ELISAs were performed in triplicate for IL-6 TIMP-1, and MMP-1 levels. In some experiments cells were pretreated with 100 uM of WRW4, a specific formyl peptide receptor inhibitor with A-SAA. Also we used fibroblasts derived from a patient with a genetic non sense mutation that results in no IRAK-4 protein production and hence a halt in TLR signalling. IRAK-4 is a central downstream mediator of Toll-Like Receptor mediated signalling and is crucial for such signalling.

Results Healthy human dermal fibroblasts incubated with A-SAA secreted high levels of IL-6 compared to untreated control cultures. Moreover A-SAA induced increased levels of TIMP-1 both at the mRNA levels and also the protein levels as determined by ELISA. Levels of the target of TIMP-1, MMP-1 protein levels were not altered at all. Thus the effect leads to a shift in the ratio of TIMP-1 to MMP-1 favouring ECM deposition. Pretreatment with WRW4 prior to A-SAA did not alter IL-6 or TIMP-1 expression levels, showing that the formyl peptide receptor plays no role in TIMP-1 induction mediated by addition of A-SAA. Furthermore cells derived from a gene deleted IRAK-4 patient with no IRAK-4 protein stimulated with A-SAA compared to control fibroblasts had TIMP-1, IL-6 levels compared to non-treated (media alone) dermal fibroblasts.

Conclusions A-SSA induces TIMP-1, but importantly does not alter levels of TIMP-1 target MMP-1, thus shifting the TIMP-1/MMP-1 ratio. IL-6, a classic proinflammatory cytokine involved in SSc pathogenesis, is also elevated by A-SAA treatment in vitro. The signalling involved IRAK-4, a critical downstream messenger of TLR mediated signalling, but not formyl peptide receptors.

A2.3 ANTI-APOPTOTIC IGG ANTIBODIES FROM PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS INHIBIT THE PHAGOCYTOSIS OF APOPTOTIC CELLS

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Background and Objectives Recent studies in our laboratory have revealed that a significant portion of SS patients manifests significantly impaired phagocytosis of apoptotic cells (ApoCell-phagocytosis), in a manner similar to SLE, a fact which probably leads to the inflammatory and autoimmune responses that characterise these two disorders. Furthermore, our data indicate that approximately 80% of sera from patients with SS and SLE, but not those with RA and healthy individuals, inhibit the clearance of early apoptotic cells from healthy monocytes. In the present study, we sought to investigate the role of IgG immunoglobulins from patients with SLE and SS in the phagocytosis of apoptotic cells.

Materials and Methods Total IgG immunoglobulin was isolated from the serum of patients with SS (n = 24), SLE (n = 12) and RA (n = 8) and healthy donors (n = 11) using Melon Gel Resin columns. Apoptotic Jurkat cells (induction of apoptosis by UV-radiation) were incubated with purified IgG (50 μg/ml) in PBS/BSA 1%. The binding of IgG on the surface of apoptotic cells was assessed by flow cytometry and analysed with the binding index (% positive cells × mean fluorescence intensity). ApoCell-phagocytosis was assessed by flow cytometry using monocyte-derived macrophages (MDM) from healthy individuals (n = 3) that were incubated (90 min) with CFSE-labelled early apoptotic Jurkat cells which had been opsonised with IgG from patients or healthy individuals or with PBS (control).

Results IgG from patients with SS and SLE exhibited high binding levels in apoptotic cells compared to healthy donors and patients with RA (all for $p < 0.0001$). Pre-incubation of apoptotic cells with IgG from patients with both SS and SLE had a significant inhibitory effect on the phagocytosis process (comparative ApoCell-phagocytosis: after pre-incubation with PBS [x = 100%], with IgG from healthy individuals [n = 11, x = 100%], with IgG from RA patients [n = 4, x = 100%], with IgG from SS patients [n = 17, x = 75%, $p < 0.0001$], with IgG from SLE patients [n = 10, x = 71%, $p = 0.0002$]). Finally, negative correlation was found between the apoptotic cell binding levels of IgG from patients and healthy individuals and the results of ApoCell-phagocytosis assays using apoptotic cells pre-incubated with the corresponding IgG (n = 42, $p < 0.0001$).

Conclusions Our results indicate that the impaired phagocytosis of apoptotic cells observed in patients with SS and SLE is primarily due to the presence of antibodies reactive with the surface of apoptotic cells. Further studies need to focus on the mechanism by which immunoglobulins exert such blocking effect, as well as its immunological consequences.

A2.4 ASSOCIATION OF CD55 WITH RETICULAR FIBRES IN THE SYNOVIAL LINING IN RHEUMATOID ARTHRITIS

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Background and Objectives CD55 (decay-accelerating factor) is a complement-regulating protein, expressed at unusually large amounts by fibroblast-like synoviocytes (FLS) in the intimal lining in rheumatoid arthritis (RA). CD55 is also a ligand for CD97, an adhesion-type G protein-coupled receptor, broadly expressed by immune cells invading the inflamed synovium. We previously reported a protective effect of lack of CD55 in experimental models of RA (Hoek *et al*, Arthritis Rheum 2010; 62(4): 1036–42). Current data cannot explain the high expression of CD55 by FLS. Therefore, we explored in detail the pattern of CD55 expression in RA synovial tissue and 3-D organ cultures.

Materials and Methods Synovial tissue was obtained by miniarthroscopy and analysed by immunohistochemistry staining to visualise CD55 and collagen type III, a constituent of extracellular matrix. Reticular fibres were visualised with Gomori silver staining. Expression of CD55 mRNA in synovial tissue was detected using antisense locked nucleic acid (LNA) oligomers. CD55 expression on 2-D-cultured RA-FLS and on blood cells of healthy individuals was detected by flow cytometry and related to mRNA levels measured by qPCR. 3-D micromasses of RA-FLS were generated in matrigel and analysed by immunohistochemistry for CD55 and reticular fibres.

Results CD55 was highly expressed in the synovial lining of RA tissue, both at the mRNA and the protein level. Notably, CD55 showed an extracellular staining pattern, which coincided with Gomori silver staining of reticular fibres on sequential sections. CD55 expression on 2D-cultured FLS was less abundant and comparable to PBMCs. In 3D-micromasses of RA-FLS, CD55 was upregulated after 3–4 weeks of culture and showed an extracellular distribution that resembled reticular fibres.

Conclusions CD55 mRNA and protein is abundantly expressed by FLS in the intimal lining of RA synovial tissue. We provide evidence that FLS-derived CD55 is deposited in extracellular matrix structures such as reticular fibres, where it may contribute to the synovial stromal address code that facilitates the recruitment of immune cells.

A2.5 CD11c+ DENDRITIC CELLS PLAY AN IMPORTANT PROINFLAMMATORY ROLE IN INFLAMMATORY ARTHRITIS

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Backround Dendritic cells (DCs) play an important role in bridging the innate and the adaptive immune response by serving as

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?

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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

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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