Pharmacological induction of type I IFN activity following therapy with rituximab determines clinical response in rheumatoid arthritis

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Introduction
Rheumatoid Arthritis (RA) is a chronic systemic autoimmune disease characterized by inflammation of the joints. Rituximab, a chimeric monoclonal antibody against CD20, has shown to deplete effectively a large part of the B cell population thereby effectively modifying the symptoms of RA in patients who failed on anti-TNF treatment. Despite the fact that rituximab directly depletes specific B-cell populations in all patients treated, the existence of interindividual differences in clinical outcome of rituximab therapy has raised questions regarding the mechanism of action. In order to provide insight in the biological basis for the clinical response towards rituximab we evaluated the pharmacological effects of rituximab using genome-wide gene expression technology in whole blood of RA patients.

Aim
1. Investigate pharmacological response to B cell depletions on gene expression level in peripheral blood of RA patients
2. Identification of biological processes underlying non-responsiveness to treatment in order to identify non-responders at forehand.

Methods
RNA was isolated and gene expression profiles were obtained from the 15 patients at baseline (t=0) and three (t=3) and six (t=6) months after start of treatment. Gene-expression profiling was performed using illumina HumanHT 12- vs bead chips. Clinical responder status was determined after six months by change in disease activity score (ΔDAS).

Results
Pharmacological induced changes in IFN response activity correlate with clinical responsiveness

For the identification of gene patterns that associate with responder status we studied the correlation between the pharmacological response, which is the induction of gene expression levels over three months (ratio t=3/t=0) and clinical responder status at 6 months following start of therapy.

Figure 1: Cluster diagrams of genes that were differentially regulated by rituximab between RA patients (A) Unsupervised (two-way) hierarchical cluster analysis of a set of 154 genes revealed a marked interindividual variation in the pharmacological response to rituximab between RA patients. (B) Supervised (one-way) cluster analysis revealed a set of 1 type I IFN-response genes associated with clinical outcome. Patients were stratified based on changes in Disease Activity Score (ΔDAS) at 6 months after the start of therapy. (C) Cluster of type I IFN-response genes, which is related to clinical responder status.

Figure 2: Differential regulation of type I IFN-response gene activity upon rituximab therapy is related to clinical responder status (A) Comparison of the type I IFN response score in peripheral blood cells in responders compared to non-responders (ratio t=3/t=13) (n=13). (B) and (C) Patients were divided into two groups based on rituximab induced changes in type I IFN-response score (ratio t=3/t=13) at a cut-off of 1.1-fold (> 0.15 log2 based). The groups were compared to each other with respect to ΔDAS improvement (B) or EULAR responder status (C). (D) The expression levels of RSAD2 were determined by qPCR in peripheral blood cells from RA patients of an independent validation cohort (n=8) and for each patient the level of induction (ratio t=3/t=13, log2 space) was calculated.

Figure 3: Pharmacodynamics of the IFN type I-response activity during rituximab treatment reveals marked differences between responders and non-responders. Shown are pharmacodynamic measurements in 13 RA patients of a set of 6 type I IFN response genes (IFI44, IFI44L, HERC5, RSAD2, LY6E, and Mx1) (left y-axis) and B-cell counts based on CD19 cytomery (right y-axis) at baseline, 3 (t3) and 6 months (t6). Patients were stratified in responders and non-responders based on ΔDAS. Marked differences between responders and non-responders for baseline IFN-response activity (ΔDAS p = 0.0062; EULAR p = 0.016, respectively) and the rituximab induced increase in IFN type I-activity at 3 months (ratio t=3/t=0) (ΔDAS p = 0.049) were observed. The change in IFN response activity during rituximab therapy negatively correlated with the corresponding baseline level, although no significance was reached (p = 0.0576 and R = 0.53).

Conclusion
An increase in IFN-response activity during rituximab treatment in RA patients is associated with a favorable response and may provide insight in the biological mechanism underlying the therapeutic response.

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