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GENERATION AND CHARACTERISATION OF MONOCLONAL ANTIBODIES FROM SINGLE RA SYNOVIAL B CELLS

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Background and objectives Anticitrulline protein antibodies (ACPA) are a hallmark of HLA-associated rheumatoid arthritis (RA), but it is still not established whether these antibodies represent a cause or a consequence of arthritis. It is however clear that such antibodies can enhance murine arthritis, but a corresponding effect in patients is difficult to prove. In this study the authors aimed to assess the specificity

and the immunoglobulin gene characteristics of B cells derived from RA synovial fluid of RA by using a method that allows in vitro production of monoclonal antibodies derived from single human memory B cells.

Materials and methods The authors have cloned and expressed antibodies from single flow cytometry purified CD19+IgG+ B cells from synovial fluid of two ACPA+ and two ACPA- RA patients and tested, by ELISA, the generated recombinant monoclonal antibodies for reactivity to different known citrullinated antigens.

Results On the molecular level, striking differences were found between ACPA+ and ACPA- patients taking into consideration the mutational pattern of the Ig genes. Indeed, based on DNA sequences, the authors could demonstrate that B cells from ACPA+ patients did not have more total mutations than ACPA- patients but when focusing on the CDR1, two and three regions of the Ig variable region, the ACPA+ clones displayed more replacement mutations than did the ACPA- clones.

Regarding the specificity of the generated monoclonal antibodies, the authors have analysed clones from one ACPA+ and one ACPA negative patient. Our results so far show that 10 out of 23 antibodies generated from an ACPA+ RA patient, proved to bind, to variable degrees, to cyclic citrullinated peptide and different citrullinated antigens including citrullinated α -enolase, fibrinogen, vimentin and type II collagen. In contrast, no ACPA positive clones were identified amongst the antibodies derived from the ACPA- RA patient.

Conclusions Our data suggest that citrulline-reactive B cells are common in synovial fluid and the Ig molecules bear clear signs of antigen-driven maturation.