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-1β or TNFα caused rapid downregulation 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-1β. Inhibition of c-Jun N-terminal kinase (JNK), but not other MAPKs, prevented downregulation of FoxO1 expression and binding by IL-1β, and blocked IL-1β-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-36α IS EXPRESSED BY SYNOVIAL PLASMA CELLS AND INDUCES CYTOKINE PRODUCTION IN HUMAN FIBROBLASTS

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Background and Objectives The interleukin (IL)-36α 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 TNFα; 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-36α expression in PsA compared to RA and osteoarthritis (OA).
Materials and Methods Synovial tissue obtained from arthritis patients were stained for IL-36α, IL-36 receptor (IL-36R) and IL-36R antagonist (IL-36Ra) by immunohistochemistry and immunofluorescence. Lysates were tested for IL-36α by immunoblotting. Synovial fibroblasts (FLS) cultured in the presence of IL-36α were assessed for cytokine expression by quantitative real time PCR and Multiplex assay. IL-36α-induced signal transduction in FLS was analysed by immunoblotting.
Results The IL-36R and its ligands IL-36α and IL-36Ra could be detected in inflammatory arthritis in the synovial lining layer as well as cellular infiltrates. IL-36α 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-36α was confirmed by western blot analysis. IL-36α induced expression of IL-6 and IL-8 in FLS. CD138-positive plasma cells were defined as a major cellular source for IL-36α. Functionally, IL-36α induced the expression of IL-6 and IL-8 in FLS through NF-κB/p38-activation.
Conclusions Here, we describe that the novel cytokine IL-36α, 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 spondyloarthritides 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

patients however show a lack of clinical response to this treatment. The use of robust predictive markers of response to identify individuals who are likely to respond to biological treatments may provide guidance in optimising treatment strategies and lead to lower costs. The ability of MRP8/14 serum complexes, a major granulocyte and monocyte protein associated with inflammation in patients with RA, was tested to differentiate between responders and non-responders to various biological treatments and to monitor disease activity in these RA patients.

Materials and Methods 170 RA patients were treated with adalimumab, infliximab or rituximab and were categorised into responders (n = 123) and non-responders (n = 47) according to the European League Against Rheumatism (EULAR) response criteria. Serum concentrations of MRP8/14 complexes were measured at baseline, week 4 and week 16 and divided in low and high MRP8/14 serum complexes level groups based on the median level for each treatment group. Non-parametric tests were used to analyse the data.

Results Before initiation of adalimumab, infliximab or rituximab treatment, responders showed significantly higher levels of MRP8/14 serum complexes compared to non-responders. ($p = 0.010$, $p = 0.001$ and $p < 0.001$, respectively). Logistic regression analysis showed that having a high level of MRP8/14 serum complexes at baseline increased the odds of being a responder by a factor of 3.3 till 55. MRP8/14 serum complexes levels decreased after 4 weeks with respectively 46% and 60% (respectively median delta changes Δ400; IQ 160–895 and 840; IQ170–1170) and 16 weeks with 61% and 68% (Δ730; IQ220–1120 and Δ970; IQ530–1830) of treatment in responders to adalimumab and infliximab, while MRP8/14 serum complexes levels were stable in non-responders. In patients treated with rituximab, MRP8/14 serum complexes decreased with 61% (Δ1670; IQ959.5–3520) after 16 weeks in responders ($p = 0.0005$) and increased with 94% (Δ960; IQ405–1135) after 16 weeks in non-responders to treatment ($p = 0.0039$).

Conclusions MRP8/14 serum complexes can be used as a biomarker predictive of the response to biological therapy in RA patients.

A10.20 ON THE ORIGIN OF THE TYPE I INTERFERON ACTIVITY IN RHEUMATOID ARTHRITIS

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Background A role for type I interferon (IFN) activity is suggested in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). While the mechanism of induction of the IFN activity in SLE is rather known, this remains to be determined for RA. This study aims to characterise the source of IFN activity in RA serum.

Methods Healthy PBMCs were exposed to serum from RA (n = 18) or SLE (n = 25) patients. IFNα protein production was measured in an immunoassay after 20 h incubation with 5% patient serum. Samples were also co-cultured with apoptotic or necrotic cell material, as this has proven to enhance IFNα production by SLE serum. Moreover, expression of IFN response genes (IRGs), IFNα and IFNβ mRNA was determined by qPCR after 4 h and 8 h incubation with 25% patient serum. To study the involvement of new protein synthesis, part of the samples was co-cultured with 2 μg/ml cycloheximide. All cultures were performed with healthy donor serum (NHS) as a negative control.

Results As expected, SLE serum induces more IFNα protein production compared to NHS ($p = 0.0006$). This increases even further in the presence of dead cell material. RA serum does not show more IFNα protein inducing activity than NHS, although a small, nonsignificant increase was observed in the absence of dead cell material.

With respect to IRG induction, both RA and SLE sera induced higher levels compared to NHS. SLE serum showed IRG induction at 4 h, which remained high after 8 h. The IRG induction at 8 h was not decreased by CHX treatment, indicating that it occurs independently of new protein synthesis, supporting a proposed direct effect by IFNα. RA serum induced IRG induction only after 8 h, which was inhibited upon CHX treatment, suggesting an indirect induction process. The IRG induction by RA serum was positively correlated with IFNβ mRNA induction at 4 h and 8 h ($p = 0.0023$ and $p = 0.0130$, respectively), but not with IFNα mRNA induction.

Conclusions Altogether, these results indicate different mechanisms underlying the induction of type I IFN activity between SLE and RA.

A10.21 TOLL-LIKE RECEPTOR TRIGGERING OF HUMAN BASOPHILS MAY SYNERGISE WITH IGE-MEDIATED ACTIVATION IN ACPA+ RA

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Background and Objectives Antibodies against citrullinated proteins (ACPA) are highly specific for rheumatoid arthritis (RA). Recently, we described a cellular immune response against citrullinated antigens that was only present in ACPA+ RA patients. This response was mediated via crosslinking IgE-ACPA bound to basophils, and suggests a major role for FcεRI-positive cells in the pathogenesis of RA. However, other mechanisms could also contribute to basophil activation in RA, for example through endogenous TLR ligands present in synovium, which are thought to contribute to chronicity of RA. As only limited information is present on TLR-expression and function in human basophils, it is not known whether such mechanisms could activate basophils. Therefore we studied the activation of basophils via TLRs in combination with activation via IgE. Because recent studies in mice suggested that Th2-associated immune responses might be protective against arthritis, we also studied the effect of activated basophils on skewing of Th cells.

Materials and Methods Basophils were isolated from healthy donors. Real-time quantitative PCR was used to evaluate RNA expression of TLRs. For TLR-mediated stimulation, basophils were stimulated with pathogen-associated TLR ligands, such as LPS. Activation of basophils was measured using flow cytometry and cytokine assays (multiplex assays and ELISA). Naïve T cells were stimulated in the presence of basophil supernatant to evaluate the effect of TLR- and IgE-mediated activation of basophils on T cell skewing.

Results We show the presence of mRNA for TLR1-8 in human basophils, with transcripts of TLR-4 being most abundant. Basophils responded to TLR triggering with cytokine production, but not with degranulation. Remarkably, simultaneous triggering of basophils via TLR-ligands and IgE greatly enhanced cytokine production. Such synergy in cytokine production by basophils led to great enhancement of Th2 skewing.

Conclusions Our data show that human basophils functionally express TLRs and that the activation via these receptors can synergise with IgE-mediated activation. These findings provide a new perspective on the role of basophils and IgE-ACPA in combination with endogenous TLR ligands as a contributor to Th2 responses in RA patients.