

## Decreased CD20 expression in rheumatoid arthritis synovium following 8 weeks of rituximab therapy

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Dr. Walsh's work is supported by a clinical research training fellowship funded by the Health Research Board of Ireland.

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Received on June 4, 2007; accepted in revised form on December 14, 2007.

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**Key words:** Rituximab, rheumatoid arthritis, synovium.

### Conflict of interest:

Dr. D.J. Veale has received unrestricted educational grants and support for nursing staff. The other co-authors have declared no competing interests.

### ABSTRACT

**Objective.** To characterise the effects of rituximab on synovial tissue of patients with refractory rheumatoid arthritis (RA).

**Methods.** Arthroscopic biopsy of knee joint synovium was performed on 6 patients with seropositive RA prior to commencing rituximab. Four patients underwent repeat biopsy eight weeks following completion of their rituximab infusion schedule. Cryostat sections of synovium were prepared and stained with mouse monoclonal specific antibodies including CD20, plasma cell antibody and CD68.

**Results.** Eight weeks after treatment mean DAS28 fell from  $6.6 \pm 0.43$  to  $4.7 \pm 0.49$  ( $p=0.068$ ). Mean CRP fell from  $86.7 \pm 27$  mg/L to  $20.5 \pm 7$  mg/L ( $p<0.05$ ). Subsynovial CD20<sup>+</sup> B cells were demonstrated in all six patients at baseline. B cells were completely depleted in two patients at follow-up biopsy. Complete depletion was associated with excellent clinical response. No change in subsynovial B cells was seen in one patient. One patient's follow-up arthroscopy yielded inadequate tissue. A reduction was also seen in subsynovial plasma cells and CD68<sup>+</sup> cells after treatment.

**Conclusion.** B cells were present in synovial tissue of all patients with refractory RA. Complete depletion of B cells was associated with an excellent clinical response. These preliminary results suggest that early depletion of synovial B cells precedes a decrease in local inflammation leading to clinical improvement.

### Introduction

Rituximab is a chimeric anti-CD20 monoclonal antibody, which is often effective in the treatment of rheumatoid arthritis (RA) refractory to both disease modifying anti-rheumatic drugs (DMARDs) and anti-tumour necrosis factor-alpha (anti-TNF- $\alpha$ ) therapies (1-4). Rituximab depletes circulating B cells (5, 6) and depletes salivary gland B cells in patients with Sjögren's syndrome (7) but little is known of its effects on synovial tissue B cells.

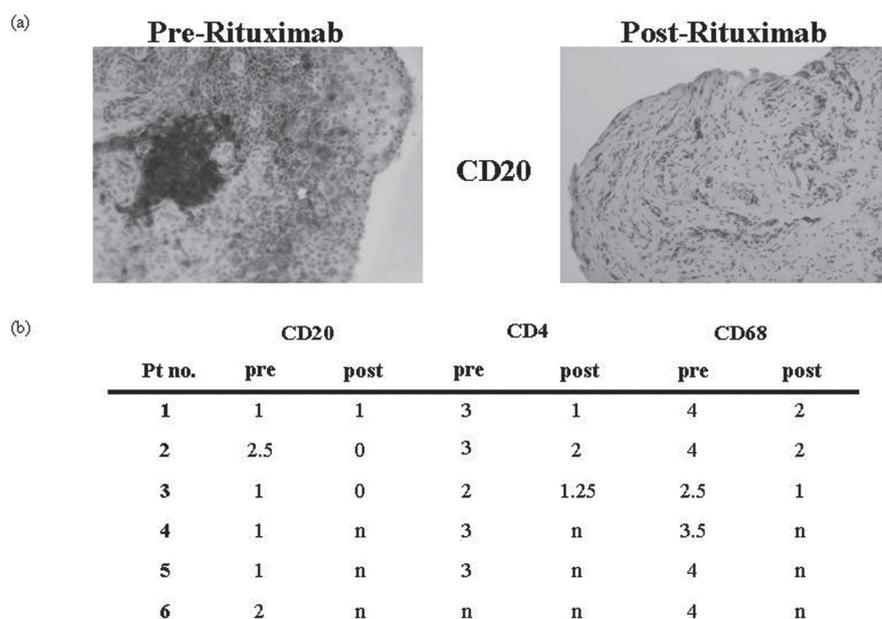
### Materials and methods

Arthroscopic biopsy of knee joint synovium was performed under local

anaesthetic on 6 patients (3 female) with seropositive RA immediately prior to commencing rituximab. All had active knee joint synovitis. To minimise sample error, synovial biopsies were obtained under direct visualization from a site of inflamed tissue pre and post treatment, snap-frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . Patients gave written and fully informed consent and ethical approval was obtained from the Medical Research and Ethics Committee at St. Vincent's University Hospital. Mean age was 55 years (range 50-62); disease duration 10.5 years (range 2.5-26). All patients had failed at least two DMARDs including methotrexate. In addition, all had demonstrated an inadequate response to anti-TNF- $\alpha$  therapies, two had failed two anti-TNF- $\alpha$  therapies and one had failed three. At baseline, three were receiving stable dose corticosteroids ( $<10\text{mg}$  prednisolone/day).

Patients received rituximab 1 gram intravenously (IV) on day 1 and 15. All patients received premedication with methylprednisolone 100 mg IV and continued a reducing dose of oral prednisolone (60mg/day to baseline dose). Four patients (unable to tolerate methotrexate) also received adjunct IV cyclophosphamide on days 3 and 17. Two patients continued regular methotrexate therapy. Infusions were well tolerated and no serious adverse events were reported. Four patients agreed to a repeat biopsy eight weeks following completion of their rituximab infusion schedule.

Cryostat sections were prepared and stained with mouse monoclonal specific antibodies against CD20, plasma cells, CD68, CD3, CD4 and CD8 (DAKO, Denmark) as described previously (8). All sections had at least a moderately intense mononuclear infiltrate and an intact lining layer which was assessed by two independent blinded observers using an established and validated semi-quantitative scoring method (0-4): 0 = no positively stained cells, 1 =  $<24\%$  cells positively stained, 2 = 25-50% cells positively stained, 3 = 50-74% cells positively stained and 4 = 75-100% cells positively stained (9, 10). Statistical analysis of clinical data was performed



**Fig. 1.** (a) synovial tissue CD20 staining before and after rituximab therapy in sample patient (patient no. 2) and (b) semi-quantitative analysis (0-4) before and after therapy in individual patients (n=not available).

using SPSS version 11.0. Differences were assessed using the Wilcoxon signed-ranks test for paired data, where  $p < 0.05$  was deemed significant.

## Results

Eight weeks after treatment, five patients demonstrated meaningful clinical improvements (fall in DAS28 > 1.2). Mean baseline DAS28 ( $\pm$ SEM) fell from  $6.6 \pm 0.43$  to  $4.7 \pm 0.49$  ( $p = 0.068$ ), tender joint count from 18.2 to 7.2 ( $p = 0.068$ ) and mean swollen joint count from 15.2 to 6.6 ( $p = 0.11$ ). The mean CRP fell from  $86.7 \pm 27$  mg/L to  $20.5 \pm 7$  mg/L ( $p < 0.05$ ).

At baseline, subsynovial CD20+ cells were demonstrated in all six patients (mean score  $\pm$ SEM  $1.4 \pm 1.25$ ) (Fig. 1). Follow-up tissue sections were inadequate for analysis in one patient. At 8 weeks, subsynovial CD20+ cells were completely depleted in two, including one who demonstrated a Grade III B cell aggregates (>10 B cells in maximum radius) at baseline (11). Total depletion of B cells was accompanied by an excellent early clinical response with falls in DAS28 from 7.4 to 4.2 and from 7.0 to 4.0. No change in sublining B cells was seen in one patient.

The mean baseline and 8-week sublining plasma cell scores were  $3.6 \pm 0.2$  and  $3.0 \pm 0.6$ , respectively. The mean

sublining CD68+ cell score fell from  $3.7 \pm 0.3$  to  $1.7 \pm 0.3$ . Mean CD3+ cell score fell from  $1.8 \pm 0.2$  to  $1.0 \pm 0$  and CD4+ cells from  $2.8 \pm 0.2$  to  $1.4 \pm 0.3$ . No change was seen in CD8+ cell infiltrate (mean 1.0).

## Discussion

In this limited study, we observed B cells in synovial tissue biopsies from all patients with active RA who had responded inadequately to a range of DMARDs and anti-TNF- $\alpha$  therapies. We observed early clinical responses to rituximab at 8 weeks. A rapid clinical response was associated with total depletion of synovial B cells. Vos *et al.* demonstrated a significant and specific reduction in sublining B cells 4 weeks after rituximab infusion before demonstrable change in DAS28 or other infiltrating cell subtypes (including T cells and macrophages) had occurred (12). An association between the clinical response to rituximab and synovial B cell depletion has been highlighted in other studies (13, 14). In our study we examined the effect of rituximab on synovial tissue 8 weeks after completion of the infusion course which may account for the depletion of synovial B cells being non-specific. In particular, the clinical response to rituximab was also associated with a marked reduction in

sublining CD68+ cells, consistent with the suggestion that sublining CD68 is a biomarker of the therapeutic response (15). Taken together, these preliminary studies of rituximab therapy suggest that early depletion of synovial B cells precedes a decrease in local inflammation leading to clinical improvement.

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