitory ODN was used as con-
trol and PBS served as placebo. Arthritis was scored using estab-
lished scoring systems, inflammation and bone erosion were
quantitatively analysed by histology. Serum and cell culture cyto-
osome levels were measured by ELISA.

Results While the TLR7 inhibitor and the control ODN showed
no effect on arthritis development and severity, the TLR9 antagon-
ist 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.