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A unique antibody gene signature is prevalent in the central nervous system of patients with multiple sclerosis*

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Abstract

B cells isolated from the CSF of patients with multiple sclerosis (MS) have a unique accumulation of somatic hypermutation, within the B cell receptor, termed the antibody gene signature (AGS). The focus of this study was to investigate whether the AGS could also be detected in MS brain tissue. Genetic analysis of B cells isolated from post-mortem CNS tissue samples from four MS brains demonstrated that signature enriched B cells are present at the site of tissue injury as well as in the circulating CSF.

Keywords

Multiple sclerosis; B lymphocytes; Antibody gene rearrangement; Somatic hypermutation; CNS tissue

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1. INTRODUCTION

The involvement of B cells in the pathogenesis of multiple sclerosis (MS) has been reviewed elsewhere (Antel and Bar-Or, 2006; McFarland, 2008; Owens et al., 2006) and is supported by the therapeutic efficacy of the B cell depleting anti-CD20 drug rituximab in patients with MS (Hauser et al., 2008). This finding supports the concept that the B cell pool in MS patients harbors a subset that contributes to disease pathology. We hypothesized that if the cellular pool in MS patients is dysregulated, one would expect that antibody genes utilized by B cells circulating within the cerebrospinal fluid (CSF) would display a pattern of somatic hypermutation not found in healthy donors or patients with other neurological diseases. Indeed, our laboratory has recently identified a novel pattern of somatic hypermutation that is unique to MS CSF B cells and not found in control derived sequences (Cameron et al., 2009). We investigated the utility of this antibody gene signature (AGS) as a molecular genetic tool to identify CIS patients at risk to develop MS that would subsequently convert to definite MS. Application of the AGS tool demonstrated the ability to predict conversion to definite MS with an accuracy of 91% (Cameron et al., 2009). The goal of this current study was to determine whether this MS-specific AGS identified in the CSF is also present in the CNS tissue of patients with MS.

2. MATERIALS AND METHODS

2.1. Specimens

CNS tissue was dissected at autopsy from four subjects with clinically definite MS. Specimens were immediately snap-frozen then stored at −80°C. Table 1 summarizes the clinical features of each subject. All human subject research was approved from the local human research internal review boards.

2.2. Immunoglobulin variable region cloning

B cell immunoglobulin variable region libraries were assembled from tissue sections prepared on a cryostat. RNA was extracted from tissue sections 14-μm thick using the RNAeasy Mini Kit (Qiagen) according to the manufacturer's instructions. From the total RNA, cDNA was synthesized and human Ig variable region genes were amplified as described previously (Willis et al., 2009).

2.3. Analysis of the B cell repertoire

A database containing 918 heavy chain sequences was compiled from the CNS tissue and analyzed using a Perl based program developed by the Bioinformatics lab in the Pathology Department at The University of Texas Southwestern. The program utilizes the IMGT/V-QUEST tool as a basis for extracting the sequence information (http://imgt.cines.fr) (Lefranc, 2001). Databases containing the gene and mutational information of each of the sequences were created using this program.

2.4. Antibody Gene Signature

The 71 unique VH4 sequences in the CNS tissue heavy chain sequence database were used to calculate antibody gene signature (AGS) scores as previously described (Cameron et al., 2009). AGS scores were calculated for each individual patient specimen.

3&4. RESULTS AND DISCUSSION

The calculated AGS scores derived from the four subjects are listed in Table 1. We had previously established (Cameron et al., 2009) that the AGS scores of CSF B cells from patients with MS ranged from 7.6 to 11.9 (average combined AGS score of 10.9) (Table 1).

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The AGS scores for the CNS tissue antibody repertoires ranged from 10.0–14.5 (average combined AGS score of 11.9) (Table 1). These data demonstrate that the AGS is not unique to the CSF but is also present in CNS tissue of MS patients. Of note, the average AGS score of CD19+ CSF B cells from three patients with other neurological diseases was 4.5 and the average AGS score of CD19+ peripheral blood B cells from 3 MS patients was 2.0 (Cameron et al., 2009).

The presence of a strong AGS score in this CNS tissue antibody gene repertoire database provides important corroboration of our principal hypothesis that AGS enriched B cells are present at the site of the disease process in MS, as well as in the circulating CSF. Our observations are in keeping with the current conceptualization of MS pathogenesis, which includes the matriculation of brain-reactive B cells from the periphery into brain tissue via the circulating CSF (Lassmann et al., 2001; Lassmann et al., 2007; Meinl et al., 2006; Pittock and Lucchinetti, 2007; Ransohoff et al., 2003; Serafini et al., 2004; Uccelli et al., 2005). Thus, if high AGS scores are a common feature of CSF B cells from MS patients, it should also represent a common characteristic of B cells localized to CNS tissue, as we have demonstrated here. A limitation in this current study is the low number of patient samples evaluated, however it provides a preliminary look into the localization of the AGS and justifies further research into the area. Ultimately, the AGS prevalence of B cells in CSF and CNS tissue of MS patients supports the hypothesis that a restricted population of B cells are involved in the biological underpinning of the disease process in MS. Further characterization of these AGS enriched B cells in MS is currently under active investigation.

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Table 1

Clinical and demographic data of patient specimens.

Subject	Age and Gender	Disease duration (years)	MS course	AGS score[1]
MS-1	38/F	NA	RRMS	10.0
MS-2	65/M	NA	CPMS[2]	14.5
MS-3	43/F	20	CPMS[2]	11.9
MS-4	39/F	13	CPMS[2]	11.0
CSF-MS[3]	41/F	7	RRMS	10.9[4]
CSF-OND[5]	58/2F:1M	NR	NR	4.5[6]
PB-MS[7]	41/F	<1	RRMS	2.0[8]

Abbreviations: MS: multiple sclerosis, CSF: cerebrospinal fluid, OND: other neurological disease, PB: peripheral blood, F: female, M: male, NA: not available, NR: not relevant, RRMS: Relapsing-remitting MS, CPMS: Chronic progessive MS,

[1]Number of VH4 sequences in AGS calculations: MS-1=20; MS-2=12, MS-3=9, MS-4=30, CSF-MS=128, CSF-OND=15, PB-MS=40

[2]Pathology reports for these patients state “chronic progressive” with no additional history provided to determine whether these patients had primary or secondary progressive MS.

[3]CD19+ CSF B cells were collected from 10 RRMS patients and 1 PPMS patient as published in (Cameron et al., 2009).

[4,6,8]AGS score analysis from (Cameron et al., 2009). Scores represent averages of each cohort.

[5]CD19+ CSF B cells were collected from 3 OND patients as published in (Cameron et al., 2009).

[7]CD19+ peripheral B cells were collected from 3 RRMS patients as published in (Cameron et al., 2009).