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.