COMPLETE T AND B CELL RECEPTOR REPERTOIRE ANALYSIS IN RHEUMATOID ARTHRITIS USING HIGH THROUGHPUT SEQUENCING

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Background T cells and B cells are likely to have important roles in the pathogenesis of rheumatoid arthritis (RA). Previous attempts to investigate the role of T and B cell clones in RA by screening the T/B cell receptor (TCR/BCR) repertoires were hampered by the sheer size and complexity of the repertoires. Here the authors use a novel high throughput sequencing-based protocol which overcomes current technological limitations and produces DNA sequences of >100 000 receptors in a single experiment. Using this technique, the authors performed the first quantitative high-resolution analysis of the complete TCR and BCR repertoires in a patient with RA.

Objectives To describe the complete BCR and TCR repertoires in synovial tissue (ST) and peripheral blood (PB) samples of a patient with RA and to screen for dominant T and B cell clones.

Methods mRNA was isolated from PB and ST simultaneously taken from an aCCP+ RA patient with active disease despite treatment with methotrexate. A multiplex linear amplification with primers for all V(ariable)-families of the receptor β-chain (TCR) or heavy-chain (BCR) was performed. The samples were analysed on a Genome Sequencer FLX (Roche) resulting in 14 000 reads/samples for TCR and 35 000 for BCR analysis, each containing the full CDR3 sequence to identify clones. Bioinformatics algorithms were used to identify gene segments and correct for sequencing errors.

Results TCR-repertoire: In ST, most TCRs contained a Vβ6 (46%), 10 (19%) and 27 (13%) gene segment, while in PB Vβ29 (32%) and 7 (26%) were most frequent. The TCR repertoire was dominated by low-frequency clones (>95%), both in the PB and
ST. However, several clones were clearly expanded (up to 217 copies/clone in PB and 121 copies/clone in ST). Interestingly, dominant clones in ST differed from those in PB. BCR-reertoire: The ST sample showed preferential usage of the VH2, 5, 6 and 7 families (total 50%) when compared with published data in PB1 (20%). Several clearly expanded clones (up to 1892 copies) were found against a background of low-frequency clones.

**Conclusions** This is the first high-resolution analysis of the TCR and BCR repertoire in RA, providing a detailed insight into the presence of T and B cell clones. The authors observed clear differences between the TCR repertoire in ST compared with PB in a patient with RA. Several expanded clones were found only in ST, suggesting proliferation or local retention of T cells. The BCR repertoire also showed expanded clones within the ST. Further studies will elucidate the role of these clones in RA.

**REFERENCE**