antigen presenting cells and are therefore implicated in the initiation of chronic autoimmune diseases, including rheumatoid arthritis. Using the K/BxN serum transfer arthritis, a model of human rheumatoid arthritis, which depends only on the innate immune system, allowed us to investigate the innate role role of dendritic cells in inflammatory arthritis.

Methods KBxN serum transfer arthritis was induced in CD11c-diphteria toxin receptor (DTR) transgenic mice, which express the human diphtheria-toxin receptor under the CD11c promoter. This allows for specific depletion of CD11c+ cells by administration of diphtheria toxin (DT). DT or PBS were given on day -1, 3, 6 and 9 and the severity of arthritis was determined clinically and histologically. In addition, serum transfer arthritis was induced in wild type animals who also received DT.

Results Efficient depletion of DCs from the spleen after injection of DT was confirmed by flow cytometry and histological analysis. Clinical scores of arthritis showed that CD11c-DTR transgenic mice had significantly reduced paw swelling and loss of grip strength compared to PBS treated animals. In contrast, wild type animals receiving DT showed identical clinical signs of arthritis as PBS treated animals, excluding unspecific effects of DT in mice. Histological analysis found that CD11c-DTR transgenic mice that had received DT displayed decreased synovial inflammation and a trend towards reduced local bone destruction.

Conclusions These data show that dendritic cells are involved in innate reactions leading to inflammatory arthritis and suggest that dendritic cells could be an important target for rheumatoid arthritis therapy.

A2.6 CELL-SPECIFIC TYPE I IFN SIGNATURES IN AUTOIMMUNITY AND VIRAL INFECTION: WHAT MAKES THE DIFFERENCE?

doi:10.1136/annrheumdis-2013-203215.6

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Background Gene expression profiling experiments using peripheral blood mononuclear cells (PBMCs) revealed a crucial role of type I interferon (IFN) in the pathogenesis of systemic lupus erythematosus (SLE). However, it is almost unknown how particular leukocyte subsets contribute to the overall type I IFN signature described for PBMCs. Furthermore, a detailed analysis of how IFN signatures differ in autoimmune disease from that observed after viral infection is missing so far. Therefore, we compared expression levels of 2442 IFN signature genes in peripheral CD4+ T helper cells and monocyte (Mo) subsets isolated from patients with SLE, healthy donors (ND) and ND vaccinated against yellow fever by global gene expression profiling.

Materials and Methods Peripheral blood from 8 patients with SLE and 4 ND were recruited. Same ND were examined before and after immunisation by yellow fever vaccine. After sorting cells, isolated RNA were applied to Affymetrix Human Genome U133 Plus 2.0 Array. Data analysis was done using BioRetis database, Genesis Software and Ingenuity Pathway Analysis (IPA).

Results 98/165/173 probe sets (CD4+ T cells/CD16– inflammatory Mo/CD16+ resident Mo, respectively, fold change ≥2, ≤–2) were detected as a "common" IFN signature observed both in autoimmunity and in immunised ND. 111/164/120 probe sets were detected as an "autoimmune-specific" IFN signature, whereas only 0/8/5 probe sets were detected to be specific for the "virus-induced" IFN signature. Expression pattern of these IFN signature genes clearly distinguished patients with SLE from immunised ND by hierarchical cluster analysis. Although major IFN signature genes were commonly expressed in CD4+ T cells and Mo of patients with SLE and immunised ND, expression magnitudes of them were higher in patients with SLE compared to immunised ND. In SLE, in addition to the typical "viral-induced" IFN signature, genes that are involved in apoptosis signalling, antiviral PKR signalling, Fcγ receptor-mediated phagocytosis and IL-10-/IL-9-/IL-15-mediated JAK/Stat signalling pathways were identified by IPA.

Conclusions This study demonstrated that IFN signature in autoimmunity and that in viral infection are quite different in the number of IFN-related genes activated and their expression magnitudes. Autoimmunity is characterised by a much stronger expression of IFN signature genes and is obviously modulated by a separate set of co-regulated genes defining the "autoimmune-specific" IFN signature. "Common" and "autoimmune-specific" IFN signature genes can be applied as a clinical biomarker to diagnose SLE flare discriminating from viral infection.

A2.7 EFFECTS OF VAGUS NERVE STIMULATION ON THE CENTRAL PROSTAGLANDIN SYSTEM AND SUBSTANCE P FOLLOWING PERIPHERAL INFLAMMATION

doi:10.1136/annrheumdis-2013-203215.7

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Background and Objectives Activation of cholinergic anti-inflammatory pathway (CAP) has shown to be important for regulation of arthritis, and ongoing trials show promising effects of vagus nerve stimulation (VNS) in RA. While peripheral mechanisms have been thoroughly investigated, central effects remain elusive. We showed recently that central nervous inflammation is a feature of RA (Lampa *et al*, PNAS 2012), and also coupled to autonomic activity. Moreover, prostaglandin E2 (PGE_2) may act as an important neuromediator in this context and we have earlier shown impaired CAP in knockout mice for the PGE_2 inducing enzyme mPGES (Le Maître *et al*, EWRR 2012). Here, we aimed to study the effects of VNS on central prostaglandin system and neuropeptides associated with inflammation.

Materials and Methods After VN isolation, we injected lipopolysaccharide (2 mg/kg) intraperitoneally. The VN was either electrically stimulated for 5 minutes (VNS) or left unstimulated (SHAM). After 6 hours, mice were sacrificed and brains were collected. Expression of the inducible enzymes COX2 and mPGES-1 in frozen brain sections was quantified by immunohistochemistry. mRNA levels of c-FOS and substance P (SP), a key central neuropeptide, were analysed by in situ hybridisation. Investigated areas include Hippocampus (Hi), Hypothalamus (Hy), periaqueductal grey (PAG), Cingulate Cortex (CC) and Dorsal raphe nuclei (DRN).

Results c-FOS mRNA level significantly increased in vagus related areas such as Hi (75.3 ± 5.7 (mean grey value; SHAM) versus 105.0 ± 1.7 (VNS); $p < 0.001$) and Hy (73.8 ± 9.4 versus 102.2 ± 6.7; $p < 0.05$). Hi and Hy as well as all other regions displayed a strong trend to VNS-induced increase in mPGES-1 protein, (Hi 0.66 ± 0.29 versus 0.88 ± 0.25 and Hy 0.72 ± 0.44 versus 1.49 ± 0.57). COX2 protein tended to decrease in all areas except CC. Interestingly, VNS exhibited strong inhibitory effects on the SP mRNA expression (Hi 119.9 ± 4.9 versus 98.0 ± 4.2 $p < 0.05$; Hy 114.0 ± 6.5 versus 83.1 ± 8.2; $p < 0.05$).

Conclusions These data indicate a role for prostaglandins and mPGES in central mechanisms of the CAP. The decreased brain COX2 expression may be related to the suppression of systemic inflammation caused by peripheral CAP action, while the up regulation of mPGES-1 in vagus-related brain areas seems to be directly related to central CAP action. These effects may be of clinical importance both in the coming VNS RA trials as well as in the

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)

doi:10.1136/annrheumdis-2013-203215.8

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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 immunofluoresence 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 ± 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) (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-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 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

doi:10.1136/annrheumdis-2013-203215.9

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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 ± 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 μm^2 ± 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 μm^2 ± 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 μm^2 ± 49.3 to 309.4 μm^2 ± 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

doi:10.1136/annrheumdis-2013-203215.10

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