Methods Phenotypic analysis of peripheral blood B-lymphocytes was made by flow-cytometry in a cohort of SLE patients treated with belimumab. SLE-disease activity was assessed by SLEDAI-2K score. BAFF was tested by ELISA. SPSS was used for statistical analysis.

Results The relative change of BAFF levels at 6 and 12 months from baseline showed linear correlation with the percentage of naïve B-cells (Pearson correlation=0.645, $p=0.044$ and 0.639, $p=0.002$, respectively) and of transitional B-cells (Pearson correlation=0.768, $p=0.009$ and 0.623, $p=0.055$, respectively). The percentage and absolute number of naïve B-cells showed a progressive decrease during time (ANOVA, $p=0.013$ and $p=0.001$ respectively). In terms of response prediction, a significant association of SLEDAI percentage improvement at 12 months with the decrease of total number of B-cells within the first 6 months of therapy was observed (Log regression $r=0.707$, $p=0.05$).

Conclusions BAFF inhibition induces B-cell number modifications in a SLE cohort. The reduction of total number of B-cells within the first six months shows predictive value for SLEDAI response after the first year of therapy.

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Disclosure of Interest None declared.

P037

ANTI-CARBAMYLATED PROTEIN ANTIBODIES : ARE THEY USEFUL FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS?

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Career situation of first and presenting author Resident.

Introduction The current EULAR-2010 diagnostic criteria for Rheumatoid Arthritis (RA) relies heavily on the presence of auto-antibodies. So far only anti-citrullinated peptide antibody (ACPA) or rheumatoid factor (RF) are accounted for however, novel auto-reactivities have been described notably anti-carbamylated protein (anti-CarP) and anti-oxylized peptide antibodies.

Objectives To investigate the potential of anti-CarP antibodies for the diagnosis of RA.

Methods 402 patients were recruited from an early arthritis register (ethically approved) with a diagnosis recorded over 24 months. 95 healthy controls were included. All participants provided written informed consent. An anti-CarP antibody ELISA was performed using carbamylated FCS as target antigen.¹ Results were expressed as aU/mL. Statistical analysis used ROC and AUC analysis established using SPSS V22.

Results We first determined the 95% CI of the distribution of ELISA results in HCs to define a negative/positive cut-off for the test, established at >250 aU/mL.

195/402 patient were classified as RA at inclusion; 28 developed RA over time while 56 remained undifferentiated arthritis; 83 had non persistent symptoms and 40 other inflammatory joint diseases.

Anti-CarP ELISA results were clearly much higher in RA (median 250 [IQR 25–762], $p<0.0001$) with 49% of patients being positive as well as in the group that progressed to RA (25% positive, 82 [13–235], $p=0.001$), compared to all other EAC patients together (8% positive, 26 [5–80]) or HC (5% positive, 41 [1–106]).

In this particular cohort, anti-CarP had an odd ratio OR=5.55 for predicting RA with a sensitivity SEN=91.5% and positive predictive value PPV=87%, compared to ACPA which had an OR=8.78 (SPE=92%, PPV=92%) and RF with an OR=4.18 (SPE=85%, PPV 84%).

Titres were not associated with other variables (age, gender, JC, CRP or DAS). A ROC analysis determined that linear data (aU/mL) for anti-CarP were not better than a positive/negative status at predicting RA.

A score counting the number of auto-antibodies (0–1–2–3) had a high predictive value (AUC=0.840, $p<0.0001$ SEN/SPE of 65%/95% and PPV/NPV of 94%/68%).

We then established that a combination of ACPA+antiCarP was superior at predicting RA (OR=25.1 SEN/SPE=43%/98.5%, PPV/NPV=97%/58%) than the ACPA+RF combination (OR=11.9, SEN/SPE=54%/95%, PPV/NPV=94%/62%), although less sensitive.

Conclusions Although RF is more frequently observed than anti-CarP, it is also less specific for RA. A combination of ACPA+anti CarP may prove more useful at classifying early arthritis patients for RA than an ACPA+RF combination. Future work needs to establish a standardised assay for anti-CarP and replicate this data.

Disclosure of Interest None declared.

P038

IN VIVO DEMONSTRATION OF TMTNF REVERSE SIGNALING: SIGNIFICANCE IN THE THERAPEUTIC RESPONSE TO ANTI-TNF AGENTS DURING MURINE ARTHRITIS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Anti-TNF agents are widely used in rheumatoid arthritis (RA). Their effect on inflammation results from the neutralization of soluble TNF (sTNF), but also supposedly from the induction of reverse signaling through their binding to membrane TNF (tmTNF). Despite possible clinical relevance, reverse signaling has been described only *in vitro* but has not been proven *in vivo*.

Objectives In this study we aim to demonstrate for the first time the existence of tmTNF reverse signaling *in vivo* and its importance in anti-TNF response during arthritis.

Methods Triple transgenic mouse model (3TG), KO for TNFR1/TNFR2 and KI for tmTNF, thus secreting no sTNF was developed. To analyze reverse signaling, mice were injected

either with etanercept (ETA, 10 mg/kg), an anti-mouse TNF antibody (MP6-XT22, rat IgG1, 10 mg/kg) or an anti-human IL17 antibody (secukinumab, SEC, 10 mg/kg) as a control. Daily clinical evaluation of K/BxN serum induced-arthritis was performed in 3TG as well as WT mice. Polarization of bone marrow-derived macrophages (BMDM) and cytokine production from non-arthritic WT and 3TG mice under the action of anti-TNF *in vitro* was evaluated by RT-qPCR, CBA and ELISA.

Results *In vivo*, the administration of anti-TNF (ETA or MP6-XT22) decreased arthritic scores in WT mice ($p=0.005$) as well as in 3TG mice ($p<0.001$), unlike SEC which had no effect, proving that anti-TNF binding of tmTNF decreased arthritis. *In vitro* effect of anti-TNF on BMDM from WT as well as 3TG mice induced a decrease in the expression of genes specific of inflammatory macrophages (CD38, GpR18 and FpR2), and an increase in the expression of genes specific of alternative macrophages (Arg1, EgR2, c-Myc). We also observed an inhibition of the secretion of pro-inflammatory cytokines (IL12p70 and IL-6) and an early peak of IL-10 secretion demonstrating an effect of reverse signaling on macrophage polarization and activation. This suggested a switch in macrophage polarization as a probable mechanism for modulation of inflammation during K/BxN serum-induced arthritis.

Conclusions Our work provides *in vivo* evidence for the involvement of reverse signaling in the anti-TNF-mediated modulation of arthritis. Reverse signaling is expected to result in the modulation of macrophage polarization from an inflammatory to an alternative functional phenotype in arthritic mice. Our data prompt us to consider new interpretation of the effects of anti-TNF in the treatment of RA.

Disclosure of Interest None declared.

P039/O03 CIRCULATING FOLLICULAR HELPER T CELLS ARE INCREASED IN SYSTEMIC SCLEROSIS AND PROMOTE PLASMABLAST DIFFERENTIATION THROUGH THE IL-21 PATHWAY WHICH CAN BE INHIBITED BY RUXOLITINIB

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Career situation of first and presenting author Student for a master or a PhD

Introduction Systemic sclerosis (SSc) is an autoimmune disease characterized by widespread fibrosis, microangiopathy and autoantibodies. Follicular helper T (Tfh) cells CD4⁺CXCR5⁺PD-1⁺ cooperate with B lymphocytes to induce the differentiation of plasmocytes secreting Immunoglobulins (Ig). Circulating Tfh (cTfh) cells are increased in several autoimmune diseases.¹ Tfh cells can infiltrate the skin of SSc patients, and induce fibrosis *in vitro*.²

Objectives The aim of this study was to perform a quantitative and functional analysis of cTfh cells in SSc.

Methods Using flow cytometry, we analyzed cTfh cells from 52 SSc patients with no immunomodulatory treatment and 38 healthy controls (HC). *In vitro* coculture experiments of sorted cTfh and B cells from 13 SSc patients and 6 HC were performed for functional analysis. IgG and IgM production were measured by ELISA. BCL-6, Blimp-1, CXCL13 and IL-21 expression were analyzed by RTqPCR.

Results We observed that cTfh cell numbers are increased in SSc patients compared with HC. Furthermore, the increase in cTfh cells was more potent in patients with severe forms of SSc such as diffuse SSc and in the presence of arterial pulmonary hypertension. cTfh cells from SSc patients present an activated Tfh phenotype, with high expression of BCL-6 and increased capacity to produce IL-21 in comparison to HC. *In vitro*, cTfh cells from SSc patients had higher capacity to stimulate the differentiation of CD19⁺CD27⁺CD38^{hi} B cells and their secretion of IgG and IgM through the IL-21 pathway than cTfh cells from HC. Blocking IL-21 or using the JAK1/2 inhibitor ruxolitinib reduced the Tfh cells' capacity to stimulate the plasmablasts and Ig production.

Conclusions Circulating Tfh cells are increased in SSc and correlate with SSc severity. The IL-21 pathway or JAK1/2 blockade by ruxolitinib could be a promising strategy in the treatment of SSc.

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Disclosure of Interest None declared

P040

THE EUROPEAN CONSENSUS FINDING STUDY GROUP ON AUTOANTIBODIES 2017/18 INVESTIGATION. CHARACTERISATION OF AUTOANTIBODY CONTENT IN A NEW INTERNATIONAL REFERENCE STANDARD FOR DENSE FINE SPECKLED 70KD (DFS70) AUTOANTIBODIES

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Career situation of first and presenting author Instructor.

Introduction The European Consensus Finding Study Group on autoantibodies (ECFSG) a.k.a. the EULAR autoantibody study group has been active for 30 years.

Objectives To reach consensus about autoantibody measurements in clinical practice, and to evaluate upcoming autoantibody standard reagents concerning autoantibody content.

Methods ECFSG focus on evaluating difficult to interpret serum samples, where differences between assays can be clearly visible. Ten unknown samples are distributed yearly to European laboratories, and analyzed broadly. Results are collected with information about laboratory techniques used, and discussed in relation to clinical information on the donating patients during EWRR. The 2017/2018 investigation contained nine patient samples, and a not yet launched pooled standard for anti-dense fine speckled 70kD antibodies, an ANA reactivity with specific nuclear staining on HEp-2 cells that can be confounded with homogenous ANA, but that is not associated with autoimmune disease.

Results Acceptable consensus was reached for the clinical samples. Anti-DFS70 pattern was reported from 32/38