Rituximab modulates the expression of IL-22 in the salivary glands of patients with primary Sjogren's syndrome

We have recently demonstrated that interleukin (IL)-22, mainly produced by T-helper 17 effector cells, natural killer (NK)p44 +NK cells and epithelial cells, may be potentially involved in the pathogenesis of primary Sjogren's syndrome (pSS).[1] The IL-22/IL-22R pathway is known to play a role in the emergence of T and B-cell lymphoma[2 3] and pSS is considered a risk factor for the development of lymphoma.[4]

Rituximab, which has historically been used for the treatment of B-cell lymphoma,[5] has also been considered to be effective in the therapy of pSS.[6]

Ten consecutive patients with pSS (eight women and two men, with a mean duration of disease of 48±18 months), diagnosed according to the American–European Consensus Group criteria for pSS,[7] who were treated with two courses of intravenous infusions of 1000 mg rituximab (Roche, Woerden, The Netherlands) at days 1 and 15, at baseline and then after 6 months, were considered for this study. After 48 weeks the patients again underwent salivary gland biopsy. The demographic, clinical and histological characteristics of the patients are shown in table 1. Ethics approval was granted and written consent was obtained from all patients. Evaluation of the clinical efficacy of rituximab treatment was done by measuring the improvement between day 1 and week 48 in the values of salivary and lacrimal gland function. Unstimulated whole, parotid and submandibular/sublingual saliva samples were collected, as described by Meijer *et al*.[8] Lacrimal gland function was evaluated by performing the Schirmer's test. Immunohistochemistry for IL-22 was performed, as previously described, on paraffin-embedded salivary gland biopsies by using a rabbit anti-human IL-22 antibody.[1] The number of IL-22$^+$ cells was determined by counting, in a blinded fashion before and after rituximab treatment, IL-22 immunoreactive cells on photomicrographs obtained from three random high-power microscopic fields (400× magnification). Intense IL-22 staining was observed among infiltrating mononuclear cells, epithelial and myoepithelial cells. Rituximab treatment significantly reduced the number of IL-22$^+$ cells infiltrating the salivary glands of pSS patients (table 1 and figure 1A,B). The expression of IL-22 by epithelial cells was also reduced in those patients displaying a lower focus score (table 1 and figure 1C,D). Interestingly, rituximab treatment specifically abolished the production of IL-22 by myoepithelial cells (figure 1E,F). The whole saliva flow rate and the lacrimal gland function showed a significant improvement after rituximab therapy from baseline to week 48 (whole saliva flow, ml/min (mean±SD) 0.24±0.12 at baseline and 0.45±0.15 at week 48, p=0.0003; Schirmer's test, mm/5 min (mean±SD) 4.7±1.5 at baseline and 8.7±2.11 at 48 weeks, p=0.00011)). A trend (although not statistically significant) towards a major improvement in salivary and lacrimal glands was detected in those patients with a major reduction in the numbers of IL-22$^+$ cells after rituximab therapy.

Although we cannot exclude the possibility that the reduction in IL-22 may be due to the natural history of the disease, these preliminary results suggest that rituximab therapy may modify the immunological micro-environment of inflamed salivary glands of pSS patients reducing the local expression of IL-22, providing an additional immunological explanation for rituximab efficacy in pSS. The immunological effects of rituximab therapy beyond B-cell depletion have recently been demonstrated. In this regard, a reduction in the T-helper 17 response in the synovial tissues of patients with rheumatoid arthritis has been observed after rituximab therapy.[9] Even if a possible explanation for these immunological changes could reside in the reduction of the presentation of antigen from B to T cells, the exact mechanisms by which rituximab therapy modifies the IL-22 response in pSS remain to be elucidated. As with the role of IL-22/IL-22RA1 in the pathogenesis of B and T-cell lymphoma,[2 3] the rituximab-dependent reduction of IL-22 expression may also be of relevance in reducing the risk of the evolution of pSS towards lymphoma.

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Table 1 Clinical and histological characteristics of the pSS patients

	Age/ sex	Disease duration (months)	ENA anti-RoSSA/ LaSSB	Focus score pre/ post-rituximab	N of IL-22$^+$ infiltrating cells pre/ post-rituximab	N of IL-22$^+$ myoepithelial cells pre/post-rituximab	Saliva flow rate ml/min pre/post-rituximab (mean)	Schirmer's test, mm/5 min (mean)
Patient 1	38/F	44	+/+	3/2	41/22	30/18	0.2/0.4	5/10
Patient 2	44/F	22	+/–	4/4	54/24	22/16	0.3/0.5	6/12
Patient 3	52/F	36	+/+	4/3	44/33	18/13	0.13/0.33	5/8
Patient 4	43/F	28	+/+	3/3	41/18	21/9	0.18/0.5	4/9
Patient 5	45/F	48	–/–	2/2	34/19	18/7	0,25/0.6	7/8
Patient 6	39/F	96	–/–	3/3	28/21	24/11	0,2/0.3	6/6
Patient 7	54/F	60	+/+	2/2	23/16	13/6	0.16/0.33	3/7
Patient 8	42/F	96	+/+	3/2	22/11	18/9	0.13/0.45	2/9
Patient 9	72/M	60	–/–	4/3	18/14	23/10	0.4/0.6	4/12
Patient 10	52/M	48	+/–	3/3	28/21	26/14	0.5/0.88	5/9

pSS, primary Sjogren's syndrome; ENA, Extractable Nuclear Antigen.

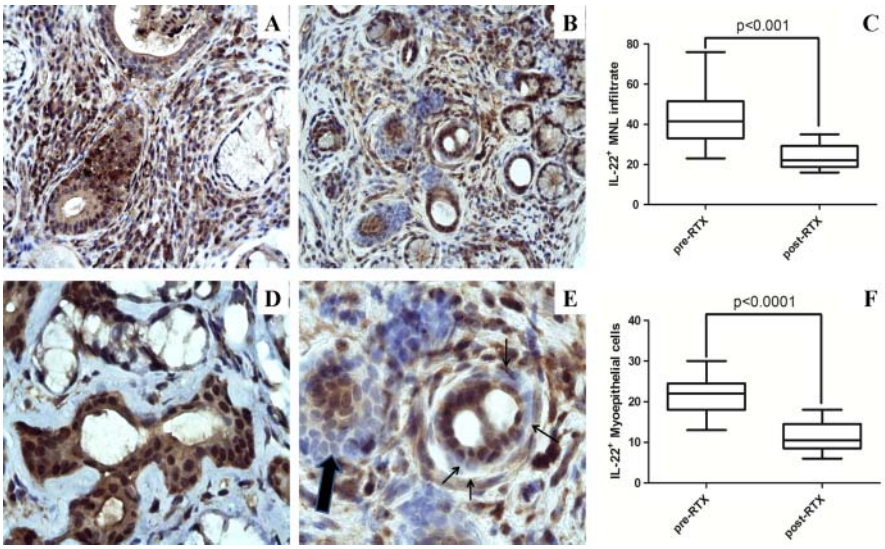

Figure 1 IL-22 expression in salivary glands of patients with primary Sjogren's syndrome (pSS) is modified by rituximab (RTX) treatment. Representative immunostaining for IL-22 in the pSS patient number 3 before and after therapy with rituximab. Positive infiltrating mononuclear cells observed before therapy (A) were significantly reduced after rituximab treatment (B) The intense immunostaining observed at baseline in myoepithelial cells (D) was also drastically reduced in rituximab-treated patients (arrows) (E). (C–F) immunohistochemical quantification of IL-22$^+$ infiltrating cells (C) and myoepithelial cells (F) in salivary gland biopsies from pSS before and after rituximab therapy. Results are expressed as the number of positive cells per field. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the 10th and 90th percentiles. (A, B) original magnification × 25; (D, E) original magnification × 63.

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