

(SC35, SRp40, SRp55, SRp20 and hnRNPA1), and the ratios between exon4 inclusion/exclusion were evaluated by RT-PCR. Second, we examined the effects of different cytokines on  $\Delta$ 4BAFF induction.

**Results** RAMOS cells presented exon 4 skipping (ratio inclusion/exclusion: 6.8) after minigene transfection. Following co-transfection of the minigene with coding plasmids for splicing proteins, only the overexpression of SC35 showed effect in the splicing of exon 4, promoting exon 4 inclusion (ratio:  $>30$ ). Incubation of different cell lines with several cytokines showed that IFN- $\gamma$  was able to induce  $\Delta$ 4BAFF-transcript. Thus, after IFN- $\gamma$  stimulation in the minigene model, the ratio inclusion/exclusion markedly decreased (1.5). IFN- $\gamma$  modifies the balance between SC35 and another member of hnRNPs family (hnRNP C1/C2) favouring the alternative splicing of exon 4.

**Conclusions** These results demonstrated that IFN- $\gamma$  induces  $\Delta$ 4BAFF, modifying the function of SC35 protein and increasing the hnRNPC1/C2. Our study provides an expanded conceptual view of BAFF gene regulation, and contributes to a better understanding of the mechanisms involved in BAFF up-regulation in autoimmunity.

#### A5.11 DETECTION OF ACPA PRODUCING B-CELLS BY A CITRULLINE PEPTIDE PANEL

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**Background and Objectives** Anti-citrullinated protein/peptide antibodies (ACPAs) are the most sensitive and specific serological markers of RA. To identify the optimal epitopes that detect different subgroups of RA patients with high sensitivity and specificity, we have investigated citrulline and arginine containing peptides derived from filaggrin, collagen or vimentin. We have identified a citrulline-containing peptide panel that was recognised by RA sera with high specificity. Our aim was to compare this peptide panel with the conventionally used serological assays and to detect peptide-specific ACPA producing B-cells in in vitro cultures.

**Materials and Methods** Previously selected citrulline- and arginine-containing filaggrin, vimentin and collagen peptide epitopes were investigated. We compared the recognition of these peptides by RA and control sera using indirect ELISA. B-cells were purified from peripheral blood by negative selection, IgG production was stimulated by B-cell activators (R848 and recombinant human IL-2) provided with the human ELISPOT kit. Antibody producing cells were enumerated after 4 days culture by using peptide-specific ELISPOT assay.

**Results** Sera samples from 247 RA and 148 age-matched (57  $\pm$  14 years) healthy controls were collected. The citrulline peptide panel detected approximately 80% of RA patients, including 20% of seronegative/CCP negative patients as well. Individual peptides detected different subgroups of RA patients. The more peptides recognised by a particular RA serum sample, the more severe the disease of the patient was. In vitro cultured B-cells from selected RA patients synthesised multiple citrulline-containing peptide-specific antibodies after polyclonal stimulation, while B-cells from healthy blood donors did not.

**Conclusions** The citrulline peptide panel can detect 20% of ACPA negative RA patients thus may have a prognostic value. Furthermore, the panel is suitable to detect citrulline peptide-specific

antibody producing cells, thus enables us to study ACPA producing B-cells of RA patients.

#### A5.12 DISAPPEARANCE AND REAPPEARANCE OF IGG, IGA AND IGM AUTOANTIBODY ISOTYPES AND IMMUNE COMPLEXES IN RITUXIMAB-TREATED SLE PATIENTS

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**Background and Objectives** We have earlier investigated the content of specific IgG autoantibodies in SLE immune complexes (IC; Åhlin *et al*, Lupus 2012; 21:586). For that purpose we have developed a line blot technique for the quantification also of non-classical (IgA and IgM) SLE autoantibody isotypes. In SLE patients, IgG autoantibody levels drop after institution of Rituximab therapy. The objective was to investigate parallel changes IgG, IgA and IgM autoantibody isotypes in parallel to IC levels.

**Materials and Methods** Nine SLE patients initially treated with two infusions of Rituximab were followed with repeated samplings at baseline and after 1, 3, 6 and 12 months. Thawed samples were investigated simultaneously concerning rheumatoid factor (RF) isotypes and C1q-binding IC with enzyme immunoassays. All samples from patients with ANA-associated autoantibodies (6/9) were investigated concerning IgG/A/M autoantibodies with line blot quantitated with densitometry and concerning IgG autoantibodies with ALBIA/Luminex technique. Significant changes were defined either as  $\geq 33\%$  drop or as  $\geq 50\%$  increase, compared to the lowest levels experienced during the follow-up period.

**Results** ALBIA measurements showed significant initial drop in anti-dsDNA in 4/6 patients but also significant drop in levels of anti-histone, anti-SSA/Ro60, anti-Sm and anti-Sm/RNP in individual patients. Late increases in IC and antibodies against dsDNA, SSA/Ro52, SSA/Ro60, SSB, Sm, Sm/RNP ribosomal P protein and histones were associated with clinical relapse. Late increase in IgA/IgM anti-DNA, anti-histones and anti-nucleosomes was also found in one patient with persistent kidney disease treated with mycophenolate mofetil at 10 months. Non-classical autoantibody isotypes showed late increases that often were not paralleled by the corresponding IgG autoantibodies. Two patients showed late increase in RF isotypes in parallel to clinical relapse. Different autoantibodies/isotypes showed different kinetics of appearance/disappearance. All ANA autoantibody positive patients initially had increased IC levels, which dropped significantly after therapy in 4/6 patients. The autoantibody negative patients never had increased IC levels and showed no significant changes in RF.

**Conclusions** Measurement of non-classical isotypes of RF and ANA-associated autoantibodies might yield clinically useful information when monitoring SLE patients treated with B cell depleting therapy.

#### A5.13 EFFECT OF RITUXIMAB ON B CELL SUBPOPULATIONS EXPRESSING THE 9G4 IDIOTYPE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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**Background and Objectives** Antibodies encoded by the V<sub>H</sub>4-34 gene are inherently autoreactive, binding to red blood cell

determinants. An idiotype present on the majority of immunoglobulins derived from the V<sub>H</sub>4–34 gene can be detected using the rat monoclonal antibody 9G4. 9G4 also allows the detection of B cells expressing B cell receptors derived from V<sub>H</sub>4–34 gene. We therefore determined the effect of B cell depletion therapy with rituximab (RTX) on B cell subpopulations expressing the 9G4 idiotype in peripheral blood of patients with rheumatoid arthritis (RA) as a means of following the fate of a specific autoreactive B cell population.

**Materials and Methods** B-cell subpopulations were characterised by flow cytometry using combinations of IgD, CD5, CD27 and CD38 in healthy controls (HC) (n = 7), patients with RA before (n = 10) and at clinical relapse after RTX (n = 17). The frequency of each 9G4+ B cell subpopulation was calculated after gating on 9G4+ cells.

**Results** No significant differences were observed in the frequency of total 9G4+ B cells between both patient groups and HC, although the levels of 9G4+ B cells tended to be higher at relapse after RTX treatment. After RTX treatment, repopulating total B cells were predominantly transitional (IgD+CD38++) and naïve (IgD+CD38+), with lower frequencies of most memory B-cell subpopulations. At relapse, RA patients had significantly more 9G4+IgD–CD38– memory B cells compared to HC. This B cell subset was positively correlated with a 9G4+CD5+CD27+ subpopulation. The frequency of 9G4+IgD–CD38+ memory B cells also tended to be increased after RTX compared to HC and pre-RTX. Lower levels of 9G4+IgD+CD38+ naïve, 9G4+IgD+CD38– memory and 9G4+CD5–CD27+ memory B cells were found after RTX treatment compared with HC and pre-RTX. No significant differences were observed in 9G4+IgD+CD38++ transitional, or 9G4+IgD–CD38++ plasmablasts in RA patients before and after RTX treatment in comparison with HC. However, there was a positive correlation between the frequency of 9G4+IgD–CD38++ plasmablasts and time after RTX treatment.

**Conclusions** Alterations in the frequency of memory B cell subpopulations expressing 9G4 idiotype occur in clinically relapsing RA patients after RTX treatment, particularly in IgD–CD38– memory B cell subset. It is possible that this 9G4+ B cell subpopulation escapes B cell depletion therapy, being rescued in niches such as secondary lymphoid organs, or in inflammatory sites. Our results also suggest that the normal tolerance mechanisms preventing 9G4+ B cells from becoming antibody producing cells is defective in patients with RA.

#### A5.14 HOMOCITRULLINE-REACTIVE ANTIBODIES CAN BE GENERATED FROM SYNOVIAL B-CELLS FROM ACPA-NEGATIVE RA PATIENTS

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**Background and Objectives** Roughly two-thirds of rheumatoid arthritis (RA) patients carry antibodies, so-called ACPA, against peptides containing citrulline, a post-translationally modified version of the amino acid (AA) arginine in their sera and/or affected joints. A recent study reported the presence of antibodies against a different kind of post-translationally modified AA, homocitrulline, also known as carbamylated proteins (CarP), where lysine residues are altered by a non-enzymatic reaction involving cyanate. The authors reported IgG antibodies recognising homocitrullinated fibrinogen in the sera of >45% of RA-patients and that 16% of ACPA-negative patients carried such anti-CarP antibodies.

Furthermore, in vivo studies describe that immunisation of mice with homocitrulline-containing peptides induced erosive arthritis. Thus, homocitrulline represents an interesting immune target in the context of RA. Therefore, we aimed to assess the proportion of anti-CarP antibodies sourced from the joints of RA patients with active disease.

**Materials and Methods** Using a single B cell-based cloning technology to isolate the immunoglobulin (Ig) genes from joint-derived IgG+ CD19+ B-cells, we then co-transfected the paired heavy and light chains into a human cell line and produced recombinant monoclonal antibodies from each individual B cell. This approach has previously allowed for the generation of citrulline-reactive monoclonal antibodies. Here, we analysed for reactivity to carbamylated (homocitrullinated) fibrinogen and compared to its counterpart unmodified fibrinogen, by ELISA. So far, we have analysed 42 monoclonal antibodies from three ACPA-positive patients and one ACPA-negative patient, for homocitrulline reactivity.

**Results** Between six and eleven antibodies were tested for homocitrulline activity for the ACPA-positive patients and eleven were examined for the ACPA-negative patient. All clones screened for CarP-reactivity were negative for citrulline binding. Antibodies were made up to concentrations of 5 ug/ml down to 0.63 ug/ml in a 4-step dilution. So far, one antibody displayed reactivity to the carbamylated peptide and this antibody originated from the ACPA-negative patient.

**Conclusions** Although the frequency of anti-CarP reactivity amongst the 42 antibodies examined does not match that previously reported for sera, the finding of one anti-CarP antibody from an ACPA negative patient, does support the earlier finding of significant frequencies of anti-CarP antibodies amongst ACPA-negative patients. It will be of great interest to expand the investigation for anti-CarP specificities, particularly among ACPA-negative patients and determine whether these antibodies could have pathological effects in RA patients.

#### A5.15 HOW MICRORNAS CONTROL PLASMA CELLS

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**Background and Objectives** Our long-term goal is to understand how one class of interfering non-coding RNAs, the so-called microRNAs (miRNAs), regulates and fine-tunes the differentiation of mature B cells into effector cells, i.e., memory B cells and antibody-secreting plasma cells. miRNAs control the expression of specific target genes at the post-transcriptional level by binding to target sequences e.g., in the 3'-untranslated region of mRNAs, which, depending on the degree of the binding, results either in a block of translation or an accelerated degradation of the respective target mRNA. Lineage-specific deletion of the miRNA-processing DICER protein as well as of individual miRNAs revealed the importance of miRNA pathway in early steps of central B cell maturation. However, the mechanisms by which miRNA-dependent circuits control the antigen-induced phase of B cell activation of mature naïve B cells and their subsequent differentiation into effector cells remain largely elusive.

**Methods** To change this situation we established a transgenic knock-in mouse line with a floxed allele of DGCR8, an essential subunit of the nuclear miRNA processing complex. B cell-specific deletion of DGCR8 resulted in a complete maturation block at the pro-B stage, indicating that miRNA processing is essential for central