

proliferation and more importantly less divisions compared with WT animals. FACS analysis of WT and Flt3L<sup>-/-</sup> mice synovium at the acute phase of CIA showed that CD11b+ DC are present and increased in arthritic animals compared with immunised but not arthritic animals. CD103+ DC are only present in WT animals, and increased in animals with a high clinical score.

**Conclusions** Our data shows that antigen presentation in Flt3L<sup>-/-</sup> mice is impaired. As CD103+ DC are important in presenting and cross-presenting antigens our data reveals an important role for CD103+ DC in both induction and maintenance of CIA. Specifically targeting CD103+ DC could provide a novel antirheumatic strategy.

#### A9.5 IDENTIFICATION AND VALIDATION OF A PROTEIN COMBINATION INCLUDING S100A9 ABLE TO PREDICT THE RESPONSE TO THE MTX/ETANERCEPT ASSOCIATION IN RHEUMATOID ARTHRITIS PATIENTS

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**Background** The number of biologic agents in Rheumatoid Arthritis (RA) is continuously increasing. However, clinicians observe that around 30 to 40% of treated patients fail to respond to TNF $\alpha$  blocking agents. One way to optimise the drug prescription is to identify predictive markers of drug responsiveness.

**Objectives** To identify a combination of serum proteins whose expression profile would predict the RA patients responses to the association of methotrexate (MTX) and etanercept (ETA) by mass spectrometry-based quantification methods and ELISA.

**Methods** A "cohort discovery phase" of 23 patients with active RA was treated by a subcutaneous injection of. The clinical efficacy of these drugs was evaluated with the DAS28 score after 6 months of treatment according to the EULAR response criteria. For proteomic analysis, a serum sample was collected in patients prior to treatment exposure. A "label free" approach on the whole proteome was performed by mass spectrometry on the 25 sera. Accordingly, the proteome of each sample was extracted and in-gel digested. The resulting peptides were analysed by LTQ Orbitrap® (ThermoFisher). Differential analysis between responder and non responder samples was performed with LCMS ProGenesis® (Non-linear Dynamics). To validate these results a relative quantification of selected protein was performed on the second "cohort validation phase" by ELISA. The proteome of peripheral blood mononuclear cells (PBMC) from a second cohort of seven patients with similar characteristics has also been studied by the same label free approach.

**Results** The label free approach revealed 12 differentially expressed serum proteins according to patient response. This combination of proteins was used to build a Random Forest statistical model to predict the patient's status. This model was validated by a blind test on a panel of seven patients. Moreover, these results have shown the protein S100A9 overexpression in both the serum and the PBMCs from responder's patients and this expression was confirmed by ELISA.

**Conclusions** The label free approach has identified a combination of predictive markers of response to MTX treatment/ETA. Thus, using sera samples collected in patients prior to treatment exposure, it is possible to predict response to treatment with a small error. These proteins represent interesting candidate biomarkers of response that must be validated in a larger population. Already identified as a diagnostic and prognostic biomarker of RA, the S100A9 protein has been identified as a predictive biomarker of response both in serum and in PBMCs.

#### A9.6 IDENTIFICATION OF NEW POTENTIAL THERAPEUTIC TARGETS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS: ENTPD1 (CD39) AND 5NTE1 (CD73)

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**Background and Objectives** Adenosine and ATP are known to have important immunomodulatory properties. Extracellular ATP has multiple roles in inflammation and can act as a damage-associated molecular pattern (DAMP) that can activate the immune system. Conversely, adenosine is primarily anti-inflammatory and can inhibit the production of pro-inflammatory molecules by immune cells. The modulation of ATP and adenosine levels are an essential part of the induction and resolution of an inflammatory response. ENTPD1 (CD39) is a membrane-bound ectonucleoside triphosphate diphosphohydrolase enzyme that converts ATP and ADP to AMP 5NTE1 (CD73) is a 5' ecto-nucleotidase that dephosphorylates AMP to form adenosine. We investigated the role of genes in the adenosine pathway in patients with rheumatoid arthritis (RA) and determined whether expression of CD39 and CD73 would have an effect in an in vitro inflammation model.

**Materials and Methods** Gene expression analysis using 43k cDNA microarrays (Stanford Functional Genomics Facility) was performed on total RNA extracted from RA synovial tissues obtained by arthroscopy. Adenosine pathway gene expression was compared between high-inflammation versus low-inflammation tissue type synovial biopsies. ATPase levels were measured in synovial fluid (SF) from RA (n = 10) or osteoarthritis (OA) (n = 6) patients. Adeno-associated viral (AAV) vectors expressing CD39 or CD73 were generated and used to transduce HEK 293 cells or RA fibroblast-like synoviocytes (FLS) and these transduced cells were co-cultured with LPS-activated human monocytes (THP-1) in the presence of ATP. Pro-inflammatory cytokine/chemokine (IL-6, CCL2) production was measured by ELISA.

**Results** Genes involved in the ATP:adenosine pathway, including CD73, were differentially expressed in high-inflammation synovial tissues, consistent with the hypothesis that there is skewing of the ATP:adenosine balance during inflammation. The half-life of ATP was significantly increased in SF from RA patients compared with OA ( $t_{1/2}$  = 8.0 versus 4.5 min.,  $p$  < 0.05), indicating that there was a significant decrease in ATPase activity in RA SF HEK 293 cells and RA FLS cell lines transduced with CD39-and/or CD73-expressing AAV5 vectors demonstrated high CD39 and CD73 activity. THP-1 cells stimulated with LPS showed lower levels (>80% reduction  $p$  < 0.05) of IL-6 and CCL2 secretion when co-cultured with CD39 and CD73 expressing HEK293 cells or FLS cells in the presence of ATP.

**Conclusions** Together, these data suggest that synovial inflammation in RA is characterised by skewing of the ATP:adenosine balance. This could be reversed by overexpression of CD39 or CD73. Thus, these data show that the ATP:adenosine pathway may be a novel therapeutic target for the treatment of RA.

#### A9.7 LOSS OF PTEN IN MYELOID CELLS CONTROLS INFLAMMATORY BONE DESTRUCTION BY REGULATING THE OSTEOCLASTOGENIC POTENTIAL OF MYELOID CELLS

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