11 AFFINITY PURIFICATION AND CHARACTERISATION OF HUMAN ACPAS

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Background and objectives Autoimmunity in rheumatoid arthritis (RA) is characterised by autoantibodies to citrullinated proteins/peptides (ACPA). These antibodies, present in 60–70% of patients, antedate clinical onset and associate with an erosive disease course, suggesting a direct pathogenic involvement in disease initiation and progression. With this study, the authors aimed to develop an efficient method for the purification of human ACPA, and to characterise their frequency and fine-specificity pattern in synovial fluid (SF) and plasma of RA patients.

Materials and methods SF and plasma samples were collected with informed consent and ethical approval from patients (fulfilling the American college of rheumatology criteria for RA) with high anti-CCP antibody levels. SF samples (n=36) were first treated with hyaluronidase to decrease viscosity, then proteins were precipitated with ammonium sulphate, dissolved and further dialysed against PBS, before the IgG fractions were purified on Protein G columns (GE Healthcare, Uppsala, Sweden). Plasma samples (n=10) were diluted in PBS before applied to the Protein G column. ACPAs were further purified using CCP2 affinity columns, kindly provided by Euro-Diagnostica. Recovery and purity of total IgG and anti-CCP immunoglobulin G (IgG) were analysed using SDS-PAGE, Nanodrop (Thermo Scientific, Wilmington, DE, USA) and the CCP2-ELISA kit. Fine-specificity of the purified ACPAs were investigated using inhouse ELISAs, with peptides from citrullinated α -enolase (CEP-1), -vimentin (Cit-vim), -fibrinogen (Cit-fib) and -collagen type II (Cit-C1).

Results Anti-CCP IgG could efficiently be purified from SF and plasma, using ProteinG-, followed by CCP2-, columns. No CCP IgG response could be detected in the flow-through fractions. Higher concentrations of total IgG were found in plasma (13.6 mg/ml) compared to SF (4.2 mg/ml), while a higher percentage of CCP-specific IgG was detected in SF (3%), compared to plasma (2%). The purified anti-CCP IgG fractions cross-reacted with CEP-1, Cit-vim, Cit-fib and Cit-C1, while no reactivity to these citrullinated antigens were detected in the IgG flow-through fractions. Anti-CCP IgG dilution curves (starting at 10 μ g/ml of purified antibodies), demonstrated differences in affinity between patients, which may correspond to the different ACPA-fine specificity patterns seen in patients.

Conclusions The described methodology efficiently purifies ACPAs with multiple specificities, which will allow for their use in in vivo and in vitro studies, to further elucidate their arthritogenic and pathogenic capacity. In addition, the ACPAs will be tools for future immunoprecipitation-, immunoblotting- and immunohistochemistry experiments.