A148 IN RHEUMATOID ARTHRITIS HIGHLY EXPANDED B CELL CLONES CAN BE FOUND IN THE EARLY SYNOVITIS STAGE

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10.1136/ard.2010.149005.15

Background B cells are thought to play an important role in the pathogenesis of rheumatoid arthritis (RA). We previously observed that highly expanded B-cell clones (HECs) were specifically present in inflamed synovial tissue (ST) compared to peripheral blood (PB) in a patient with established RA (ESRA). These findings suggest involvement of B cells via their B cell receptor (BCR), and local retention and/or proliferation of B cell clones in ST. Here we extend our patient group and include patients with early synovitis (ERA).

Objective To analyse the presence of expanded B-cell clones in paired samples from PB and ST during different stages of disease using a newly developed High-Throughput Sequencing (HTS) protocol.

Methods Six ACPA+ patients with active RA were included. Three patients were disease-modifying antirheumatic drug naïve and had synovitis for less than 1 year (ERA). Three patients had active disease despite methotrexate treatment and had a disease duration of more than 10 years (ESRA). mRNA was isolated from paired samples (PB and ST biopsies from arthritic ankle/knee), and linear amplification of the B-cell receptor heavy chain was performed with primers for all V-gene families. The amplified products encode the CDR3 of all analysed B cells, used as a 'fingerprint' for each clone. The samples were analysed using a Genome Sequencer (454). The frequency of clones was determined by custom bioinformatics algorithms identifying the CDR3 of each receptor (up to 10.000 receptors per sample). Clones with a frequency of ≥1% were arbitrarily considered as HECs.

Results HECs were detected in ST of both ERA and ESRA patients, with comparable frequencies in both stages (9 vs 11 clones resp.). Frequencies of HECs were as high as 53% in ERA, while this was 34% in ESRA. These HECs were either absent, or present in very low frequency in PB-samples. Surprisingly, HECs were also found in PB-samples of both groups (up to 30% in both groups), but the HECs recovered in PB were not found in ST.

Conclusion This is the first analysis of B cell clones in ST- and PB-samples comparing RA patients with different disease duration, using novel HTS technology. Our data suggests that ST-specific HECs can already be detected in early stages of synovial inflammation, and that the number of HECs does not change during disease. These findings suggest that specific BCRs are involved in the early phase of RA and provide a rationale for early anti-B cell therapy.