proliferation and more importantly less divisions compared with WT animals. FACS analysis of WT and Fk5L−/− 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 Fk5L−/− 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 antarthritic 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

doi:10.1136/annrheumdis-2013-203223.5

A Obry, T Lequerer, J Hardouin, O Boyer, O Vittecoq, P Cosette. INSERM 905, Institute for Biomedical Research, University of Rouen, Rouen; UMR 6270 CNRS, Plate-Forme d’Analyse Protéomique PIS SARAO, Faculté des Sciences; Department of Immunology, Rouen University Hospital; Department of rheumatology, Rouen University Hospital

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 TNFα 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 ETA and MTX. One way to optimise the drug prescription is to identify predictive markers of drug responsiveness.

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 proteins to be independent markers 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 5NT1 (CD73)

doi:10.1136/annrheumdis-2013-203223.6

Jonathan D Finn, Lisa van Baaren, Jan van Ittersum, Ciska Braam, Maria C Lebre, Paul T Tak, Margriet J Vervoordeldonk. Arthrogen BV, Amsterdam, The Netherlands

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 and 5NT1 (CD73) is a 5′-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 45k 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 (t1/2 = 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 AAV 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 OSTEOCLASTIC GENETIC POTENTIAL OF MYELOID CELLS


Stephan Blüml, Gernot Schubauer, Martin Friedrich, Antonia Puchner, Victoria Saferding, Emine Sahin, Tobias Lohmeyer, Josef Smolen, Kurt Redlich. Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Austria; Institute for Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology, Medical University Vienna, A-1090 Vienna, Austria; Institute of Immunology, Biomedical Sciences Research Center (BSRC) "Alexander Fleming," Vani, Greece

Background Objectives In the present study, we investigated the role of PTEN in synovial inflammation in RA patients. PTEN is a negative regulator of cellular growth and a key component of the PTEN/PI3K/AKT pathway. In this study, we aimed to investigate the expression of PTEN and its role in the inflammatory response in RA patients.