

A10.14 IDENTIFICATION OF NOVEL ACPA TARGETS IN RHEUMATOID ARTHRITIS SYNOVIAL TISSUES USING 2D GEL ELECTROPHORESIS AND MASS SPECTROMETRY

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Background and Objectives Rheumatoid arthritis (RA) is an inflammatory autoimmune disease characterised by synovial joint inflammation and pannus formation that leads to degradation of cartilage and the underlying bone. Presence of anti-citrullinated protein/peptide antibodies (ACPA) in 60–70% of patients with RA is one of the major characteristics of the disease and associates with a more aggressive disease course, suggesting a direct pathogenic involvement of ACPA in disease initiation and progression. ACPA recognises several citrullinated proteins like fibrinogen, α -enolase, vimentin, and collagen II. In this study, we aim for the identification of novel ACPA targets in synovial tissues of patients with RA.

Materials and Methods RA synovial tissues were obtained from patients undergoing joint replacement surgery for rheumatoid arthritis of the knee or elbow at the Karolinska University Hospital, Stockholm, Sweden. Synovial tissues were frozen in liquid nitrogen shortly after resection and stored at -80°C . All procedures were approved by Northern Stockholm Ethical Review Board and tissues were obtained with informed patient consent. Proteins, extracted from pulverised frozen synovial tissues and solubilised in lysis buffer, were resolved in 2D PAGE. Separated proteins were directly transferred to a nitrocellulose membrane and probed with human ACPA pool obtained using CCP2 affinity columns, kindly provided by Euro-Diagnostica, as described previously. [1] Human IgG and CCP2 flow-through fraction were used as control antibodies. Silver stained gel spots, corresponding to WB signals, were extracted from 2D gels, in-gel digested using Lys-C, and resulting peptides were identified using mass spectrometry.

Results By combining 2D gel electrophoresis with mass spectrometry, we have identified several novel potential ACPA targets as well as already characterised proteins. It remains to demonstrate if these proteins are citrullinated.

Conclusions Here we demonstrate an extensive ACPA reactivity against novel proteins in RA synovial membranes. The results encourage further exploration of the role of these proteins/peptides in rheumatoid arthritis both as additional biomarkers as well as their potential roles in the pathogenesis of RA.

Reference

1. Ossipova E, Cerqueira C, Reed E, *et al*, Affinity purification and characterization of anti-citrullinated protein/peptide antibodies from plasma and synovial fluids of patients with rheumatoid arthritis (submitted).

A10.15 IGA RHEUMATOID FACTOR IS MORE PREDOMINANT THAN ANTI-CCP IN SUDANESE RHEUMATOID ARTHRITIS PATIENTS, WHEREAS IGG RF IS A STRONG PROGNOSTIC MARKER AND ASSOCIATED WITH EARLY ONSET

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Background and Objectives The aim was to investigate the diagnostic and prognostic impact of the conventionally used autoantibodies (IgG) anti-CCP and IgM rheumatoid factor (RF) as well as IgA and IgG RF in the first ever collected cohort of Sudanese rheumatoid arthritis (RA) patients.

Materials and Methods 264 consecutive RA patients (87% females) diagnosed according to the 1987 ACR criteria attending two rheumatology centres in Khartoum between December 2008 and September 2010 were included, together with 168 healthy Sudanese blood donor controls. Autoantibody levels were investigated in Uppsala, and RF specificity levels aligned to the anti-CCP specificity (97.6%).

Results Anti-CCP was elevated in 52% (131/252) of the patients, a figure not different from what has been found in Sweden (57%, Rönnelid ARD 2005; $p = 0.2$). Among the Sudanese RA patient, 57.2%, 51% and 49.8% had IgA, IgM and IgG RF, respectively. The areas under the Receiver Operator Characteristics (ROC) curves were 0.94 for anti-CCP, and 0.95, 0.82 and 0.85 for IgA, IgG and IgM RF, respectively.

IgG RF was associated with young age ($p = 0.0005$) and lower age of disease onset ($p < 0.0001$), as well as with higher total number of affected joints ($p = 0.03$). Hand deformities like swan neck deformity ($p = 0.0001$) and boutonnière deformity ($p = 0.02$) were also primarily associated with IgG RF. Association with the other investigated autoantibodies were weaker or absent. The prognostic impact of IgG RF was not secondarily dependent on anti-CCP, as the correlation between anti-CCP was stronger for IgM RF ($r = 0.49$) and IgA RF ($r = 0.31$) than for IgG RF ($r = 0.23$).

Conclusions The occurrence of anti-CCP in Sudanese RA patients does not differ from Sweden. In contrary to what has been found in Caucasian RA populations, IgA RF is a diagnostically more sensitive marker than anti-CCP IgG RF is the strongest marker for bad prognosis, and associated with early disease onset.

A10.16 INFLAMMATORY CYTOKINES DOWNREGULATE FoxO1 BY JNK-DEPENDENT ACCELERATION OF MRNA DEGRADATION TO PROMOTE SURVIVAL AND PROLIFERATION OF RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Background and Objectives Aberrant regulation of proliferation and survival of immune and stromal cells contributes to the pathogenesis of rheumatoid arthritis (RA). Forkhead box O (FoxO) transcription factors integrate extracellular signals to modulate expression of genes regulating cell cycle and apoptosis, and alterations in activity and expression of FoxOs have been reported in several inflammatory diseases, including RA. In this study, we examined the relationships between inflammation and FoxO expression in RA, and analysed the mechanisms and biological consequences of cytokine-mediated regulation of FoxO1 expression in RA fibroblast-like synoviocytes (FLS).

Materials and Methods RNA was isolated from synovial biopsies obtained by arthroscopy from 20 RA patients and expression of FoxO1, FoxO3a, FoxO4 and IL-6 was measured by quantitative PCR (qPCR). FoxO1 DNA binding, FoxO1 expression and mRNA stability were measured by ELISA-based assays and qPCR in RA FLS stimulated with IL-1 β , TNF α , or LPS in the absence or presence of mitogen-activated protein kinase (MAPK) or protein kinase B (PKB) inhibitors. RA FLS were transduced with adenovirus encoding control GFP or constitutively active FoxO1ADA to examine the effects on cell viability and gene expression.

Results In RA synovial tissue expression of FoxO1 negatively correlated with clinical parameters of disease activity: serum C-reactive protein ($R = -0.771$, $P = 0.0008$), erythrocyte sedimentation rate ($R = -0.739$, $P = 0.0003$), and DAS28 ($R = -0.575$, $P = 0.01$), as well as synovial IL-6 mRNA levels ($R = -0.628$, $P = 0.004$). In vitro, RA FLS stimulation with IL-1 β or TNF α caused rapid down-regulation of FoxO1 mRNA levels, followed by reduction of FoxO1 protein expression and DNA binding. This effect was independent of PKB signalling, and was associated with acceleration of FoxO1 mRNA degradation in the presence of IL-1 β . Inhibition of c-Jun N-terminal kinase (JNK), but not other MAPKs, prevented down-regulation of FoxO1 expression and binding by IL-1 β , and blocked IL-1 β -induced reduction of FoxO1 mRNA stability. Overexpression of constitutively active FoxO1 in RA FLS induced apoptosis associated with altered expression of genes regulating cell cycle and apoptosis: BIM and p27^{Kip1} were induced while expression of Bcl-XL was suppressed in cells expressing active FoxO1.

Conclusions Collectively, our findings suggest that suppressed synovial FoxO1 expression is strongly associated with RA pathology and demonstrate that reduction of FoxO1 expression might contribute to perpetuation of inflammation in RA by promoting FLS survival and proliferation. Our data also identify JNK-mediated modulation of FoxO1 mRNA stability as an important mechanism underlying regulation of FoxO1 by inflammatory cytokines.

A10.17 INTERLEUKIN-36 α IS EXPRESSED BY SYNOVIAL PLASMA CELLS AND INDUCES CYTOKINE PRODUCTION IN HUMAN FIBROBLASTS

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Background and Objectives The interleukin (IL)-36 α is a recently described member of the IL-1 cytokine family with pro-inflammatory and clearly pathogenic properties in psoriasis arthritis (PsA). The majority of patients with PsA and rheumatoid arthritis (RA) benefit from cytokine blocking therapies against TNF α ; however, despite novel developments, subgroups of patients do not respond to this therapy. Therefore it is necessary to get a better understanding of the pathogenesis of synovitis in PsA and RA to learn more about the complex cellular interplay and to develop new treatment approaches. Therefore, we wanted to determine the IL-36 α expression in PsA compared to RA and osteoarthritis (OA).

Materials and Methods Synovial tissue obtained from arthritis patients were stained for IL-36 α , IL-36 receptor (IL-36R) and IL-36R antagonist (IL-36Ra) by immunohistochemistry and immunofluorescence. Lysates were tested for IL-36 α by immunoblotting. Synovial fibroblasts (FLS) cultured in the presence of IL-36 α were assessed for cytokine expression by quantitative real time PCR and Multiplex assay. IL-36 α -induced signal transduction in FLS was analysed by immunoblotting.

Results The IL-36R and its ligands IL-36 α and IL-36Ra could be detected in inflammatory arthritis in the synovial lining layer as well as cellular infiltrates. IL-36 α was significantly higher expressed in PsA and RA synovium compared to OA ($p = 0.0011$ and $p < 0.0001$, respectively). No differences were seen in IL-36R and IL-36Ra. The expression of IL-36 α was confirmed by western blot analysis. IL-36 α induced expression of IL-6 and IL-8 in FLS. CD138-positive plasma cells were defined as a major cellular source for IL-36 α . Functionally, IL-36 α induced the expression of IL-6 and IL-8 in FLS through NF- κ B/p38-activation.

Conclusions Here, we describe that the novel cytokine IL-36 α , mainly expressed by plasma cells, is upregulated in PsA and RA synovium and leads to IL-6 and IL-8 production by synovial fibroblasts. This finding needs further studies to determine if the IL-36 family can function as a potential target for arthritis therapy.

A10.18 LACK OF ASSOCIATION OF SERUM INTERLEUKIN-17 AND INTERLEUKIN-23 LEVELS WITH DISEASE ACTIVITY IN PATIENTS WITH ANKYLOSING SPONDYLITIS IN LATVIA

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Background Ankylosing spondylitis (AS) is a clinically well-known chronic inflammatory disease of the axial skeleton and peripheral joints. The pathogenesis of this disease still remains a challenge. Determination of cytokine profile and its role involved in AS pathogenesis give an opportunity to extend the targeted therapeutic approach. Interleukin-17 (IL-17) and interleukin-23 (IL-23) are cytokines of interest in the investigation of the pathogenesis of spondyloarthritis although their importance in AS is not clearly defined.

Objectives to investigate levels of IL-17 and IL-23 in a group of AS and in a demographically matched group of healthy subjects and its association with the disease activity measured by relevant clinical and biochemical parameters.

Materials and Methods 39 AS patients classified by the modified New York and ASAS criteria were assessed clinically and 6 ml of serum were collected from each patient. 39 healthy subjects as control group were included in this study. The serum IL-17 and IL-23 levels were tested using xMAP multiplex immunobead assay technology. At the same time the disease activity was measured by using Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score (ASDAS) using C-reactive protein (CRP), erythrocyte sedimentation rate (ESR).

Results The mean serum IL-17 and IL-23 level in AS group was respectively 18.9 (SD 39.6) and 194.6 (SD 261.4) pg/ml. In the healthy control group the mean serum IL-17 level was 15.4 (SD 26.0) and IL-23 level – 200.3 (SD 256.3) pg/ml. The serum levels of IL-17 and IL-23 were not statistically significantly different from the healthy subjects and the levels did not correlate with the disease activity measured by BASDAI and ASDAS (using the CRP and ESR).

Conclusions These results suggest that IL-17 and IL-23 are not major components of the pathogenesis of inflammation in AS patients. Our data differ from Chen W S *et al*, in 2012 published data of the serum IL-17 and IL-23 level association with the disease activity in Chinese patients with AS. This difference is probably due to the various genetic aspects characterising AS as geographically matched disease.

A10.19 MRP8/14 SERUM COMPLEXES AS PREDICTOR OF RESPONSE TO BIOLOGICAL TREATMENTS IN RHEUMATOID ARTHRITIS

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Background and Objectives Biological therapy has dramatically improved the treatment of rheumatoid arthritis (RA). One-third of