RITUXIMAB THERAPY MARKEDLY REDUCES CSF T AND B CELL POPULATIONS IN MS

MSRCNY

Niamh B O'Hara, Jacqueline Dinzey, Jessie Kerr, Nicola R Donelan and Saud A. Sadiq

Multiple Sclerosis Research Center of New York

INTRODUCTION

Rituximab, a monoclonal antibody directed against CD20+ B-cells, significantly reduces disease activity in MS as indicated by brain MRI gadolinium scans (AAN 2007). Although humoral immune responses are increasingly implicated in the pathogenesis of MS, the degree of reported efficacy in early clinical trials is difficult to explain on the basis of isolated B-cell antagonism. However, if B-cells are involved as antigen presenting cells to T-cells, a marked reduction in B-cell population would inhibit CNS T-cell recruitment and amplification responses thereby decreasing CSF T-cell numbers. This pilot study investigated intrathecal B- and T-cell responses to rituximab therapy.

DESIGN AND METHODS

Patient Sample

Five patients with treatment resistant clinically definite MS were selected as shown in Table 1.

Patient	Disease Type	Age	Sex	Disease Duration (years)	EDSS	Previous Treatment
1	SPMS	47	M	18	7.0	β-IFN, steroids, mitoxantrone, natalizumab.
2	SPMS	41	F	11	6.5	copaxone, β-IFN, steroids, IVIG, natalizumab.
3	PPMS	50	M	17	6.5	copaxone, β-IFN , steroids, methotrexate.
4	PPMS	54	F	8	7.0	β-IFN, steroids, IVIG, mitoxantrone, methotrexate, natalizumab.
5	SPMS	45	F	24	6.5	β-IFN, steroids, mitoxantrone, methotrexate.

Table 1. Patient demographics

All disease modifying agents were discontinued three months prior to this study. Patients 1, 4 and 5 received mitoxantrone more than five years prior to this study. All five patients had significant spasticity and were treated with intrathecal baclofen via surgically implanted pumps. These pumps were implanted at least 3 years prior to this study. This allowed for easy and frequent access to CSF and obviated the need for frequent lumbar punctures.

Treatment Protocol

Informed consent was obtained for the IRB approved study. Rituximab was given at a dose of 1000 mg intravenously over six hours on day 1 and day 14/15. Peripheral blood lymphocyte counts and cerebrospinal fluid (CSF) B- and T- cell counts were determined by FACS analysis prior to rituximab treatment and at 2,4,8 and 12 weeks post-treatment. All peripheral blood analysis was done commercially.

CSF Analysis

Cells from the CSF were isolated for FACS analysis by centrifugation at 200 g for 15 minutes. The cells were brought up in 100 ul of 2% FBS in PBS and a cell count was done by hematocytometer. Cells were blocked for 2 minutes at room temperature using mouse IgG. Immunostaining was conducted using 5 ul of CD3-FITC and CD19-APC antibodies/fluorochromes on 60% of the cells, while 20% of the cells were used for an unstained control and 20% of the cells were frozen. During staining cells were incubated on ice for 30 mins and then brought up to 500 ul using 2% FBS. FACS analysis was conducted, using the unstained control cells to determine baseline fluorescence.

Results

Peripheral Blood Results

All patients had normal pre-treatment total lymphocyte and B and T cell subset values. As anticipated, following rituximab treatment, B lymphocytes were essentially completely eliminated. Peripheral T cells fluctuated in individual patients within the normal range. The mean values for the 5 patients are shown in Table 2.

Cell Type	Pre-treatment (cells/cu.mm)	Post-treatment (cells/cu.mm)	Normal values (cells/cu.mm)
CD3+ cells (T-cells)	1082	935	840-3060
CD19+ cells (B-cells)	533	1	110-660

Table 2. Pre and post-treatment peripheral lymphocyte counts (mean of n=5).

CSF Results

B-cell CSF count in all five patients pre-treatment were relatively low and became undetectable in 4 of 5 patients post-treatment. In the other patient as shown in Figures 1 and 2, a steady decline in B-cells was seen. Post-treatment, the decrease in CSF B-cell counts appeared to lag behind the precipitous fall in peripheral B-cell counts.

A. B.

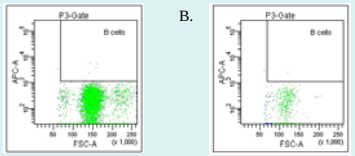

Figure 1. FACS analysis scatter plots of CSF B-cell lymphocyte counts for patient 1. (A) Pre-treatment (B) Eight weeks Post-treatment

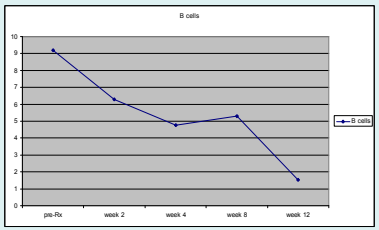

Figure 2. CSF B-cell lymphocyte counts depicting temporal decline post-treatment (patient 1).

CSF T-cell counts steadily declined post-treatment in patient 1 as shown in Figures 3 and 4. In addition, two other patients had a similar pattern of steady T-cell decline, but the decline was most noticeable 4 weeks post-treatment 1 and then maintained for the study period. In 2/5 patients the T-cell counts appeared to fluctuate with a paradoxical increase seen at 2 and 4 weeks followed by a reduction by week 8-12. (Data not shown) In contrast, to the effect on B-cells where the peripheral blood and CSF numbers decreased post-treatment, the CSF T-cells counts appeared independent of the corresponding peripheral numbers.

A. B.

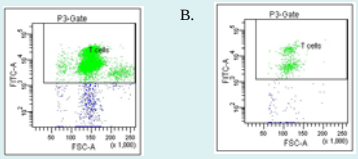

Figure 3. FACS analysis scatter plots of CSF T-cell lymphocyte counts for patient 1. (A) Pre-treatment (B) Eight weeks Post-treatment

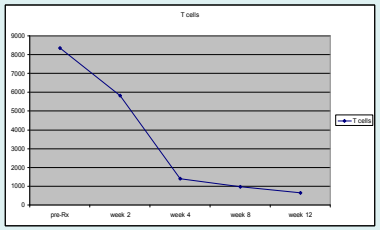

Figure 4. CSF T-cell lymphocyte counts depicting temporal decline post-treatment (patient 1).

Summary of CSF Results

Rituximab treatment had a profound effect on CSF lymphocytes in all 5 treated patients as shown in Table 3. The decrease in cell counts persisted for the study period.

Cell Type	Pre-treatment (total cells)	Post-treatment (total cells)	% Reduction
CD3+ cells (T-cells)	3061	565	82
CD19+ cells (B-cells)	7	<1	100

Table 3. Pre and post-treatment mean CSF lymphocyte count of 5 patients.

CONCLUSIONS AND RELEVANCE

1. As expected rituximab, a monoclonal antibody directed at CD20 (B-cells), reduces both peripheral and CSF B lymphocytes.
2. In our pilot study rituximab treatment reduced CSF T-cell counts by over 80%, whereas peripheral T-cell counts appeared unchanged.
3. These preliminary findings suggest that the therapeutic efficacy of rituximab may not solely be a result of B-cell depletion.
4. The mechanism by which CNS T-cell depletion occurs needs further investigation, but may be a consequence of the lack of B-cell antigen presentation.
5. These findings need to be validated in a larger number of patients in a study of greater duration.