particles are targeted by autoantibodies from RA and other systemic rheumatic diseases.

Methods: Using a protein microarray we identified JKTBP in humans and animal models of inflammatory rheumatic diseases. Bacterially expressed recombinant JKDBP proteins were used to confirm the obtained data. Epitope, TLR7/9 and MyD88 dependency was determined by ELISA. JKTBP expression in cultivated cells and synovial tissue was analysed by indirect immunofluorescence, immunoblot and immunohistochemistry.

Results: Anti-JKTBP autoantibodies were detected in 46% of the patients with systemic lupus erythematosus (n=103), in 20–30% of the patients with rheumatoid arthritis (n=286), in 10% of the patients with mixed connective tissue disease (n=20) or spondyloarthropathy (n=20), and in <10% of patients with other autoimmune disorders (n=382). Sera positive to JKTBP as well as hnRNP-B1, revealed nearly two thirds of the RF IgM/ CCP2-seronegative patients as early RA patients. Combining sensitivities to all autoantigens tested (JKTBP, AUF1, hnRNP-B1), it was possible to identify 92% of the early RA patients (n=91). In the MRL/lpr mouse model of SLE, mice deficient of MyD88 or TLR7/9 lacked anti-JKTBP autoantibodies, whereas mice deficient of SIGIRR/TIR8 showed enhanced anti-JKTBP autoantibody production. These results show that autoantibody generation against JKTBP, AUF1, hnRNP-B1 is dependent on TLR 7 and TLR9 like rheumatoid factor different to TLR 7 dependent generation of snRNPs. For all tested autoantigens either their titer or generation are dependent on the activation of innate immunity genes MyD88 and SIGIRR/TIR8 gene.

In localization, experiments anti-JKTBP autoantibodies specifically stained stress granules (SG) in the cytoplasm. Immunohistochemical studies revealed JKTBP to be highly expressed in SG in the cytoplasm of RA synovial tissue different from OA and normal control tissue

Conclusions: These data identify SG as targeted particle in RA and JKTBP as a novel autoantigen in RA, SLE patients and mouse models of inflammatory rheumatic diseases. In combination with the hnRNPs AUF1 and hnRNP-B1, JKTBP autoantibodies close the sensitivity gap in RA left by rheumatoid factor and anti-CCP2 antibodies.

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SAT0005 DNA METHYLATION INHIBITORS PRODUCE SUSTAINED REMISSION OF ARTHRITIS IN MICE AND PROMOTE REGULATORY T CELL RESPONSES

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Background: Dysfunction of Tregs results in a breakdown of immunological tolerance and has been implicated in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis (RA) and type 1 diabetes. Treg function is regulated by epigenetic factors and we have previously reported the presence of Tregs expressing an aberrant DNA methylation profile in RA.

Objectives: The aim of this study was to assess the potential utility of DNA methylation inhibitors for the treatment of RA, using collagen-induced arthritis (CIA) as an animal model.

Methods: DBA/1 mice were immunised with bovine type II collagen emulsified in complete Freund's adjuvant. The mice were treated with zebularine (400 mg/kg), decitabine (1 mg/kg) or psammaplin A (10 mg/kg) for 4 days, starting on the day of arthritis onset. Treatment was then stopped and the disease was monitored up to day 10 of arthritis. The expression of Treg genes was measured in lymph nodes on day 10 by qPCR. To assess the effect of DNA methylation inhibitors on generation of Tregs, naïve CD4⁺CD25⁻ T cells were cultured with mitomycin C treated APCs plus IL-2, TGFβ and anti-CD3 in the presence or absence of DNA demethylating agents and numbers of CD4⁺FoxP3⁺ Tregs were determined by FACS after 72h.

Results: Treatment with zebularine resulted in a sustained reduction of arthritis severity, accompanied by an increase in the expression of Treg associated genes, *Foxp3*, *Ctla4* and *Tgfb1*, in draining lymph nodes. Treatment with decitabine produced a more profound reduction in disease severity whereas the therapeutic effect of psammaplin A was more transient. All three DNA methylation inhibitors could convert CD4⁺CD25⁻ T cells into CD4⁺FoxP3⁺ Tregs in a dose-dependent manner *in vitro*.

Conclusions: This study has shown that pulse treatment with DNA demethylating drugs produces a sustained reduction in the severity of arthritis and promotes the generation of Tregs. The findings raise the possibility that epigenetic drugs can be used on a short-term basis for re-setting tolerance and boosting Treg responses in human autoimmune disease.

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Sjogren is a co-founder of Idogen and a co-inventor of a patent on the use of zebularine for the treatment of autoimmune diseases., Z. Xue: None declared, L. Salford Shareholder of: Leif Salford is a co-founder of Idogen and member of the board of Idogen and a co-inventor of a patent on the use of zebularine for the treatment of autoimmune diseases, A. Sundstedt Employee of: Anette Sundstedt is an employee of Idogen, R. Williams Consultant for: Dr. Richard Williams is on scientific board of Idogen

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SAT0006 ANTI-TNF AGENTS INDUCE MACROPHAGES WITH PRO-RESOLVING PHENOTYPE

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Background: Macrophages contribute to the rheumatoid arthritis (RA) pathogenesis. They can display various states of activation or "polarization", characterized by distinct functions in inflammation, and reversibility depending on their environment [1]. M1 polarization corresponds to the "classical", pro-inflammatory activation as identified in RA. M2 "alternative" polarizations display pro-resolving or wound-healing properties.

Data concerning the effects of RA biological drugs (bDMARDs) on macrophage polarization are scarce.

Objectives: To assess *in vitro* modulation of macrophage polarization by RA bDMARDs.

Methods: Blood monocytes from 14 healthy donors were positively sorted by CD14⁺ magnetic selection. Macrophages were Derived from Monocytes (MDM) by 5 days of culture in the presence of MCSF, and activated or not for 24h as M1 pro-inflammatory MDM (by LPS + IFNγ) or as M2 alternative MDM (by IL10 or IL4). MDM were cultured with or without bDMARDs.

We evaluated 2 anti-TNF agents (etanercept (ETA), adalimumab (ADA)), 1 anti-IL6R agent (tocilizumab (TCZ)), and 1 anti-CD20 agent (rituximab (RTX)) used as control monoclonal antibody. bDMARDs effects were assessed separately on differentiation and activation phases by flow cytometric analysis of membrane markers. Functional aspects of polarization were assessed by analysis of cytokine production in supernatants (cytometric bead array) and phagocytosis (flow cytometry).

MDM cultured in the presence of bDMARDs were compared to untreated MDM by a Wilcoxon matched pairs test.

Results: We first validated *membranous polarization markers* in our culture model: CD40 and CD80 as M1 (LPS + IFNγ) markers; CD16, CD163 and CD64 as M2 (IL10) markers, CD206 and CD200R as M2 (IL4) markers.

Anti-TNF significantly modulated surface polarization markers of MDM obtained after differentiation. CD40 decreased with both anti-TNFs. CD16 increased with ADA, whereas opposite effects on CD64, decrease with ETA, increase with ADA, were observed. CD206 increased with ADA. CD200R increased with both anti-TNF. We observed a significant increase in CD64 with TCZ. RTX induced no modulation except an increase in CD64.

Concerning the effect of bDMARDs on *M1 activation* (M1 MDM) shown in Figure, anti-TNF agents induced a significant decrease in M1 markers and a significant modulation in M2 (IL10) markers. We observed a decrease in CD40 and CD80, an increase in CD16, CD163, a decrease in CD64 with ETA and an increase with ADA. TCZ induced a slight but significant decrease in CD40 and an increase in CD64. RTX only affected CD64 as observed for differentiation.

In *M2 (IL4) et M2 (IL10) activated MDM*, anti-TNF agents induced a similar trend toward M2 (IL10) phenotype.

Anti-TNF agents led to a significant decrease in *TNFα*, *IL6*, *IL12* and *IL10* levels measured in cell culture supernatant after M1 MDM. TCZ mainly affected IL6 and TNFα productions. No significant effect was observed with RTX.

Normalized polarization surface markers on M1 MDM

Data were normalized to M1 MDM (represented as a dotted line) for each donor. Statistical comparison of M1 MDM without bDMARD (dotted line) versus M1 MDM with bDMARD, using a Wilcoxon matched pairs test → * p < 0.05; ** p < 0.005; *** p < 0.0005

Phagocytosis was superior in M2 (IL10) and M2 (IL4) activated MDM than in M1 MDM. Anti-TNF agents but not TCZ or RTX induced an increase of phagocytosis in M1 MDM.

Conclusions: Anti-TNF agents upregulate M2 alternative pro-resolving markers and downregulate M1 inflammatory markers in macrophages. Our results need to be extended by transcriptional analysis and evaluated in RA patients.

References:

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SAT0007 GROUP 3 INNATE LYMPHOID CELLS NUMBERS IN PERIPHERAL BLOOD PREDICT USTEKINUMAB (STELARA) THERAPY RESPONSIVENESS IN PSORIATIC DISEASE CASES WITH SUBCLINICAL IMAGING ENTHESOPATHY

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Background: Ustekinumab¹ targets the common p40 sub-unit of interleukin-12 (IL-12)/interleukin-23 (IL-23). In patients treated with Ustekinumab for psoriasis where patients were selected on the basis of subclinical imaging enthesopathy, we have noted an improvement in subclinical imaging enthesopathy (Savage LJ et al submitted), raising the possibility that it may be possible to find a biomarker for predicting response to therapy in psoriatic disease. Innate lymphoid cells may be centrally involved in the pathogenesis of psoriatic skin and joints disease², since they express IL-23R receptor and are associated with IL-17/IL-22 production.

Objectives: This work was performed to test the hypothesis that peripheral blood ILC perturbations may be useful in defining response in psoriasis cases with imaging confirmed subclinical enthesopathy.

Methods: Peripheral blood collected at baseline (before therapy, 24weeks, 54 weeks) from patients in the MUSTEK trial (Ustekinumab in psoriasis cases who had ultrasound imaging confirmed subclinical enthesopathy) (n=23). Cellular immunophenotyping was performed density gradient separated PBMCs. Innate lymphoid cells were identified as lineage negative (CD3- TCR $\gamma\delta$ - TCR $\alpha\beta$ - CD19- CD14- CD11c- CD1a- CD303- Fc ϵ R1- CD34- CD123-) with positive expression of CD45, CD127. ILC2 cells were identified as Lineage- CD127+ and CRTH2 positive, while ILC3 were identified as Lineage- CD127+, CRTH2 - and CD117 (c-Kit) positive and further subdivided of Nkp44+ and Nkp44-. ILC1 were identified as lineage- CD127+ CD117-and CRTH2-. For data analysis we separated cases into PASI>90% or PASI <90% responders. The subclinical enthesopathy scores also fell significantly under therapy (Savage LJ data submitted)

Results: No correlation was found with total ILCs (ILC1,2, AND 3) (R=0.104, p=321, Spearman R) and therapy response. While, The absolute numbers of baseline ILC3s was inversely correlated in with the reduction in the PASI score (R -0.404, p=0308, Spearman R). The ILC3s also fell progressively under therapy. All the patients respond with reduction of PASI score mean 92.6% (range 65.8–100%), Interestingly, those patients with reduction below 90% of PASI score

has a significantly higher absolute numbers of ILC3+ cells in peripheral blood at the baseline than PASI (n=6/23) than super-responder group (n=17/23)

Conclusions: Only peripheral blood ILC3s, but not other ILCs changes, correlate with the PASI score (disease activity). Furthermore, excellent responders (PASI reduction >90%) showed strong correlated with higher ILC3 population at the baseline. This may help to use ILC3 enumeration as predictive parameter for ustekinumab clinical therapeutic response and may be relevant to assessing novel biomarkers for subclinical arthropathy in psoriasis.

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SAT0008 DRUG THERAPY ENHANCES TOLEROGENIC PROPERTIES OF DENDRITIC CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Dendritic cells (DCs) are known to contribute to the pathogenesis of rheumatoid arthritis (RA) through presentation of cartilage glycoprotein, production of proinflammatory cytokines and activation of Th1/Th17 responses. Along with stimulating activity, DCs may exhibit suppressive functions via capacity to induce T cell apoptosis/anergy and to generate regulatory T cells. Since these DCs have potential to control autoreactive T-lymphocytes, the enhancing of tolerogenic properties of DCs seems to be a new important strategy in treatment of RA. The experimental research in animals and human in vitro studies revealed the capacity of anti-rheumatic drugs to inhibit stimulating activity and to enhance tolerogenic functions of DCs. However, data concerning the in vivo influences of drug therapies on DC functions in RA patients are not available.

Objectives: The aim of our study is to investigate, whether drug therapies influence the properties of monocyte-derived DCs generated in the presence of IFN α (IFN-DCs) in RA patients, and if the effect of disease-modifying anti-rheumatic drugs differ from that of biological/pulse steroid therapy.

Methods: Thirty nine patients with RA with high and moderate activity of disease were recruited in this study. All patients fulfilled ACR/EULAR criteria (2010). Nineteen patients received methotrexate, leflunomide, sulfasalazine or their combination (RA1). Twenty patients were at pulse therapy (methylprednisolone 500mg No. 3) or biological drugs (adalimumab or rituximab) (RA 2). All studies were performed after receiving informed consent. DCs were generated from blood monocytes culturing for 5 days with GM-CSF and IFN- α in the absence and presence dexamethasone, applied on third day. LPS as maturation stimuli was added on fourth day. The expression of CD14, CD83, CD 86, B7H1, HLA-DR, TLR-2 on the surface of DCs was measured by flow cytometry. The functions of DCs were evaluated by measuring cytokine production and DC allostimulatory activity in mixed lymphocyte culture.

Results: Both DC-RA1 and DC-RA2 where shown to display impaired maturation evidenced by elevated expression of CD14 and decreased number of mature (CD14-CD83+) DCs. Wherein, DCs-RA2 demonstrated several additional differences, including increased number of intermediate CD14+CD83+ cells (compared with donors DCs), higher expression of inhibitory molecule B7-H1 (PD-1L) (compare with donors DCs and DCs-RA1) and tendency to lower expression of CD86 and higher expression of TLR-2. Besides, DCs-RA2 produced higher concentrations of IL-6 and had 2-fold lower allostimulatory activity than DCs-RA1. These differences together with phenotypic changes suggested more pronounced tolerogenic properties of DCs-RA2. Despite the revealed DC differences in RA1 and RA2 patients both types of DCs preserved in vitro sensitivity to dexamethasone, that suppressed the production of TNF- α and reduced allostimulatory activity.

Conclusions: The data obtained indicate that, IFN-DCs from RA patients at drug treatments are characterized by tolerogenic properties, which are more pronounced in patients with biological or pulse steroid therapy.

Disclosure of Interest: None declared

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SAT0009 DISTRIBUTION AND INTRACELLULAR SETTING OF GRANULYSIN IN WOMEN WITH KNEE OSTEOARTHRITIS

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Background: The role of the cell-mediated immune response is recently recognized in osteoarthritis (OA) (1). Granulysin (GNLY) is mediator of cellular immunity and cytotoxic molecule expressed in T and NK cells in regulatory (15 kDa) and cytotoxic (9 kDa) forms (2). Cytotoxic 9 kDa form of GNLY mediates apoptosis of eukaryotic cells (3) and might be responsible for silent unscheduled apoptosis of joint tissue cells in patients with OA without clinically recognized