A2.18 THE EXPRESSION OF THE TRANSCRIPTION FACTOR PPAR-GAMMA IS SIGNIFICANTLY REDUCED IN THE SALIVARY GLAND EPITHELIAL CELLS FROM PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Background and Objectives PPAR-gamma is an essential transcription factor that apart from participating in the regulation of genes associated with lipogenesis, it exerts significant anti-inflammatory actions. PPAR-gamma has been implicated in the pathogenesis of human autoimmune diseases that are characterised by the inflammatory damage of epithelial cells. More specifically, dramatically reduced expression of PPAR-gamma has been shown in the epithelial cells of the intestinal tract and of the biliary tree of patients with ulcerative colitis and primary biliary cirrhosis, respectively. Primary Sjögren's syndrome (SS or autoimmune epithelitis) is characterised by chronic inflammatory lesions mainly affecting epithelial tissues and is associated with systemic autoimmune responses and the chronic intrinsic activation of salivary gland epithelial cells (SGEC). Thus, SGEC are probably both the target and the inducer of inflammatory responses. In this context, we aimed to investigate the levels of constitutive expression of PPAR-gamma in cultured non-neoplastic SGEC lines from SS patients and non-SS controls, as well as the patterns of PPAR-gamma expression following cellular activation.

Materials and Methods To examine the levels of mRNA expression of PPAR-g, total RNA was isolated from long-term cultured non-neoplastic SGEC and from peripheral blood mononuclear cells (PBMCs) of 18 SS patients and 11 non-SS disease controls. The expression of PPAR-gamma was studied by Real-time PCR with primers specific for PPAR-gamma and the reporter gene HPRT1 and analysed by the ddCt method. To evaluate the effect of epithelial activation in the expression of PPAR-g, SGEC from non-SS controls were stimulated with specific ligands of TLR-3 (Polyinosinic-polycytidylic acid, PolyI:C, 5 mg/ml), TLR-4 (lipopolysaccharide, LPS, 1 mg/ml) receptor and with the cytokine IFN-gamma (500 U/ml).

Results PPAR-gamma mRNA expression was significantly reduced in the SGEC from patients with SS, compared to controls ($p = 0.0001$). In contrast, no difference was found in PPAR-gamma expression in PBMCs between patients and controls. The activation of cultured SGEC by stimulation with PolyI:C, LPS and IFN-g resulted in a significant down-regulation of PPAR-gamma mRNA expression (in all cases; by ≈80% at 12 hours, $p = 0.0001$).

Conclusions Our findings indicate that the expression of PPAR-gamma in human epithelial cells is significantly reduced following activation via TLRs and the Th1 cytokine INF-gamma. Furthermore, the present study demonstrates for the first time the significantly reduced expression of PPAR-gamma in the SGEC of SS patients. This finding likely owes to the chronic intrinsic activation, which characterises the epithelia of SS patients.

A2.19 THE ROLE OF microRNA 155 IN INNATE IMMUNITY AND ARTHRITIS

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Background MicroRNA 155 (miR155) has been demonstrated to be essential for the development of collagen induced arthritis by controlling the generation of autoreactive T and B cells. However, the contribution of miR155 in innate immune cells is not known.

Materials and Methods We analysed activation and cytokine production of macrophages and dendritic cells (DCs) in vitro and in vivo. We analysed T-cells stimulatory capacity of DCs. We crossed miR155 deficient mice into hTNFtg mice and analysed arthritis development clinically as well as histologically.

Results MiR155 deficiency did not alter the expression of costimulatory molecules or MHCII expression after stimulation of macrophages and DCs in vitro and in vivo. We also Facs-sorted DCs after stimulation with LPS in vivo and determined the production of proinflammatory cytokines such as IL-23, IL-6 as well as TNF. We did not detect differences between wt and miR155$^{-/-}$ mice. In addition, the T cell stimulatory capacity of wt and miR155$^{-/-}$ was identical. When we analysed hTNFtg/miR155$^{-/-}$ mice compared to wt mice, we did not detect differences in the clinical signs and symptoms of arthritis. Histologically, we even found slightly increased synovial inflammation in hTNFtg/miR155$^{-/-}$ mice compared to wt mice.

Conclusions In contrast to the pivotal role of miR155 in autoimmunity requiring the adaptive immune system, the role of miR155 in innate immunity seems to be limited. This is emphasised by the fact that miR155 hardly influences the course of TNF-driven arthritis, which is mainly dependent on components of the innate immune system.

A2.20 THE ROLE OF SERUM FACTORS AND TOLL-LIKE RECEPTOR SIGNALING IN THE INDUCTION OF PROFIBROTIC TIMP-1 BY MONOCYTES IN SYSTEMIC SCLEROSIS

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Background and Objectives Systemic sclerosis (SSc) is an autoimmune disease characterised by fibrosis, vascular dysfunction and abnormal activation of immune cells including monocytes. Monocytes along with fibroblasts play an important role in the production of profibrotic factors such as IL-6 and TIMP-1 (tissue-inhibitor of metalloproteinase-1). TIMPs are specific inhibitors of matrix metalloproteinases (MMPs) regulating extracellular matrix (ECM) turnover. Importantly, the balance between TIMPs and MMPs is altered in most pathological stages including SSc and is associated with abnormal ECM formation. However, the exact factors which drive both profibrotic TIMP-1 secretion are not fully defined.

We aim to test whether circulating monocytes from SSc patients produce TIMP-1 in response to TLR activation and serum factors, which contributes to excessive matrix deposition and consequently disease progression.

Materials and Methods 25 patients with SSc, one IRAK4 deficient patient and 20 HC (healthy control) were included in this study. Peripheral blood monocytes were further separated by CD14+ microbeads. Production of TIMP-1, IL-6 by monocytes was determined by ELISA, qRT-PCR or functional assay in response to either panel of conventional TLR agonists or HC and SSc sera. Skin section from SSc patient was stained with CD14+ and TIMP-1 antibodies and further analysed by confocal microscopy.

Results TIMP-1 production by monocytes was observed in the SSc skin section and was upregulated in SSc patients compared to HC. Incubation of HC monocytes with SSc sera resulted in functionally active TIMP-1 production. However, pre-treatment with MyD88 inhibitor, but not control peptide, decreased TIMP-1 secretion. Furthermore, SSc-mediated TIMP-1 induction by monocytes was attenuated when FcγR was blocked and also when SSc sera were treated with DNA/RNA endonuclease prior to stimulation. This indicates that SSc sera contain RNA/DNA agonists inducing TIMP-1 production. Indeed, direct treatment of HC and SSc