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The effects of intrathecal rituximab on biomarkers in multiple sclerosis

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ABSTRACT

Objectives: Clinical trials of IV-rituximab have proved successful. It is unclear whether intrathecal (IT)-rituximab is more efficacious at lower doses. We examine its effects on B-cell biomarkers.
Methods: MS patients received IT-rituximab at 3 time-points. CSF and serum samples were obtained at up to 5 time-points (days 0, 7, 14, 56 and 112).

Serum and CSF BAFF and CXCL13, and CSF kappa and lambda free light chains (FLC) were measured. Flow cytometry was performed, examining effects on lymphocytes, CD3-19+ and CD3-20+ cells.
Results: CSF BAFF fell following rituximab ($p=0.0091$ absolute values, $p=0.0284$ change from baseline) whilst serum BAFF increased across time-points 1–4 ($p=0.0005$ absolute values, $p=0.0017$ change from baseline).

There were significant reductions in CD20+ and CD19+ cells in blood from baseline ($p<0.0001$) but not in CSF. CSF kappa FLC levels significantly increased ($p=0.0480$).
Conclusions: BAFF levels fall in CSF but increase in serum following IT-rituximab. Rituximab appears to act peripherally with dramatic decreases in peripheral CD20+ and CD19+ cells. It is likely that CSF B-cell counts were too low to enable differences to be seen. The rapid reduction in B-cells suggests rituximab has immediate effects. The profound depletion of B-cells, despite low doses of rituximab, underlines rituximab's efficacy.

1. Introduction

Increasing evidence suggests that the pathology of Multiple Sclerosis (MS) is not solely T-cell mediated, with B-cells appearing to contribute. The discovery of intrathecal oligoclonal immunoglobulin (Ig) production in around 90% of MS patients (Kabat et al., 1948; Cross and Waubant, 2011; Dobson et al., 2013) led to research centering on humoral immune responses. Since then, B-cell aggregations into lymphoid-like structures have been discovered in the brain, meninges and demyelinating lesions of MS patients (Serafini et al., 2004; Aloisi and Pujol-Borrell, 2006; Serafini et al., 2007; Willis et al., 2009), and a correlation between localisation of ectopic follicles and severe cortical pathology and clinical deterioration has been described in patients with secondary progressive MS (Franciotta et al., 2008; Magliozzi et al., 2007). Latent EBV infection was found in a considerable proportion of these B-cell and plasma cell accumulations by one group (Serafini et al., 2007), but this finding has not been replicated by other groups (Willis et al., 2009). B-cell infiltration short of follicle development has also been widely described, and is thought to be a common feature of disease (Pikor and Gommerman, 2012). This evidence, alongside the success of B-cell targeted therapies, such as Phase I/II/III clinical trials of intravenous (IV) rituximab (Cross et al., 2006; Bar-Or et al., 2008; Hauser et al., 2008; Naismith et al., 2010) (an anti-CD20 monoclonal antibody), demonstrates potential clinical benefits in targeting B-cells in MS.

CD19 and CD20 cells are phosphoproteins expressed on the surface of B-lymphocytes, pre-B cells and mature B-cells, during B-cell development (Cross et al., 2006; Reff et al., 1994; Dobson et al., 2011). Plasma cells do not express CD20 or CD19 in contrast to plasmablasts which express both cell surface markers. Memory B-cells express CD20; but unlike naive B-cells, memory B-cells also express toll-like receptor 9 through which they can be non-

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Table 1
Patient characteristics.

Patient	Age/gender	MS type	Disease duration (years)	Baseline EDSS	Therapy prior to RTX		Criteria of resistance to ongoing therapy		
					First line	Second line	Increase EDSS >0.5 point	$\geq$ 1 relapses within 12 months	New T2 lesions (MRI)
1	47/M	RR	4	6.50	INFb-1a 44		x	x	x
2	37/M	SP	12	7.00	INFb-1b 8,6ME	MTX	x	x	x
3	42/F	SP	6	8.00	GA		x	x	x
4	36/F	RR	5	4.00	GA			x	x
5	37/F	SP	9	6.00	GA		x	x	x
6	54/F	SP	19	6.00	INFb-1b 8,6ME			x	x
7	36/F	SP	11	5.00	GA	MTX	x	x	x
8	42/F	RR	3	4.00	INFb-1b 8,6ME			x	x
9	47/M	RR	4	4.00	INFb-1b 8,6ME			x	x

x=Yes, RR – relapsing remitting, SP – secondary progressive INF=Interferon, GA=Glatiramer Acetate (Copaxone), MTX=Mitoxantrone IV.

specifically reactivated. The mechanisms involved in the effects of rituximab remain unknown. Current theories include antibody-dependent cell-mediated cytotoxicity involving apoptosis, cell lysis through complement activation, or activation of macrophages, monocytes or natural killer cells (Reff et al., 1994; Anderson et al., 1997; Golay et al., 2000; Silverman and Weisman, 2003; Beum et al., 2008).

Studies have shown that treatment with rituximab in relapsing remitting MS (RRMS) depletes CD20+ B-cells, with subsequent reductions in CD4+ and CD8+ T-cells, as well as in proliferative and pro-inflammatory cytokine responses, such as Th1 and Th17. This suggests a possible effect via reduced T-cell trafficking into the CNS (Cross and Waubant, 2011; Bar-Or et al., 2010). Whether this is a direct or indirect effect of B-cells remains questionable. It has been suggested that B-cells of the CNS may alter the blood brain barrier (BBB) or secrete chemokines promoting T-cell recruitment (Cross et al., 2006) possibly explaining the reduced T-cell response to diminished circulating B-cells as a result of rituximab treatment.

BAFF is a B-cell activating factor that regulates B-cell survival, differentiation, development, production of Immunoglobulins (Ig) and size of peripheral B-cell pool. BAFF levels are increased in MS patients in the serum, and within the CSF in some studies however, the latter still remains questionable and varies between studies. In MS brains, BAFF may promote BAFF-R-expressing B cell survival, enabling expansion of CNS B-cells, contributing to plasma cell survival (Krumbholz et al., 2005). Both interferon-beta (Krumbholz et al., 2008) and alemtuzumab (Tompson et al., 2010) increase serum BAFF when used in the treatment of MS. CXCL-13 is alternatively known as B lymphocyte chemoattractant factor, and has a key role in regulating B-cell trafficking into the CNS (Gunn et al., 1998). Both CSF and serum levels are increased in patients with MS (Michalowska-Wender et al., 2008); first-line disease modifying treatment has not been shown to affect CXCL-13 levels. CXCL-13 appears to significantly decline after IV rituximab treatment (375 mg/m² weekly for 4 doses) in both blood and CSF (Piccio et al., 2010).

Previous clinical trials of intravenous (IV) rituximab in MS have shown dramatic reductions in gadolinium enhancing brain lesions at doses of 1000 mg on days 1 and 15 (Bar-Or et al., 2008; Hauser et al., 2008) and a repeat course administered at weeks 24 and 26 (Bar-Or et al., 2008), with one or two trials involving smaller doses of 375 mg/m^2 IV weekly for 4 doses (Naismith et al., 2010; Monson et al., 2005). Current trials are beginning to emerge in which 1000 mg rituximab has been administered both intravenously and intrathecally in secondary progressive MS (SPMS) NCT01212094, 2012. There is an ongoing open label trial of low dose intrathecal rituximab (ClinicalTrials.gov identifier NCT01719159) in progressive MS (Svenningsson et al., 2015) and a single case report of intrathecal use in a patient with severe progressive MS (Studer et al., 2014). It remains uncertain whether intrathecal rituximab is more efficacious at lower doses than are administered peripherally.

We therefore set out to evaluate the efficacy of intrathecal rituximab at three low doses (5, 10 and 15 mg), and studied the effects on B-cell biomarkers BAFF, free light chains (FLC) and CXCL13, and on CSF and peripheral B-cell populations. These B-cell biomarkers were chosen as representative markers for B-cell survival [BAFF], B-cell trafficking [CXCL13] and B-cell activation [FLC].

2. Methods

2.1. Recruitment

A total of 9 patients with clinically definite MS (4 RRMS and 5 SPMS; EDSS 4-8, Table 1) were recruited. Subjects were recruited at the MS Centre (City Clinical Hospital 31) in Saint-Petersburg, Russia, using specific inclusion and exclusion criteria documented in Tables 1 and 2 in Appendix 1 (full criteria documented in Appendix 2). Ethical approval for this study was granted by the Research Council of Pavlov State Medical University, St. Petersburg. All subjects provided written informed consent. Details regarding clinical history and previous MS medications are given in Table 1.

2.2. Rituximab administration and dosage

Intrathecal rituximab was administered to all patients at 3 timepoints at increasing doses: 5 mg on D0, 10 mg on D7 and 15 mg on D14 [Appendix 1 Table 3]. Serum and CSF samples were taken at up to 5 timepoints; baseline and days 7, 14, 56 and 112 (immediately prior to the initial and subsequent intrathecal rituximab treatments administered on D0, D7 and D14). Expanded Disability Status Scores (EDSS) were documented throughout the trial at 5 timepoints (Baseline, D7, D14, D56, D112).

2.3. Flow cytometry of peripheral blood and cerebrospinal fluid cells

Flow cytometry was performed on peripheral blood and CSF, at the MS Centre (City Clinical Hospital 31) in Saint-Petersburg, Russia, to examine the effects of IT rituximab on total white blood cell count, lymphocytes, CD3-CD19+ and CD3-CD20+ cells, using BD FACSAria II and BD FACSDiva Software (BD Biosciences, USA). CSF samples were taken by lumbar puncture immediately prior to rituximab administration and standard CSF analysis was performed. Peripheral blood was taken at the same time. Cells were