B-cell activity predicts response to glatiramer acetate and interferon in relapsing-remitting MS

# Appendix e-2 Summary of studies using an ELISPOT assay of B-cell brain-tissue reactivity in patients with multiple sclerosis

The enzyme-linked immunospot (ELISPOT) assay is based on the fact that resting B cells, when stimulated with suitable mitogens in vitro, convert into antibody-producing plasmablasts and plasma cells.1 Their antigen specificity can then be measured by incubating the cells in ELISPOT wells coated with the antigen(s) of interest. Antigen-specific antibodies are captured around the B cells that secrete those antibodies and can be visualized using a conjugated secondary antibody and substrate dye. In our studies of B cells from patients with multiple sclerosis (MS) we have used whole brain lysate as the antigenic target on the basis that patients’ B cells are likely to recognize a range of different CNS tissue antigens.

In an initial study2 we used the ELISPOT technique to investigate the CNS reactivity of B cells from patients with clinically isolated syndrome (CIS; n=15), MS (n=67), other non-inflammatory neurological (n=12), inflammatory neurological (n=16), or autoimmune diseases (n=13), and healthy donors (n=127). All patients were recruited from the University Hospitals of Cologne. As in the present study, peripheral blood mononuclear cells (PBMCs) were isolated from all participants and, before being applied to the ELISPOT plates, were polyclonally stimulated with R-848, interleukin-2, and β-mercaptoethanol for 96 h, so that resting B cells converted into antibody-producing plasma cells. The ELISPOT plates were coated overnight with whole human brain lysate, which was isolated from fresh frozen tissue (30 μg/mL; Novus Biologicals, Littleton, CO). Anti-human immunoglobulin (Ig)κ at 10 μg/mL was used to coat the wells for a positive control. Each sample was plated in duplicate using 1 million polyclonally stimulated PBMCs/well and the plates incubated for 26 h at 37°C and 7% CO2. All plates were incubated with the biotinylated anti-human IgG monoclonal antibody as in the current study, and were developed with CTL True Blue substrate. Spots were counted on an ImmunoSpot® Series 6 Analyzer (Cellular Technology Limited, Shaker Heights, OH). Nine of 15 patients with CIS (60.0%) and 53 of 67 patients with MS (79.1%) showed 4.5–150 CNS-specific B cells. None of the healthy controls or other patients showed increased numbers of CNS-specific B cells in the blood, exceeding 1 in 106 in the stimulated PBMCs. When the MS cohort was divided into disease subtypes, 48 of 60 (80.0%) with relapsing-remitting MS (RRMS) and 5 of 7 (71.4%) patients with secondary progressive MS had a positive CNS-specific B-cell response. The test was repeated in 20 patients with MS 6 months later. In 16 of these patients the CNS-specific B-cell response was comparable at the two time points tested. Four patients who tested negative in the first test showed a positive response in the second test, suggesting that a conversion to B-cell-dependent MS can occur. The number of CNS-specific B-cell-positive patients with CIS and MS was independent of the treatment status.

In a second study,3 PBMCs from 30 patients with MS (24 with RRMS, six with secondary progressive MS) who were experiencing an acute MS relapse were plated on whole human brain lysate in ELISPOT wells, as above. The PBMCs from each patient were plated both immediately after separation from the blood sample without any prestimulation, and after 96 h of polyclonal stimulation. As above, B-cell-secreted antibodies were captured on the ELISPOT plates and visualized as spots corresponding to the numbers of brain antigen-specific B cells. Based on the observed ELISPOT B-cell response, MS relapses could be categorized into three different patterns. A brain-specific B-cell response was observed in unstimulated PBMCs in nine patients (pattern I); in six patients a B-cell response was only seen after polyclonal B-cell stimulation (pattern II); and 15 patients showed no B-cell response to brain antigens (pattern III). Patients who displayed brain-reactive B-cell responses both immediately ex vivo and after polyclonal stimulation (pattern I) were significantly younger than patients in whom only memory B-cell responses were detectable or in whom B-cell responses were absent (patterns II and III, respectively). Longitudinal analysis showed that in one patient, conversion to a positive B-cell response directly ex vivo and also after polyclonal stimulation was associated with the subsequent clinical relapse. Evaluation of the predictive value of a brain antigen-specific B-cell response showed that seven of eight patients (87.5%) with a pattern I response experienced clinical relapse during the observation period of 10 months compared with two of five patients (40%) with a pattern II and three of 14 patients (21.4%) with a pattern III response (*p* = 0.0005; hazard ratio 6.08; 95% confidence interval 1.87–19.77). The results suggest that detection by ELISPOT of the presence of brain-specific B cells in blood may be associated with an increased risk of relapse.

Following on from these studies, our third study4 was conducted: (i) to investigate whether the presence of a brain-specific B-cell response in patients with MS, as determined by the ELISPOT assay, was associated with responsiveness to glatiramer acetate (GA) treatment; (ii) to evaluate whether these findings were specific for GA or also applied to interferon-β (IFN β); and (iii) to investigate any relationship between the brain-specific B-cell response in patients and their disability status. PBMCs were obtained from patients with RRMS recruited from the University Hospitals of Cologne and Würzburg, Germany; the Department of Neurology, Klinikum Augsburg, Augsburg, Germany; and the NeuroCure Clinical Research Center, Charité-Universitätsmedizin Berlin, Berlin, Germany. Patients had been treated with GA (n = 34) or IFN-β (n = 23) for at least 6 months. Disability was graded using the Expanded Disability Status Scale. ELISPOT assays using wells coated with human brain lysate were conducted using polyclonally stimulated PBMCs as described in the previous studies. This study found that the presence of brain antigen-specific B cells in the blood of patients with RRMS correlated with their responsiveness to GA. The ELISPOT responders (n = 22) showed a strong positive correlation between the duration of GA treatment and the time since last relapse (*r* = 0.66; *p* < 0.001). Conversely, there was no correlation between GA treatment duration and the time since last relapse in the ELISPOT non-responder group (n = 12). In contrast to the GA-treated cohort, among patients treated with IFN-β, ELISPOT non-responders (n = 13) displayed a very strong correlation (*r* = 0.93; *p* = 0.0001) between duration of IFN-β treatment and the time since last relapse, while the ELISPOT responders did not show any relationship between these parameters. Evaluation of the GA-treated ELISPOT responder group according to their disability status revealed a strong association between treatment duration and time since last relapse in the cohort with a mild disease course (n = 16; *r* = 0.79; *p* < 0.001), but no such correlation was found in patients who experienced a more severe disease course. These results led us to believe that the presence of brain-specific B cells is a categorical variable by which to identify GA responders and that IFN-β effectiveness might be compromised in patients who have brain-reactive B cells.

**References**

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