A12 DETECTION OF GENUINE ANTI-CITRULLINATED PROTEIN ANTIBODIES IN MICE REVEALS THEIR PRESENCE IN BALB/C BUT NOT DBA/1 AND SJL MICE HYPERIMMUNISED WITH CITRULLINATED COLLAGEN

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Background Antibodies against citrullinated proteins (ACPA) occur in 60–70% of all rheumatoid arthritis (RA) patients with a specificity of 95%. The role of the immune response against citrullinated proteins in the pathophysiology of RA remains unknown, because studies in experimental models yielded contrasting results. It is unclear whether mice develop antibodies crucially dependent on citrullination of the epitope. The authors aimed to develop a reliable method to detect ACPA in mice and to assess the development of ACPA in different mouse strains.

Methods Bovine collagen type II was citrullinated in vitro using peptidyl arginine deiminase enzyme (PAD). BALB/c, DBA/1 and SJL mice were immunised with 100 µg native or citrullinated collagen in complete Freud's adjuvant at day 0 and 40. Sera were analysed with ELISA and Western blotting, using different citrullinated substrates and their native counterparts as control.

Results Sera were analysed by ELISA using a cyclic citrullinated peptide as coating, as used in the commercial anti-CCP ELISA (detecting ACPA in human sera). Positivity was observed in 7 out of 12 BALB/c mice immunised with citrullinated collagen versus 5 out of 12 BALB/c mice immunised with collagen. Using the arginine peptide as control yielded the same reactivity, indicating that the signal is not dependent on citrullination of the epitope. Therefore, the authors evaluated the use of vitro citrullinated proteins as ELISA coating. Control experiments using PAD coated plates indicated that mice immunised with in vitro citrullinated proteins developed anti-PAD reactivity. To distinguish between the reactivity against PAD and citrullinated epitopes, the authors used citrullinated versus native human fibrinogen as substrate on western blots. The authors could differentiate anti-PAD reactivity (70 kDa) and ACPA reactivity (58 kDa). The authors observed ACPA reactivity in BALB/c mice immunised four times with citrullinated collagen within 56 days. These mice did not develop arthritis and there was no difference in anti-collagen antibodies in animals immunised with citrullinated versus native collagen. Both DBA/1 and SJL mice developed arthritis but no genuine ACPA. Both strains displayed no difference in incidence, severity of the arthritis, or anti-collagen antibodies in animals immunised with citrullinated versus native collagen.

Conclusions The use of ELISA for ACPA detection leads to false positive findings in mice. Using an optimised western blotting approach, the authors demonstrated the presence of genuine ACPA in BALB/c but not DBA/1 or SJL mice immunised multiple times with citrullinated bovine collagen type II. These ACPA were not associated with higher anti-collagen antibody levels or arthritis.