whether changes in these levels and/or presence associates with SDFR.

Methods In sera of 399 seropositive RA patients in the IMPROVED study,¹ we measured, at 4 month intervals over the first year of treatment: IgG, IgM, and IgA isotypes for anti-cyclic citrullinated peptide-2 (anti-CCP2) and anti-carbamylated protein antibodies (anti-CarP), IgM and IgA for rheumatoid factor (RF), and IgG autoantibodies against 4 citrullinated and 2 acetylated peptides. We investigated whether changes in antibody levels and seroconversion from positive to negative for each individual antibody was favourable for SDFR (drug-free DAS44 <1.6 lasting ≥1 year until last follow-up).

Results For all 14 antibodies, median levels decreased significantly between baseline and 4 months and then stabilised. Most seroconversion to negative happened within the first 4 months of treatment (with prednisone and methotrexate), after which some patients converted back to seropositive. The prevalence of seroconversion varied substantially depending on the autoantibody. The percentage of baseline autoantibody positive patients that became seronegative by 12 months was: anti-CCP2 IgG (2%), IgM (29%), IgA (29%); RF IgM (21%), IgA (31%); anti-CarP IgG (51%), IgM (49%), IgA (66%); anti-acetylated-lysine-vimentin IgG (24%), anti-acetylated-ornithine-vimentin IgG (10%); anti-cit-vimentin 59–74 IgG (21%); anti-cit-fibrinogen α27–43 IgG (45%), anti-cit-fibrinogen β36–52 IgG (20%), and anti-cit-enolase 5–20 IgG (22%). We hypothesised that greater level decreases and seroconversion to negative, reflecting disappearance of the underlying immunopathology, might be favourable for the long-term outcome SDFR, but surprisingly, greater median decreases in levels were not associated with higher chance of SDFR for any antibody. Furthermore, we found no evidence that rates of SDFR were higher in patients who seroconverted to negative compared those who stayed seropositive, for any of the 14 antibodies analysed.

Conclusions Autoantibody levels decrease and seroconversion from positive to negative occurs under treatment, but these changes do not translate to apparent clinical long-term benefits with regard to SDFR. This suggests that the disappearance of measurable serological autoimmunity does not lead to eradication of disease.

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Disclosure of interest None declared

P008

METHOTREXATE AND BAFF INTERACTION PREVENTS IMMUNISATION AGAINST TNF-A INHIBITORS BY INCREASING ADENOSINE AND REGULATORY B-CELLS

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10.1136/annrheumdis-2018-EWRR2018.34

Introduction TNFα inhibitors (TNFi), frequently used in patients with autoimmune diseases, can induce anti-drug antibodies (ADA) in one third of cases, leading to secondary resistance. Methotrexate (MTX) is known to decrease

immunisation against TNFi. In BAFFtg mice, a model of autoimmune disease in which immunisation against biologic drugs is high, we investigated a new way of tolerization using a single course of MTX and we identified the mechanisms involved in this tolerization.

Objectives To assess the potential interaction between BAFF and tolerization induced by MTX.

Methods BAFFtg mice treated with TNFi with or without MTX were monitored for drug concentration and ADA. WT and BAFFtg mice were compared for B-Cell surface markers involved in MTX-related purinergic metabolism, adenosine production or B-regulatory cells (Bregs). Then, BAFF levels, MTX treatment and ADA were assessed in the human ABIR-ISK cohort of patients with chronic inflammatory diseases.

Results In BAFFtg but not in WT mice, a single course of MTX prevented immunisation against TNFi and maintained drug concentration for over 52 weeks. BAFFtg mice B-cells expressed more CD73 and CD39 leading to more adenosine production and to increase in Breg. MTX-induced tolerization was reversed *in vivo* using anti-CD73 antibodies. In patients treated with TNFi for chronic inflammatory diseases, high BAFF serum level was protective against ADA formation to TNFi only in patients co-treated with MTX but not in patients on TNFi monotherapy.

Conclusions This data supports an interaction between MTX and BAFF via increase in CD73 to prevent ADA formation in mice and humans. Treatment increasing CD73 could potentialize the role of MTX to prevent immunisation.

Disclosure of interest S. Bitoun: None declared, G. Nocturne: None declared, B. Ly: None declared, R. Krzysiek: None declared, A. Pruvost: None declared, A. Paoletti: None declared, J. Pascaud: None declared, P. Dönnès: None declared, K. Florence Employee of: GSK, A. Gleizes: None declared, A. Hincelin-Mery Employee of: Sanofi, M. Allez: None declared, S. Hacin Bey: None declared, M. Pallardy: None declared, X. Mariette: None declared

P009

TRANSCRIPTOMIC ANALYSIS OF PLASMACYTOID DENDRITIC CELLS FROM RHEUMATOID ARTHRITIS PATIENTS REVEALS NOVEL TARGETS FOR THERAPY

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10.1136/annrheumdis-2018-EWRR2018.35

Introduction Reestablishing immune tolerance and long term remission represent major therapeutic goals in rheumatoid arthritis (RA). Our laboratory previously demonstrated that plasmacytoid dendritic cells (pDCs) from RA patients in remission have the ability to induce IL-10 producing regulatory T cells *in vitro*. However, the molecular pathway of RA pDC-mediated Treg induction remains elusive.

Objectives Herein, we sought to identify the molecular mechanism through which pDCs contribute to restoration of tolerance in RA.

Methods pDCs were isolated from peripheral blood of RA patients responding to anti-TNF therapy (DAS28 <5.1) and

healthy control subjects and DNA microarrays were performed. Flow cytometry and real time PCR were used to verify the expression of de-regulated genes in RA pDCs. Finally, *in vitro* cultures of pDCs activated with CpG A in the presence or absence of recombinant IL-6 (rIL-6) were performed to assess the functional importance of these gene signatures.

Results pDCs from RA patients (n=5) exhibited a differential gene signature (6741 deregulated genes) compared to pDCs from healthy controls (n=5). Notably, IL-6 receptor (IL-6R) gene, exhibited increased expression levels in pDCs isolated from RA patients compared to healthy pDCs and the surface expression levels of IL-6 receptor were verified in a subsequent cohort of patients responding to therapy (n=9) versus active patients or healthy donors. Moreover, assessment of IL-6 signalling pathway in RA patients versus healthy donors revealed a significant increase of pSTAT1 expression levels in RA patients (n=9) compared with healthy donors (n=6) (MFI±SEM, 7.98±0.8 versus 12.65±1.18, p=0.0076). Importantly, IL-6-treated pDCs exhibited a vast decrease in TNF-α production (p=0.0002) whereas no differences were found in the production of IFN-α and in their antigen presenting capacity between CpG-treated pDCs in the presence or absence of rIL-6. Moreover, confocal experiments in progress will assess the expression levels of TNF-α in pDCs isolated from RA patients in remission versus active or healthy donors. The functional importance of the previous findings will be addressed in coculture experiments of IL-6 stimulated pDCs with neutrophils isolated from healthy donors and monitor the neutrophil extracellular trap formation.

Conclusions We found that pDCs from RA patients in remission display increased IL-6R expression levels and an activated IL-6 signalling pathway. Activation of IL-6 signalling on pDCs *in vitro* significantly decreases the production of TNF-α whereas it does not alter IFN-α production and their antigen presenting capacity. This novel finding that may drive pDCs towards a previously described tolerogenic phenotype, need to be further addressed.

Disclosure of interest None declared

P010 ABSTRACT WITHDRAWN

P011 EXPANDED T-CELL CLONES ARE PRESENT IN THE SYNOVIUM BEFORE THE ONSET OF CLINICAL RHEUMATOID ARTHRITIS

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10.1136/annrheumdis-2018-EWRR2018.36

Introduction T-cells are thought to be key players in the initiation and progression of rheumatoid arthritis (RA). Earlier we showed that already at the seropositive *at-risk* stage uninfamed synovial tissue contains T-cell infiltrates.¹ In another study we showed that inflamed synovium harbours expanded T-cell clones.²

Objectives Following up on these observations, we longitudinally investigated whether the expanded T-cell clones found in

the inflamed synovial tissue at onset of RA are already present in the *at-risk* stage.

Methods Next-Generation Sequencing of the TCRβ repertoire was performed on 20 randomly selected individuals with elevated IgM-RF and/or ACPA levels. Ten individuals did not develop RA during at least 3 years of follow-up, and 10 individuals did. Peripheral blood and synovial tissue samples were analysed during the *at-risk* phase and, for individuals that developed RA, again after RA onset. T-cell clones were identified by their unique TCRβ sequence.³ For each sample 3,570 TCRβ sequences were analysed.

Results During the *at-risk* phase the TCRβ repertoire in the synovium is characterised by expanded clones. This is observed both in *at-risk* individuals that did and did not develop RA. Interestingly, a higher impact of expanded clones inversely correlated with a longer disease-free follow-up time (p=0.02). During progression to RA, the *at-risk* TCRβ repertoire is largely maintained in the tissue. Further characterisation of the synovial CDR3 sequences showed no significant differences between clones that were maintained in the tissue during progression to clinical disease and clones that were uniquely present in the *at-risk* phase or at RA onset.

Conclusions Expanded T-cell clones are present in the synovial tissue in the *at-risk* phase regardless of future development of RA, and are maintained after onset of clinical disease. The resemblance in TCRβ repertoires indicates that the process leading to disease – at least at the T-cell level – constitutes a smooth development. Elucidating the role of these synovial T cells (resident memory, regulatory or autoreactive) might help understanding the earliest pathogenic events in RA.

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Acknowledgements MED, NdV, DMG and PPT received support from BTCURE, a research project from the Innovative Medicines Initiative Joint Undertaking (grant no 115142-2), NdV from the Dutch Arthritis Foundation, and from ZonMw, the Netherlands Organisation for Health Research and Development, in the program 2Treat (Grant 436001001).

Disclosure of interest None declared

P012 PEPTIDYL ARGININE DEIMINASE IMMUNISATION INDUCES ANTI-CITRULLINATED PROTEIN ANTIBODIES IN HLA-DRB1*04:01 TRANSGENIC MICE

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10.1136/annrheumdis-2018-EWRR2018.37

Introduction Autoantibodies to citrullinated proteins (ACPAs) are highly associated with rheumatoid arthritis (RA). ACPAs are produced in the absence of T cell responses to citrullinated proteins.

Objectives Peptidyl arginine deiminase 4 (PAD4), which binds numerous different proteins and citrullinates them, is the target of autoantibodies in early RA. This suggests a model to explain the production of ACPAs in the absence of T cells to citrullinated proteins. ACPAs could arise because, at first, PADs are recognised by T cells, which, in turn help the production of autoantibodies to proteins being citrullinated by