P015

AUTOPHAGY CONTROLS TREGS TO TH17 CONVERSION AND SHAPES THE SEVERITY OF ARTHRITIS

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Introduction Macroautophagy is an important contributor of cellular homeostasis, and therefore is active and up regulated in various conditions of cellular stress and inflammation. The pathway has been implicated in shaping the innate and adaptive immune responses by acting at multiple and diverse levels including cytokine secretion and antigen presentation.

Objectives Our aim is to analyse the contribution of macroautophagy in antigen presenting cells to the adaptive immune response in the context of arthritis. We analysed the antigen induced arthritis model in mice that are deficient in autophagy in their dendritic cells.

Methods CD11c-Cre mice (C57BL/6), (Jackson Laboratory) were crossed with $Atg5^{flox/flox}$ mice (C57BL/6) provided by Dr. Noboru Mizushima (Japan). ATG5 is an essential autophagy gene, its targeted deletion in dendritic cells completely abolished a functional autophagy pathway in these cells (DC/ATG5^{-/-}). For the antigen induced arthritis mice model (AIA), mice were immunised with methylated BSA (mBSA) and CFA. Monoarthritis was induced at day 21 by injection of mBSA into the knee joint and animals sacrificed at day 28. Sections from knees were stained with hematoxylin and eosin for macroscopic scoring of the cellular infiltrate. In parallel, toluidine blue staining was used for cartilage damage. T cell function was monitored by cytokine production by ELISA, after *in vitro* re-stimulation with mBSA or CD3/CD28 for 72 hour, using single cell suspensions from lymph nodes.

Results Mice lacking autophagy in their dendritic cells (DC/ATG5^{-/-}) showed enhanced cartilage destruction and bone erosion. Interestingly, the Th1/Th17 response in DC/ATG5^{-/-} mice was significantly increased. In parallel this phenotype was linked to a decreased Foxp3 expression in the regulatory T cell (Treg) population. Using Treg transfer upon AIA we could demonstrate that regulatory T cells switch to Th17 cells in the context of inflammation.

Conclusions Autophagy deficiency in dendritic cells exacerbates the Th1/Th17 inflammatory response in the AIA model, resulting in increased cartilage destruction and bone erosion. This phenotype is linked to Tregs instability upon inflammation.

Disclosure of interest None declared

P016

BASAL LEVELS OF IL-17-PRODUCING CD4⁺ T CELLS PREDICT CLINICAL RESPONSE TO ABATACEPT IN RHEUMATOID ARTHRITIS PATIENTS

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Introduction Rheumatoid arthritis (RA) develops upon aberrant activation of the immune system mainly due to failure of

self-tolerance mechanisms.¹ Abatacept, approved for RA treatment, a recombinant fusion protein of the extracellular domain of human cytotoxic T lymphocyte antigen 4 (CTLA4), restricts T cell activation by blocking interaction of CD80/CD86 on dendritic cells (DCs) to CD28 on T cells.² In clinical practice, approximately 40–50% of RA patients treated with abatacept, respond to therapy in the first 6 months.³ Development of a predictor of response is of clinical and immunological significance.

Objectives Herein, we sought to investigate for early biomarkers of response to abatacept, based on a detailed immunological profile of peripheral blood cells and cytokines.

Methods RA patients (ACR/EULAR 2010 criteria) who started abatacept due to highly active disease, were recruited to perform immunological studies at baseline, 3 and 6 months of therapy. Peripheral blood mononuclear cells (PBMCs) were isolated and pathogenic IL-17 and IFN- γ producing CD4⁺ T cells (Th1, Th17), regulatory (Tregs) T cell subsets as well as myeloid cell populations, like DCs and myeloid derived suppressor cells (MDSCs) were characterized using flow cytometry. Response to therapy (remission or low disease activity) was assessed based on the “Swollen joints” value.

Results We studied 21 patients (mean age 60 years, 86% women, 48% rheumatoid factor or anti-citrullinated protein antibody positive). After 6 months of treatment, 45% of them attained remission or low disease activity. Notably, baseline levels of Th17 were statistically significant decreased in peripheral blood of patients in remission or low disease activity compared to those with active disease at 6 months of treatment ($1.29 \pm 0.18\%$ versus $2.44 \pm 0.41\%$, $p=0.0482$). Baseline levels of Th1 and Foxp3⁺Tregs were comparable between responders and non-responders. No significant differences in CD14^{int}CD15⁺CD33⁺ MDSCs or CD3⁺HLADR⁺ DCs were observed.

Conclusions In this cohort of RA patients treated with abatacept (CTLA4Ig), low levels of IL-17 producing CD4⁺ T cells at baseline are associated with a better response to abatacept at 6 months. This novel finding (validation to a larger cohort in progress) may be used as an early biomarker to predict clinical responses to abatacept.

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P017

RANK/RANK-LIGAND INTERACTION REGULATES PATHOGENIC T CELL RECRUITMENT IN SJÖGREN'S SYNDROME

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Introduction The RANKL (ligand)-RANK-OPG triad, members of the TNF(R) superfamily, is implicated in lymphoid organ

development and bone homeostasis. It has recently been demonstrated that RANK-activated astrocytes release CCL20 and attract T cells to the central nervous system in a model of Multiple Sclerosis and that transgenic RANK expression in the skin promotes aberrant epithelial cell proliferation and is sufficient to induce ectopic formation of tertiary lymphoid structures (TLS). Ductal epithelial cells (SGEs) have been implicated in Sjögren's Syndrome (SS) pathogenesis where they mediate immune recruitment by expression of pro-inflammatory chemokines and support the formation of pre-malignant myoepithelial lesions.

Objectives To address the role of RANK-RANKL interaction in primary (p) SS.

Methods A combination of human and mouse studies were used to explore the RANK-RANKL interaction in pSS. Salivary glands (SGs) and saliva samples from patients recruited in the OASIS cohort (University of Birmingham) were studied to evaluate this pathway in human disease. Consecutive stimulated saliva samples (n=69) were analysed using Proseque Multiplex $\text{INF}^{96} \times 96$, covering 92 unique inflammation-related protein biomarkers. Taking advantage of a viral induced model of pSS we studied the effect of this pathway with a RANKL blocking antibody and by inducing gain of function with direct cannulation in the salivary glands of recombinant RANKL. Murine SGs were studied by immunofluorescence, flow cytometry and qPCR on total tissue and sorted cells.

Results Fourteen proteins in saliva were significantly separated between pSS and sicca controls, and elevated levels of just two proteins, RANKL and $\text{TNF}\beta$, could classify pSS or sicca with 75% accuracy. Levels of salivary RANKL and CCL20 were strongly correlated ($r=0.6$; $p<0.01$). We demonstrated that both human and murine inflamed SGEs upregulate both RANK and CCL20, a chemokine known to recruit pathogenic T cells. Upregulation of RANKL was found in human Th2 cells, classically associated with humoral responses and germinal centre (GC) formation. SGs from mice treated with anti-RANKL antibody showed decreased epithelial proliferation, reduced T cell infiltration and defective TLS establishment. On the contrary, viral infected SGs treated with recombinant RANKL showed increased T cell infiltration, CCL20 expression and enhanced differentiation of GC B cells.

Conclusions *In vivo* RANK/RANKL interaction mediates recruitment of activated T cells that are skewed toward a Th2 phenotype. These, in turn, will favour the establishment of TLS in the SG. Those data were confirmed in human pSS, where expression of RANK is found in inflamed epithelium and RANKL detection in saliva is able to differentiate patients with pSS from sicca controls, thus candidates this pathway both for drug targeting and patient stratification.

Disclosure of interest None declared

P018

PROTEASE ACTIVATED RECEPTOR 2 (PAR2) EXPRESSION IN THE MYELOID COMPARTMENT IMPACTS OSTEOCLASTOGENESIS

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Introduction Protease activated receptor 2 (PAR2) is a G protein coupled receptor responsive to serine proteases, which

plays a key role in inflammation and pain reception. Rheumatoid arthritis (RA) patients have up-regulated surface expression of PAR2 in circulating monocytes which correlates with disease activity. We have previously demonstrated that *Par2*^{-/-} mice are protected from inflammation, bone erosion, and cartilage destruction in a Freund's Complete Adjuvant (FCA) induced arthritis model. However, it is unclear how PAR2 affects the composition of the myeloid compartment and osteoclastogenesis.

Objectives The aim of this study was to evaluate the impact of loss of PAR2 on the myeloid compartment and osteoclastogenesis.

Methods Bone marrow (BM) from 6–10 week old *Par2*^{-/-} and control C57BL/6 mice was cultured in pro-osteoclastogenic media for 5 days and the generated mature osteoclasts, tartrate-resistant acid phosphatase positive (TRAP) multinucleated cells, were counted. The resorption potential of these cells was assessed using osteolysis plates and the total osteo-resorption quantified after 7–14 days of culture. BM was also collected for flow cytometric analysis of the haematopoietic cellular composition (with the following markers: CD3, B220, CD11b, Ly6C, Ly6G, CD115, and CD117) and assessed for osteoclast precursors.

Results *In vitro* osteoclastogenesis revealed an increase in numbers of mature osteoclasts from *Par2*^{-/-} BM compared to WT, corresponding with increased levels of resorption. Flow cytometry of BM from both WT and *Par2*^{-/-} mice showed 3 distinct monocyte populations defined by the cell surface expression levels of CD11b and Ly6C. The overall ratio of these populations was not altered in *Par2*^{-/-} animals.

Conclusions BM from *Par2*^{-/-} mice has increased osteoclastogenic potential and overall resorptive activity. We propose that this is not due to differences in bone marrow residing osteoclast precursor numbers. This study indicates a potential role for PAR2 in osteoclast differentiation and bone remodelling.

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P019

ULTRASOUND DETECTED TENOSYNOVITIS AS A MARKER OF SUBCLINICAL INFLAMMATION PRIOR TO ARTHRITIS ONSET

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Introduction Prospective studies of individuals at increased risk of developing rheumatoid arthritis (RA) will further improve the understanding of disease development. Ultrasound emerges clinically useful in detecting subtle inflammatory changes in rheumatic diseases.

Objectives To investigate ultrasound (US) detected changes as markers for future arthritis development.

Methods Patients presenting with musculoskeletal complaints and a positive Anti-Citrullinated Protein Antibody (ACPA) test at primary care, were referred to Karolinska rheumatology clinic for further rheumatic joint disease assessments. Those lacking arthritis by clinical and US examination (defined as synovial hypertrophy with Doppler activity) were recruited