Results Out of the 73 aPL positive patients:

- 21% were positive for aβ2GPI, aDI and aDIV/V;
- 41% were positive for aβ2GPI and aDI but negative for aDIV/V;
- 4% were positive for aβ2GPI and aDIV/V but negative for aDI;
- 21% were aβ2GPI positive only;
- 4% were positive for aDIV/V;
- 9% were negative for antibodies against the whole molecule and the studied domains.

The prevalence of aDI was 74% among patients with thrombotic pAPS and 60% among women with obstetric manifestations. 40% of aPL asymptomatic carriers were positive for aDI.

We observed a strong correlation between aβ2GPI and aDI ($p << 0.01$, $r = 0.836$) but not aDIV/V ($p = 0.07$, $r = 0.216$).

Conclusions Most of the aβ2GPI positive sera displayed reactivity against DI, while aDIV/DV were detected in a low rate of patients. Our data suggest that DI is the immunodominant β2GPI epitope and that aDI are the main antibody population in APS patients. Future studies are warranted to better define the diagnostic and prognostic role of anti-DI in APS.

A5.6 ANTI-CARBAMYLATED PROTEIN ANTIBODIES ARE PRESENT IN MICE WITH COLLAGEN INDUCED ARTHRITIS

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Objective Antibodies against citrullinated proteins (ACPA) are a characteristic of rheumatoid arthritis (RA). Carbamylation is a different type of post translational modification, where a Lysine amino acid is converted into a homocitrullin. Recently we identified antibodies binding to carbamylated proteins (anti-CarP) in a subgroup of RA patients. In ACPA negative RA patients anti-CarP antibodies associate with joint damage.

The Aim of this study was to determine whether these anti-CarP antibodies are present in animal models of arthritis.

Methods Collagen induced arthritis (CIA) was induced in DBA/1 ($n = 29$) and C57Bl/6 ($n = 20$) mice by immunisation with type II collagen in CFA. Arthritis severity was monitored using a clinical scoring system. Non-immunised animals ($n = 9$) served as negative controls. After disease onset serum was harvested and antibody levels were determined by ELISA. The specificity of our anti-CarP. ELISA was validated using dotblots.

Results Whereas no anti-CarP antibodies could be detected in non-immunised DBA/1 mice, anti-CarP total Ig was present in 93% of the arthritic mice. Of those mice 39% had IgG1 and 79% had IgG2a anti-CarP antibodies. Antibodies to citrullinated proteins could not be detected. The levels of mouse collagen-specific IgG2a correlated with the clinical score. However, the levels of the different anti-CarP isotypes did not. Around 60% of the immunised C56Bl/6 mice developed arthritis. Anti-CarP IgG2c could be detected in 55% of those mice and could not be detected in the mice that did not get CIA. Anti-CarP IgG1 was detected in 28% of the arthritic mice. Interestingly, mouse collagen specific IgG2c antibodies were detected in 100% of the immunised C57Bl/6 mice. Dotblot analysis, using carbamylated and non-modified proteins confirmed the ELISA results regarding the specificity of the antibodies for homocitrulline containing proteins.

Conclusions Mice with CIA have antibodies to carbamylated proteins and their presence associated with disease development. All immunised mice have anti-mouse CII antibodies, indicating that the presence of anti-CarP antibodies could be a disease specific marker for arthritis in mice. Further studies will be required to determine the role of anti-CarP in the pathogenesis of arthritis.

A5.7 AUTOANTIBODIES TO THE FIBRIN-DERIVED CITRULLINATED PEPTIDES α36–50 AND β60–74 ARE TWO DISTINCT NON-OVERLAPPING SUBFAMILIES OF ACPA THAT TOGETHER ALMOST SUMMARISE THEIR REACTIVITY TO CITRULLINATED FIBRINOGEN AND TO CCP2 ANTIGENS

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Objectives To evaluate the proportions of Rheumatoid Arthritis (RA) sera containing autoantibodies to citrullinated proteins (ACPA) reactive to α36–50 and/or β60–74 two citrullinated peptides identified as bearing the immunodominant epitopes of their major target: citrullinated fibrin. To analyse the relationships of anti-α36–50 and anti-β60–74 autoantibodies with autoantibodies to the whole citrullinated human fibrinogen (AhFibA) and with anti-CCP2 antibodies.

Methods 617 sera from 181 established RA and 436 non-RA rheumatic diseases were tested by ELISA for AhFibA, anti-CCP2, anti-α36–50, anti-β60–74 autoantibodies, and by nephelometry for Rheumatoid Factor (RF). Diagnostic indexes, correlations and concordances between tests were analysed. Cross–reactivity between anti-α36–50 and anti-β60–74 autoantibodies was analysed with peptide absorption experiments.

Results At diagnostic specificity of 95%, the diagnostic sensitivity of AhFibA (83%) was significantly higher than that of all other tests. The diagnostic sensitivity of anti-β60–74 autoantibodies (71%) was significantly higher than that of anti-α36–50 (51%) but similar to that of anti-CCP2 (74%). Titres of RF, anti-α36–50 and anti-β60–74 autoantibodies were weakly correlated with each other, whereas titres of anti-β60–74 were strongly correlated with those of AhFibA ($rho = 0.633$) and of anti-CCP2 ($rho = 0.634$). More than 90% of AhFibA-positive or anti-CCP2-positive sera recognised the α36–50 and/or the β60–74 peptide. Absorption experiments showed that anti-α36–50 and anti-β60–74 mainly correspond to 2 non-cross reactive subfamilies of ACPA.

Conclusions Autoantibodies to α36–50 and β60–74 are two distinct non-overlapping subfamilies of ACPA that together almost summarise the ACPA reactivity to citrullinated fibrinogen and to CCP2 antigens. In established RA, anti-β60–74 autoantibodies show diagnostic indexes similar to those of anti-CCP2.

A5.8 B CELL DEPLETION THERAPY IN A COHORT OF PATIENTS WITH SEROPOSITIVE AND SERONEGATIVE RHEUMATOID ARTHRITIS

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Background B cells play a crucial role in the pathogenesis of rheumatoid arthritis (RA). They are responsible for the autoantibodies formation such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP) and the production of cytokines, act as antigen presenting cells and regulate T cell functions.

Rituximab (RTX), murine monoclonal antibody which selectively targets CD20-positive B-cells, has proved to be an effective and safe therapy for active RA. Initially it was used in seropositive RA, but considering the other functions of B cells, it is logical to think that it is also useful in seronegative forms.

Abstract A5.8 Table 1

	Mean DAS28 at baseline	Mean DAS28 at 3 months	Mean DAS28 at 6 months	Goodeular response at 3 months	Goodeular response at 6 months	DAS28 < 2.6 at 3 monts	DAS28 < 2.6 at 6 months	HAQ at baseline	HAQ at 3 months	HAQ at 6 months
RF +	5.73 ± 1.24	3.38 ± 1.30	4.13 ± 1.83	90.5%	65.2%	18.2%	17.4%	1.81 ± 0.77	1.00 ± 0.57	1.2 ± 0.74
RF –	5.52 ± 1.61	3.72 ± 0.93	4.22 ± 1.23	83.3%	57.1%	14.3%	14.3%	1.57 ± 0.78	0.83 ± 0.56	0.95 ± 0.54

Objective To evaluate the efficacy of RTX in our series of refractory seronegative and seropositive RA.

Materials and Methods Baseline characteristics and disease activity markers at baseline, and after 3 and 6 months of treatment with RTX (1g × 2 weeks), were collected in 33 patients. A descriptive study was made; and the relations between variables were analysed statistically.

Results The mean age was 52.06 ± 12.01 years, 75.8% female, 78.8% RF positive. The mean duration of illness was 7.70 ± 4.47 years. Thirty two patients (97%) had failed at least to one TNF antagonist. Most of the patients (84.8% 9) received RTX with methotrexate.

The mean DAS28 at baseline was 5.7 ± 1.30; at 3 months decreased to 3.4 ± 1.22, and at 6 months to 4.15 ± 1.69 ($p < 0.0005$).

At 3 months, 88.9% reached good eular response, and 63.3% at 6 months. Remission was obtained in 17.2% at 3 months and in 16.7% at 6 months.

It was also noted improvement in baseline HAQ, after 3 and 6 months (from 1.75 ± 0.767 to 0.96 ± 0.56 and 1.24 ± 0.70 respectively).

No significant differences were found between decreases in DAS 28 at 3 and 6 months compared to baseline between RF seronegative and seropositive patients, neither in good eular response, remission percentages or HAQ improvement. The data are shown in the table.

Discussion The efficacy and safety of RTX has been proved in several clinical trials.

The presence of RF, low baseline functional disability and no more than one previous anti-TNF are predictors of good response to RTX, as has been recently published.

Response rates in seronegative RA are slightly lower, although higher than placebo, as described in other publications.

In conclussion, the experience of RTX treatment in our patients with seronegative RA is positive, in terms of efficacy, due to the action on B cells and their different roles, with no significant differences comparing to seropositive RA.

A5.9 VALUES OF B LYMPHOCYTE SUBPOPULATIONS (HEALTHY POPULATION) USING FLOW CYTOMETRY A STARTING POINT FOR ANY STUDY

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Background Flow cytometry is a widely used technique nowadays for the determination of cell subpopulations in the study of autoimmune, infectious and tumoral diseases.

Our goal is to study the means and medians of B lymphocyte subpopulations in healthy population, to thereby have reference limits with which being able to compare when carrying out studies in population with autoimmune diseases.

Methods We studied 50 healthy patients. Male/female: 25/25. Age: 18–65 years. Previously, it was checked that none of these patients had any autoimmune diseases nor any other disease or condition causing lymphopenia or lymphocytosis. The fluorochromes used were: CD3, CD19, CD38, CD27 and IgD.

Results

Abstract A5.9 Table 1

Subpopulations	Mean	Typical deviation	Median
Naive	70.46	8.06	73.70
DN	4.05	2.08	4.66
Unswitched Memory	9.65	5.33	10.05
switched Memory	11.38	4.62	11.00
Bm1	7.82	9.29	5.10
Bm2	73.14	10.71	73.05
PreGC	2.90	1.61	2.89
Bm3 + 4	0.56	0.53	0.44
Late Bm5	3.17	2.74	2.50
Early Bm5	9.12	4.93	9.21

Conclusions Knowing the mean, medians and standard deviations of the B lymphocyte subpopulations subsets is important in helping to compare these results with those obtained in studies of patients with autoimmune diseases.

In most cases, conclusions drawn after the study with flow cytometry are based on knowing how these subpopulations vary with respect to healthy people in order to draw conclusions about what subpopulations are involved the most in the pathogenesis of the disease.

We believe, therefore, important to deepen in studies of this kind in order to clarify more situations of normality in the world of flow cytometry: a technique that is increasingly taking more importance in the understanding of autoimmune diseases.

A5.10 Δ4BAFF ALTERNATIVE SPLICING IS REGULATED BY IFN-γ AND SC35 PROTEIN

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Background The B-cell activating factor (BAFF) is a potent survival factor involved in the pathogenesis of autoimmune diseases. Recently, we reported the discovery of a new transcript for BAFF, Δ4BAFF – lacking exon 4 –, which is mainly detected in autoimmune diseases and acts as a transcription factor for its own gene. However, the mechanisms implicated in Δ4BAFF induction and upregulation are unknown. In this study we analysed the induction and regulation of Δ4BAFF.

Materials and Methods First, to study the alternative splicing of *BAFF* exon 4, we transfected a minigene construct, centred on exon 4, into RAMOS B cells. To determine the proteins implicated in exon 4 inclusion/exclusion, we co-transfected the minigene together with each of the plasmids coding for the main splicing proteins