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DISRUPTION OF DOMINANT B CELL/PLASMA CELL CLONES IN RHEUMATOID ARTHRITIS SYNOVIUM BY RITUXIMAB CORRELATES WITH TREATMENT RESPONSE

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Background Autoreactive B lymphocytes are thought to play an important role in rheumatoid arthritis (RA). B cell depletion

therapy by rituximab (RTX) has shown that targeting the humoral immune response can result in clinical improvement. Unfortunately, repopulation of B cells leads to disease relapse. Hence, analysis of the B cell/plasma cell compartment in synovium (ST) in patients undergoing RTX therapy might help to identify autoreactive cells responsible for disease persistence and relapse.

Objectives To compare the B cell/plasma cell repertoire in ST at baseline, 4 weeks and 16 weeks after RTX treatment using a newly developed next-generation sequencing protocol.

Methods Nine RA patients were included and treated with two intravenous infusions of 1000 mg RTX without additional methylprednisolone. At baseline, all patients fulfilled ACR criteria for RA, were ACPA+ and had a Disease Activity Score 28 (DAS28) >3.2. At week 24, all patients demonstrated moderate EULAR responses. mRNA was isolated from consecutive samples and full-repertoire analysis of the immunoglobulin heavy-chain was performed with primers for all V(ariable)-genes. All amplified products encoded the CDR3, a unique sequence that defines a unique clone. The number of sequences reflects the amount of immunoglobulins produced by that clone and can be used as a measure for 'dominance' of that particular clone. The samples were analysed using a GS-FLX/454 and custom bioinformatics algorithms (>10 000 sequences/sample). Clones with a frequency of >0.5% were arbitrarily considered as dominant clones.

Results Clones were detected in equal numbers at baseline and 4 weeks after RTX. However, after 16 weeks there was a significant decrease in the number of non-dominant clones compared to baseline (mean -47.7%, $p=0.03$). In line, the number of dominant clones significantly increased compared to baseline (mean +55.3%, $p=0.04$). In five patients 17.9% of the dominant clones at baseline was detectable after 16 weeks (mean, SD 8.0%), half of these were dominant at both time points (mean 8.8%, SD 4.6%). In the remaining four patients dominant clones at baseline were not retrieved 16 weeks after RTX. Interestingly, the latter group showed a better response to treatment as determined by a decrease in DAS28 score ($r^2=0.51$, $p=0.03$).

Conclusion Our observations suggest a disruption of dominant clones and a repopulation of distinctly different clones 16 weeks after rituximab. The persistence of dominant clones was associated with decreased treatment response. Further study of persisting dominant clones, especially during disease relapse, might help to identify and characterise disease-associated B cells and/or plasma cells.