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Peripheral blood memory B cell frequency predicts conversion from clinically isolated syndrome to multiple sclerosis

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ABSTRACT

Background: Starting from the first attack, activated B cells are found in multiple sclerosis (MS) patients and are associated with disease activity.
Methods: Peripheral blood cells of 17 clinically isolated syndrome (CIS) patients were collected during the first attack. CIS patients were divided as those converting to MS (CIS-MS+, n = 8) and not converting to MS (CIS-MS-, n = 9) in three years. Age-gender matched MS patients (n = 19) and healthy individuals (n = 20) were included as controls. Peripheral blood frequencies of total, immature, naive, unswitched and switched memory B cells, plasmablasts and plasma cells were measured by flow cytometry.
Results: CIS patients showed reduced unswitched memory B cell and plasma cell frequencies. CIS-MS- patients had significantly increased levels of total B cells and suppressed unswitched memory B cell and plasma cell frequencies.
Conclusion: Our results suggest that conversion from CIS to MS occurs due to the inability of the immune system to suppress effector B cell production.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system. Around 85–90% of patients are classified as relapsing-remitting multiple sclerosis (RRMS) in which disease relapses are followed by periods of remission. The clinically isolated syndrome (CIS) has been defined as the first neurological disturbance of RRMS patients beginning with an acute or subacute attack. A fraction of CIS patients progresses to MS after developing additional clinical attacks and demyelinating lesions. Currently, there is an urgent need of biomarkers that can predict conversion from CIS to MS (Miller et al., 2005).

There is growing evidence emphasizing the prominent role of B cells in the pathogenesis of MS. Demonstration of B cell aggregates reminiscent of tertiary lymphoid organs, association of these foci with disability and cognitive decline and amelioration of MS symptoms with B cell depleting treatment methods gave way to development of B cell-based pathophysiology models (Prineas, 1979, Magliozzi et al., 2007, Wekerle, 2017).

Despite accumulating data on the role of B cells in advanced stages of MS, there is relatively less information on B cell actions in earlier stages of the disease. Activated brain antigen-specific B cells, memory B cells, regulatory B cells and plasma cells have been found in peripheral blood and/or cerebrospinal fluid (CSF) of MS patients as early as during the first clinical episode (de Andrés et al., 2014, Kuerten et al., 2014, Haas et al., 2011, Lee-Chang et al., 2011, Kuenz et al., 2008). There is also evidence suggesting that, in CIS patients, CSF B cells are recruited from peripheral blood during attack episodes and this recruitment is likely mediated by CXCL13, a chemokine that is selectively chemotactic for B cells (Haas et al., 2011). Moreover, in CIS patients, CSF levels of mature B cells and plasmablasts are correlated with brain lesion load, CSF IgG, IgM, matrix metalloproteinase (MMP)-9 and CXCL13 concentrations (Kuenz et al., 2008).

Overall, these findings indicate that B cell activation occurs at relatively early stages of brain inflammation and different B cell subsets start contributing to MS lesion formation during initial MS attacks. Increased understanding of B cell functions in MS pathogenesis brings forward the question whether B cell measurements might be utilized to predict progression of CIS to MS. In this study, the peripheral blood frequencies of various B cell subsets of CIS patients were measured by flow cytometry to find out whether certain B cell subsets might predict conversion of CIS to MS in a follow-up period of three years.

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Table 1
Clinical and demographic features of clinically isolated syndrome (CIS) patients, multiple sclerosis (MS) patients and healthy controls (HC) during blood sampling.

	CIS (n = 17)	MS (n = 19)	HC (n = 20)	*p* value
Gender (women/men)	11/6	11/8	12/8	0.913*
Age	30.7 ± 12.0	34.9 ± 6.9	31.5 ± 6.1	0.464**
Age at disease onset	30.7 ± 12.0	29.6 ± 7.9	–	0.651***
Disease duration (years)	–	4.8 ± 1.7	–	–
Number of relapses	1.0 ± 0.0	6.5 ± 2.6	–	NA
Number of MRI lesions	3.1 ± 2.1	10.2 ± 5.8	–	<0.001***
Number of patients with CSF OCB	7	12	–	0.187*
EDSS score	1.5 ± 0.4	3.4 ± 1.7	–	<0.001†

CSF, cerebrospinal fluid; OCB, oligoclonal IgG bands; EDSS, expanded disability status scale; NA, not applicable.
Numerical values were signified in the form of average ± standard deviation.
* chi-square test.
** ANOVA.
*** Student's *t*-test.
† Mann–Whitney U.

2. Materials and methods

2.1. Patients

Among patients admitted to MS outpatient clinic, 17 consecutive, newly diagnosed patients fulfilling the criteria for CIS (Polman et al., 2011) were enrolled. All CIS patients were within the first three months of their first clinical episode during enrollment. CIS location was brainstem (5 patients), spinal cord (3 patients), optic nerve (4 patients) or hemisphere (presenting with hemiparesis and/or hemihypesthesia, 5 patients). Additionally, 19 age/gender matched relapsing remitting MS patients satisfying the revised McDonald Criteria (Polman et al., 2011) and 20 age/gender matched healthy controls were included as controls (Table 1). Individuals with other coexisting neurological or systemic disorders were excluded.

All CIS and MS patients underwent cranial MRI with the same 1.5 T scanner at the time of enrollment. The clinical workup to rule out other diagnoses included tests for anti-nuclear antibodies, anti-neutrophil cytoplasm antibodies, B12 vitamin and folate levels, serological tests for syphilis, Lyme disease, Mycoplasma pneumoniae and HIV. Oligoclonal IgG bands (OCB) were investigated in all CIS and MS patients. The study protocol was approved by the local ethics committee and all subjects gave informed consent.

CIS patients were followed by the same neurologist for three years with planned examinations every 6 months and whenever a relapse was suspected. Patients were divided in two groups as CIS patients that converted to MS any time within the 3-year follow-up period (CIS-MS+) as per McDonald criteria (n = 8) (Polman et al., 2011) and as CIS patients that did not convert to MS (CIS-MS-) (n = 9) within the same time frame (Table 2). None of the CIS patients had simultaneous asymptomatic contrast-enhancing and non-enhancing lesions. All CIS patients had at least one non-enhancing MS lesion during enrollment. Six of 8 CIS-MS+ patients satisfied criteria for dissemination in space but not dissemination in time. Five CIS-MS+ patients fulfilled the MS criteria by developing a new T2 or contrast-enhancing lesion during the follow-up. These patients also had a second clinical attack during the entire three-year follow-up period. Remaining 3 patients fulfilled the MS criteria by way of a second clinical attack. Clinical findings of the second attacks were consistent with hemispheric (n = 5), cerebellar (n = 1) or brainstem (n = 2) involvement. Average duration (± standard deviation) for conversion to MS was 19.3 (± 10.2) months with a range of 7–35 months. Five of 9 CIS-MS- patients satisfied criteria for dissemination in space but not dissemination in time. Remaining CIS-MS- patients had MS lesions without fulfilling dissemination in space. None of these patients developed a new lesion or a second clinical attack during the follow-up period.

Table 2
Clinical and demographic features of clinically isolated syndrome (CIS) patients who have converted (CIS-MS+) and not converted (CIS-MS-) to multiple sclerosis (MS) during blood sampling (first clinical attack).

	CIS-MS- (n = 9)	CIS-MS+ (n = 8)	*p* value
Gender (women/men)	6/3	5/3	0.858*
Age	32.7 ± 12.3	29.2 ± 7.5	0.565**
Number of MRI lesions	1.8 ± 0.9	3.4 ± 2.3	0.187**
Number of patients with CSF OCB	2/9	5/8	0.092*
EDSS score	1.5 ± 0.6	1.4 ± 0.3	0.801†
MRI lesion locations			
Total	16	27	
Hemispheric††	7	11	>0.999*
Cerebellar	3	6	>0.999*
Brainstem	3	5	>0.999*
Spinal cord	1	3	>0.999*
Optic nerve	2	2	0.621*
Symptomatic contrast-enhancing lesions	0/16	3/27	0.282*
Number of patients showing dissemination in space	5/9	6/8	0.742

CSF, cerebrospinal fluid; OCB, oligoclonal IgG bands; EDSS, expanded disability status scale.
Numerical values were signified in the form of average ± standard deviation.
* chi-square test;
** Student's *t*-test.
† Mann–Whitney U.
†† Hemispheric lesions include both periventricular and juxtacortical lesions.

2.2. Phenotypic distribution of peripheral B cells by flow cytometry

Blood samples were collected from all participants between 08.00 a.m. and 10.00 a.m.. All CIS and MS patients were in remission during blood sampling and had not received steroid treatment within the last 30 days of blood collection. None of the CIS patients were under immunomodulating drug treatment, whereas all MS patients were using immunomodulating medications (11 interferon-β, 5 glatiramer acetate and 3 fingolimod). Blood samples collected after the first clinical episode were used for CIS patients. In order to obtain standard conditions, peripheral blood mononuclear cells (PBMCs) were separated by Ficoll density gradient centrifugation, then resuspended in freezing solution and stored in liquid nitrogen. Frozen PBMCs were thawed and washed in complete medium (enriched with 10% fetal calf serum, 1% minimum essential medium vitamin, 1% L-glutamine, 1% Na-pyruvate, 1% non-essential amino acids, 1% penicillin-streptomycin). Cells were stained with anti-human monoclonal CD19-APC, CD27-FITC, IgD-APC/Cy7, CD138-PE, CD24-PerCP and CD38-Alexa fluor 700 (Biolegend, San Diego, CA) conjugates for 30 min at 4 °C, washed with PBS and re-suspended in PBS. Thereafter a 6 color immunofluorescence staining was utilized (BD FACS Aria II, Becton Dickinson, Franklin Lakes, NJ). At least 5×10^5 cells were acquired for each sample and data were analyzed using the FlowJo software (Fig. 1).

2.3. Statistical analysis

Demographic and clinical features of CIS, MS and healthy control groups were compared by chi-square test, ANOVA, Student's *t*-test or Mann–Whitney U, as appropriate. B cell subset frequencies were compared with ANOVA and Tukey's post-hoc test among study groups. $p < 0.05$ was considered as statistically significant.