Targeting the humoral immune system of patients with rheumatoid arthritis

Teng, Y.K.O.

Chapter 7

CD20 epitope masking by rituximab

Teng YKO
Ioan-Facsinay A
van Laar JM

Arthritis & Rheumatism 2008; 58: 634-636

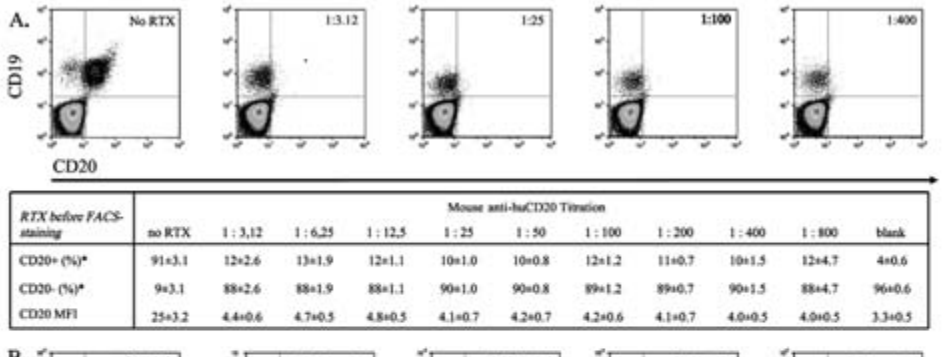
Rituximab, an anti-CD20 B-cell depleting monoclonal antibody (mAb), is increasingly used in the treatment of refractory autoimmune diseases, such as rheumatoid arthritis[1,2] and systemic lupus erythematosus[3]. Mouse anti-human mAb against the pan-B-cell markers CD20 and/or CD19 are generally used for flow cytometric and immunohistochemical analyses of blood and tissue to assess the extent of B-cell depletion. Teeling *et al.* recently reported that all mouse anti-human CD20 mAb, including rituximab, bind the large extracellular loop of the CD20 membrane protein[4]. Therefore, it is conceivable that cellular analyses of rituximab-treated patients using anti-CD20 antibodies are influenced by masking of the CD20 epitope by rituximab. In order to investigate this, we performed a flow cytometric competition assay with rituximab and a commonly used mouse anti-human mAb against CD20.

Peripheral blood mononuclear cells from 4 healthy volunteers were obtained through isolation over a Ficoll gradient and freshly processed for 2 sets of experiments, as follows. In one experiment, samples were preincubated with 0.67 mg/ml rituximab, washed vigorously in phosphate buffered saline-1% bovine serum albumin, and then stained with increasing concentrations of fluorescein isothiocyanate (FITC)-conjugated anti-CD20 (clone 2H7) in combination with an optimal concentration of phyco-erythrin (PE)-conjugated anti-CD19 (clone H1B19) (all from Becton Dickinson, San Jose, CA). In the second experiment, samples were stained with optimal concentrations of FITC-conjugated anti-CD20 (clone 2H7) and PE-conjugated anti-CD19 (clone H1B19) and subsequently incubated with increasing concentrations of rituximab. Thereafter, samples were vigorously washed, and data were collected using a FACSCalibur flow cytometer and analyzed with the Flow Jo software program (Tree Star, Ashland, OR).

Preincubation with rituximab before flow cytometric staining led to a significant decrease in the number of CD20+, CD19+ B-cells (from a mean ± SEM of 91 ± 3.1% to 12 ± 2.6%; P=0.004) and a significant reduction in the mean fluorescence intensity (MFI) of CD20 (from 25 ± 3.2 to 4.4 ± 0.6; P=0.024), even with a large surplus of clone 2H7 mAb. When B-cells were first double-stained for CD19 and CD20 and then incubated with increasing concentrations of rituximab, CD19+ B-cells gradually lost their CD20 expression (MFI 17 ± 2.4 without rituximab and 6.4 ± 1.0 after incubation with 2.67 mg/ml rituximab;

P=0.017). Importantly, the percentages of CD19+ B-cells in the pre-rituximab incubation experiments compared with the post-rituximab incubation experiments were constant (6.1 ± 2.1% and 6.8 ± 1.8%, respectively; *P*=0.34).

The effective competition of rituximab against cell-bound anti-CD20 antibody and the failure of increasing concentrations of 2H7 to dislodge rituximab after binding to B-cells indicate that rituximab binds with higher affinity to the CD20 epitope than does 2H7. Our findings suggest that data based on CD20 staining of B-cells, as in Gunnarsson and colleagues' study, should be interpreted with caution. Importantly, CD19 expression on B-cells was not influenced by rituximab, and therefore, studies using rituximab should include pan-B-cell markers, such as CD19, CD79a, or CD22, to corroborate data on CD20 expression and/or cytoplasmatic CD20 staining.



RTX before FACS-staining	Mouse anti-huCD20 Titration										
	no RTX	1 : 3,12	1 : 6,25	1 : 12,5	1 : 25	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	blank
CD20+ (%)*	91±3.1	12±2.6	13±1.9	12±1.1	10±1.0	10±0.8	12±1.2	11±0.7	10±1.5	12±4.7	4±0.6
CD20- (%)*	9±3.1	88±2.6	88±1.9	88±1.1	90±1.0	90±0.8	89±1.2	89±0.7	90±1.5	88±4.7	96±0.6
CD20 MFI	25±3.2	4.4±0.6	4.7±0.5	4.8±0.5	4.1±0.7	4.2±0.7	4.2±0.6	4.1±0.7	4.0±0.5	4.0±0.5	3.3±0.5

RTX after FACS-staining	Rituximab concentration (mg/mL)											
	0	0.0025	0.005	0.01	0.02	0.04	0.08	0.17	0.33	0.67	1.33	2.67
CD20+ (%)*	87±3.4	80±3.6	73±4.4	65±6.3	63±5.7	60±5.5	51±7.4	45±7.5	42±5.7	36±5.7	31±4.3	28±4.0
CD20- (%)*	13±3.4	20±3.6	27±4.4	35±6.3	37±5.7	41±5.5	49±7.4	55±7.5	58±5.7	64±5.7	69±4.3	72±4.0
CD20 MFI	17±2.4	15±2.5	15±2.3	12±0.5	12±0.5	11±0.3	9.2±1.0	8.6±1.1	8.1±1.2	7.4±1.1	6.8±1.0	6.4±1.0

* Depicted are percentages of CD19+ B-cells

Figure 1

References

1. Gunnarsson I et al. Histopathologic and clinical outcome of rituximab treatment in patients with cyclophosphamide-resistant proliferative lupus nephritis. *Arthritis Rheum* **56**: 1263-72 (2007)
2. Cohen SB *et al.* Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* **54**: 2793-806 (2006)
3. Anolik JH *et al.* Rituximab improves peripheral B-cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum* **50:** 3580-90 (2004)
4. Teeling JL *et al.* The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. *J Immunol* **177**: 362-71 (2006)