

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  $\Delta 400$ ; IQ 160–895 and 840; IQ170–1170) and 16 weeks with 61% and 68% ( $\Delta 730$ ; IQ220–1120 and  $\Delta 970$ ; 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% ( $\Delta 1670$ ; IQ959.5–3520) after 16 weeks in responders ( $p = 0.0005$ ) and increased with 94% ( $\Delta 960$ ; 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. IFN $\alpha$  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 IFN $\alpha$  production by SLE serum. Moreover, expression of IFN response genes (IRGs), IFN $\alpha$  and IFN $\beta$  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  $\mu\text{g}/\text{ml}$  cycloheximide. All cultures were performed with healthy donor serum (NHS) as a negative control.

**Results** As expected, SLE serum induces more IFN $\alpha$  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 IFN $\alpha$  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 IFN $\alpha$ . 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 IFN $\beta$  mRNA induction at 4 h and 8 h ( $p = 0.0023$  and  $p = 0.0130$ , respectively), but not with IFN $\alpha$  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 Fc $\epsilon$ RI-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.