P070 ABSTRACT WITHDRAWN

P071 AUTOANTIBODIES AGAINST SERUM AMYLOID A REDUCE IL-6 RELEASE FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

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Introduction Serum amyloid A (SAA) is a sensitive inflammatory marker rapidly increased during the acute phase, followed by a steady decline to physiological levels during resolution. While resolution and SAA reduction have been documented, the exact mechanism remains elusive. Although antibodies against SAA (anti-SAA) have been previously identified in healthy blood donors (HBDs) in smaller, preliminary studies, their potential function is still unclear.^{1,2}

Objectives To detect anti-SAA and anti-SAA1α in the sera of 300 HBDs using ELISA, characterise their subclasses and avidity. Additionally, we aimed to evaluate their presence in intravenous immunoglobulin (IVIG) and potential effects on released IL-6 from SAA-treated peripheral blood mononuclear cells (PBMCs).

Methods An *in-house* ELISA was adapted from Rosenau and Schur¹ and developed² for detection of anti-SAA and anti-SAA1α. Both antibody fractions were isolated from IVIG using MicroLink Protein Coupling Kit (Thermo Scientific). PBMCs were purified from 5 HBDs by density gradient centrifugation and stimulated with SAA or SAA1α (1.5 µg/ml) in the presence/absence of anti-SAA and anti-SAA1α for 5 hours, 37°C. IL-6 concentration was measured in supernatants by ELISA (Invitrogen).

Results The median (IQR) absorbance in HBDs was 0.655 (0.262–1.293) for anti-SAA and 0.493 (0.284–0.713) for anti-SAA1α. Both anti-SAA and anti-SAA1α reached peak levels between 41–50 years and diminished with age, with women exhibiting significantly higher levels than men. Good positive correlation was observed between anti-SAA and anti-SAA1α. Both antibodies were prevalently of the IgG subclass, with heterogeneous to high avidity and were detected also in IVIG. Stimulation of PBMCs with SAA significantly induced IL-6 release (mean ±SD) (389.5±184.4 pg/ml) with levels decreasing significantly upon addition of 4.5 (131.4±44.4 pg/ml) or 9.0 µg/ml (118.1±57.4 pg/ml) anti-SAA. A similar trend was also found for SAA1α and anti-SAA1α.

Conclusions Anti-SAA could play a physiological role in down-regulating proinflammatory activity of SAA and could represent an attractive, novel therapeutic option for patients with chronic inflammatory diseases.

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P072 IRF1 IS CRITICAL FOR THE INFLAMMATORY GENE EXPRESSION IN FIBROBLAST-LIKE SYNOVIOCYTES

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Introduction Fibroblast-like synoviocytes (FLS) are increasingly recognised as major pathogenic cells in synovial inflammation of patients with Rheumatoid Arthritis (RA). In response to pro-inflammatory stimuli, such as TNF, FLS produce vast amounts of cytokines and chemokines that help to recruit and activate immune cells and drive the local inflammatory process. The pathways and transcription factors that determine the inflammatory response in FLS are, however, largely unexplored.

Objectives To investigate the potential contribution of the transcription-factor IRF1 to the inflammatory gene expression in FLS.

Methods Expression of IRF1 in synovial tissue samples (12 RA and 8 osteoarthritis (OA) patients) was assessed by immunohistochemistry (IHC). Moreover, FLS were isolated according to established protocols and cultured using 2-D or 3-D culture techniques. IRF1 expression in response to TNF was determined by western blots, qPCR, immunofluorescence microscopy or IHC. FLS were also stimulated with TNF in the presence or absence of IRF1 siRNA pools. Expression of pro-inflammatory cytokines and chemokines was measured by qPCR.

Results IRF1 expression was significantly increased in rheumatoid synovial tissues as compared to patients with OA on protein level. RA-FLS stimulation with TNF *ex vivo* caused rapid upregulation of IRF1 and proved the involvement of TNF in the regulation of IRF1. Immunofluorescence analysis further revealed that IRF1 was mainly localised in the nucleus of TNF-stimulated FLS. Moreover, also chronic TNF exposure of FLS grown in a 3-D synovial tissue culture system promoted the expression of IRF1. siRNA-mediated knockdown of IRF1 in FLS significantly reduced the TNF-induced expression of pro-inflammatory cytokines and chemokines, such as IL6, CCL7, CXCL11 and TNFSF13B, which confirmed the role of IRF1 as a critical regulator of proinflammatory genes in RA FLS.

Conclusions Our data reveal that IRF1 is crucial for the inflammatory response of FLS and support the idea that IRF1 might be an exciting therapeutic target in patients with RA.

Disclosure of interest None declared

P073 REGULATION OF JOINT DESTRUCTION BY ACTIVIN A IN RHEUMATOID ARTHRITIS

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Introduction Activins and inhibins belong to the transforming growth factor β family. Activins are disulphide-linked homo- or heterodimers consisting of two inhibin β chains (βA, βB) that are expressed in many cell types. However, activin A (βA βA) is the only activin that is expressed in bone and cartilage. Moreover, activin A has been demonstrated not only to stimulate receptor activator of NF-κB ligand (RANKL)-induced