Rituximab Significantly Diminishes CD86^{+} B Cells in Systemic Sclerosis

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BACKGROUND

Rituximab (RTX) is a monoclonal antibody that targets the CD20 surface marker. This results in a reduction of CD20^{+} immune cell populations, foremost B cells. B cell depletion via RTX was found to be beneficial for patients suffering from systemic sclerosis (SSc), leading to an improvement of skin fibrosis and autoimmunity [1,2]. However, little is known about the influence of RTX on specific B subsets in SSc.

OBJECTIVE

The purpose of this study was to further characterize the effect of RTX on B cell populations in patients with SSc.

MATERIAL & METHODS

Peripheral blood samples from 37 patients suffering from SSc (mean age: 54 years ± 1.64 SEM, female ratio: 0.78) and 10 age-matched healthy participants were drawn over a sampling period of two years (Table 1). 20 SSc patients in this study group were at the time under RTX treatment and the modified Rodnan Skin score (mRSS) was measured before and after treatment start. The percentage of CD19$^{+/-}$, CD20$^{+/-}$ lymphatic cells and highly CD19^{+}CD20^{+} B cells co-expressing either CD5, CD24, CD27 or CD86 on their surface was done by staining freshly isolated PBMCs (Fig.1). A quantitative flow cytometric bead-based assay (QuantiBRITE PE kit from Becton Dickinson) was used for the estimation of CD19 antibodies bound per cell. All cytometric measurements were performed using a standardized BD LSR Fortessa platform.

Table 1 Patient characteristics

Patient characteristics	
sample size	37
Gender	
Female, n (%)	29 (78.4 %)
Age, years ± SD	54.5 ± 1.64
disease duration, , years ± SD	7.35 ± 10.41
subtypes, n (%)	
limited form	18 (48.6 %)
diffuse form	17 (46 %)
Overlap SSc/RA	1 (2.7 %)
Overlap SSc/Polymyositis	1 (2.7 %)
Clinical parameters	
SSc AS	1.1 ± 1.2
SSc SS	3.8 ± 1.1
mRSS	8.1 ± 7.0
DLCO, (%)	70.9 ± 17.0
Treatment, n (%)	
Rituximab/Mabthera (RTX)	20 (54.1 %)
mRSS before RTX treament	19.7 ± 10.7
mRSS after RTX treament	8.1 ± 7.0
DLCO before RTX treatment	69.0 ± 20.2
DLCO after RTX treatment	70.9 ± 17.0

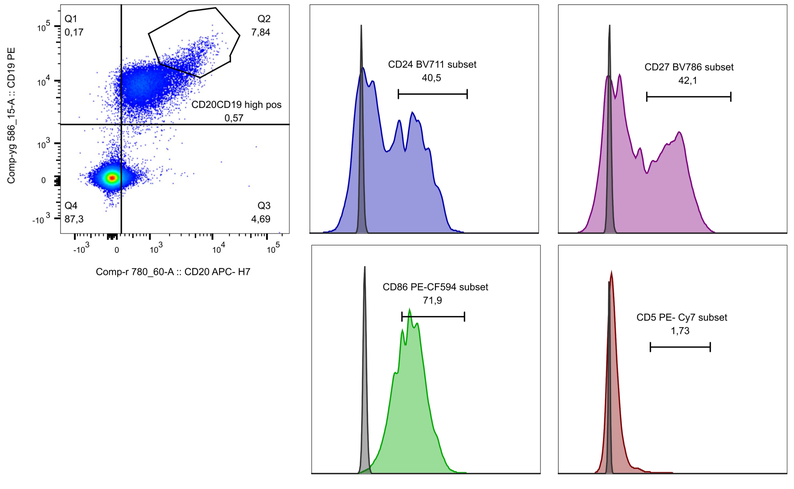

Figure 1 Gating strategy. Highly CD19^{+}CD20^{+} B cells co-expressing either CD24, CD27, CD86 or CD5 on their surface were identified using flow cytometry.

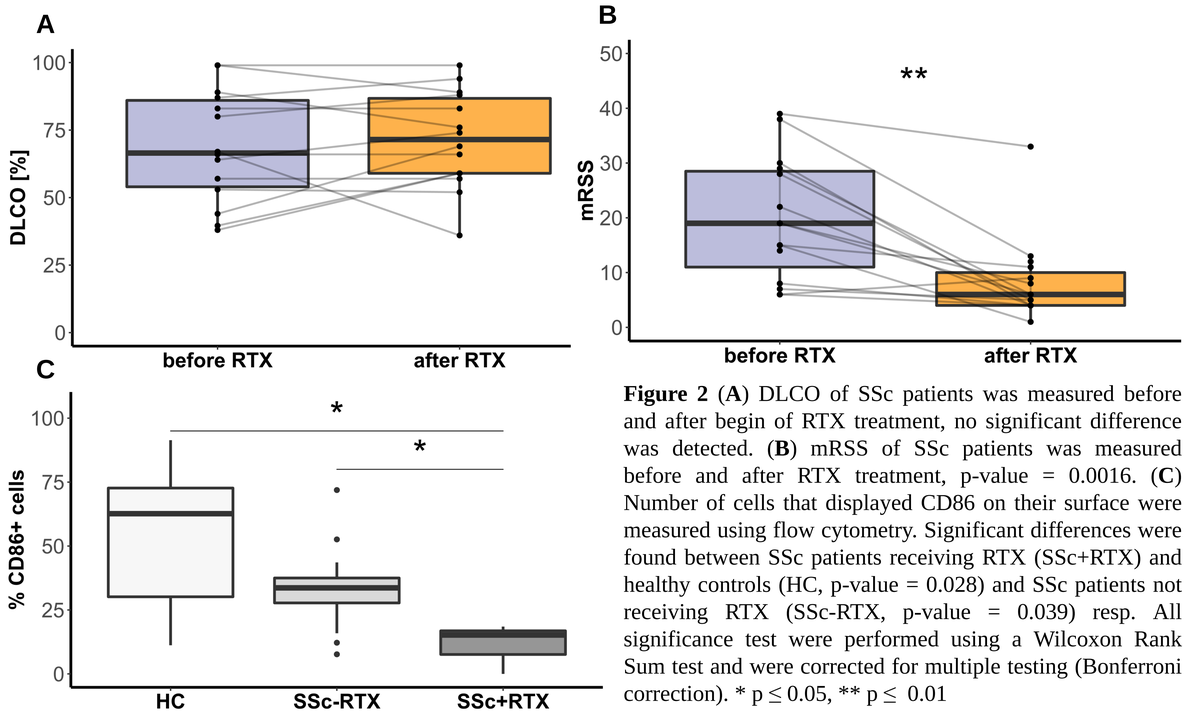

Figure 2 (**A**) DLCO of SSc patients was measured before and after begin of RTX treatment, no significant difference was detected. (**B**) mRSS of SSc patients was measured before and after RTX treatment, p-value = 0.0016. (**C**) Number of cells that displayed CD86 on their surface were measured using flow cytometry. Significant differences were found between SSc patients receiving RTX (SSc+RTX) and healthy controls (HC, p-value = 0.028) and SSc patients not receiving RTX (SSc-RTX, p-value = 0.039) resp. All significance test were performed using a Wilcoxon Rank Sum test and were corrected for multiple testing (Bonferroni correction). * $p \leq 0.05$, ** $p \leq 0.01$

RESULTS

RTX induced a significant decrease in mRSS from 19.7 ± 2.8 to 8.1 ± 1.7 (mean± SEM; p = 0.0016) (Fig. 2B) but no difference in DLCO (Fig. 2A). In addition, CD19^{-}CD20^{+} cells were significantly diminished as a result of the treatment. Thus, the frequency of CD19^{-}CD20^{+} cells in the non-treatment group was 1.3% ± 0.3% compared to 0.7% ± 0.2% (p = 0.048). Within the B cell population 33.3% ± 3.8% were positive for CD86, a checkpoint molecule for the activation of T cells during an immune response. RTX treatment significantly decreased this B cell population to 11.2% ± 4.7% (p = 0.039) (Fig. 2C).

CONCLUSION

RTX treatment in SSc might not only be effective by reducing B cells but also by down regulation of the CD86 B cell surface marker on B cells. This would indicate that B cells under RTX treatment are less capable of activating T cells.

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