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Clonal expansion of IgA-positive plasma cells and axon-reactive antibodies in MS lesions☆

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Abstract

Immunoglobulin A (IgA), the predominant immunoglobulin class in mucosal secretions, has been found in the cerebrospinal fluid of patients with multiple sclerosis (MS). In this study we examined the infiltration of clonally expanded IgA plasma cells in lesions of MS brains. Sequences of complementarity-determining region 3 of IgA variable heavy chain (V_H) genes demonstrated the clonal expansion of IgA-bearing plasma cells in MS lesions. Somatic mutations and ongoing intra-clonal mutations occurred in their V_H genes. Immunohistochemical study demonstrated infiltration of dimer and polymer IgA1- and A2-positive plasma cells in perivascular spaces, in the parenchyma of MS lesions, and in the adjacent white matter. Double immunofluorescence staining showed binding of IgA antibody on axons and walls of microvessels in the areas of chronic active and inactive demyelination. Bielshowsky's silver impregnation revealed axonal damage in these areas. These findings suggest that IgA in the CNS are localized on axons in lesions and may contribute to axonal damage in MS.

Keywords: IgA and IgG plasma cells; Clonal expansion; Axonal reactive antibody in lesion; Multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS), characterized by alteration of the blood–brain barrier, mononuclear cell infiltration of white matter and demyelination. In the animal model experimental autoimmune encephalomyelitis, autoreactive T cells are considered to initiate the disease process (Ben-Nun et al., 1981; Martin et al., 1993). In MS, the local formation of oligoclonal IgG has been emphasized in the literature (Tibbling et al., 1977; Tourtellotte, 1970). IgA, IgG and IgM-positive plasma cells are found in CSF and in lesions of MS patients (Budka et al., 1985; Esiri, 1977; Genain et al., 1999; Giles and Wroe, 1990; Henriksson et al., 1985). Sequence analyses of the variable heavy chain region

Abbreviations: IgA, Immunoglobulin A; MS, Multiple sclerosis; V_H, Variable heavy chain; CDR, Complementarity-determining region; FR, Framework; Ig, Immunoglobulin; CNS, Central nervous system; CSF, Cerebrospinal fluid; NF, Neurofilament; MALT, Mucosal-associated lymphoid tissues; NAGM, Normal-appearing grey matter; LFB, Luxol Fast Blue; PTC, Pseudotumor cerebri; MBP, Myelin basic protein; BCR, B cell receptor.

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gene have revealed a dominant B-cell clonal expansion in the CSF and in lesions of a majority of MS patients (Baranzini et al., 1999; Colombo et al., 2000; Owens et al., 1998; Qin et al., 1998, 2003). Presumably, clonally expanded B cells and plasma cells that have undergone somatic hypermutation through their antigen receptor in the germinal centers of secondary lymphoid tissue then migrated into the CNS.

IgA represents mainly mucosal secretory antibody and is the most important immune system component at mucosal surfaces. IgA has also been considered a major component of CNS immune responses in certain viral infections and in MS (Esiri, 1980; Griffin, 1981; Lamoureux and Borduas, 1966; Link and Muller, 1971; Olsson and Link, 1973).

In this study, we demonstrate clonally expanded IgA-positive plasma cells in MS lesions. These IgA plasma cells are post-germinal center cells that have accumulated somatic mutations. Intraclonal diversity provides evidence that a sustained antigen stimulation occurs in MS lesions. Immunohistochemical analysis also shows infiltration of IgA-positive plasma cells, including both subclasses of dimeric IgA1 and IgA2 containing the J-chain, in MS lesions. Double immunofluorescence staining demonstrates co-labeling of anti-IgA and anti-neurofilament (NF) on axons in both actively demyelinating and inactive MS lesions. Axonal damage was also seen in these areas. Taken together, these findings suggest that highly mutated IgA-positive plasma cells are present in MS lesions and may mediate anti-axonal immune responses thereby contributing to local tissue injury.

2. Materials and methods

2.1. Tissues preparation

Postmortem brain tissue specimens from four cases of MS were obtained from the Neurospecimen Bank at the Neurology Service, VA Greater Los Angeles Healthcare System, University of California, Los Angeles, CA. The patients had been diagnosed clinically as having MS during life and this was confirmed by neuropathological examination at necropsy (Table 1). Six plaques containing demyelinating lesions from frontal, parietal and occipital regions and four corresponding samples of normal-appearing grey matter (NAGM) from each MS brain were used. Each sample was bisected; one half was kept frozen for RNA extraction, PCR amplification and Ig gene sequencing and the other half was fixed in 10% formalin and embedded in paraffin for staining with Hematoxylin and Eosin to assess inflammation, Luxol Fast Blue (LFB) to assess myelin loss and immunocytochemistry to assess IgA immunoreactivity in the lesions. Bielshowsky's silver impregnation was used to evaluate axonal integrity.

2.2. PCR amplification of MS plaque B cells

Total RNA was extracted from postmortem tissue samples (average size $100 \times 100 \times 2$ mm) and CSF mononuclear cells from a patient with pseudotumor cerebri (PTC) served as controls for polyclonal B cells (Da et al., 2004), using an RNeasy kit (QIAGEN Inc., Chatsworth, CA). First-strand cDNA was synthesized using oligo d(T) as primer and avian myeloblastosis virus reverse transcriptase in a total volume of 40 μl. V_H of IgA genes were amplified via PCR (Saiki et al., 1988) in a final volume of 50 μl reaction buffer [50 mM Tris–HCl, pH 9.0 at 25 °C: 20 mM $(NH_4)_2SO_4$; 3.0 mM $MgCl_2$] containing 10 μl of cDNA from each sample, 2 units of recombinant Taq Polymerase, and 50 pmol primers. PCR was carried out for 40 cycles with a mixture of six 5′ VH family-specific leader primers (VHL1: 5′-CCATGGACTGGACCTGGAGG-3′, VHL2: 5′-ATGGACATACTTTGTTC CAGC-3′, VHL3: 5′-CCATG-GAGTTTGGGCTGAGC-3′, VHL4: 5′-ATGAAACACCTG TGGTTCTT-3′, VHL5: 5′-ATGGGGTCAACCGCCAT CCT-3′, VHL6: 5′-ATGTCTGTC TCCTTCCTCAT-3′), plus IgA Cα (5′-GGGTCAGCTGGGTGCTGCTGG-3′) or IgG Cγ 5′-AAGTAGTCCTTGACCAGGCAG-3′ or IgM Cμ (5′-GAATTCTCACAGGAGACG AGGGG-3′-specific primers under standard conditions (denaturation 1 min at 94 °C, annealing 2 min at 52–56 °C, extension 1 min at 72 °C). A nested PCR was performed with six 5′ VH family-specific leader primers and 3′ J_H primers (J_H1,2,4: 5′-ACT-CACGTTTGATYTCC ASCTTGGTCC-3′, J_H3: 5′-GTACTTA CGTTTGATATC CACTTTGGTCC-3′ and J_H5: 5′-GCTTACGTTTAATCTCCAGTCG TGTCC-3′) in the condition as above described. Aliquots of the PCR product were analyzed by electrophoresis in a 2% agarose gel (Sigma) containing ethidium bromide.

2.3. Sequencing Ig V_H genes

PCR products were recovered and ligated into the pGEM T vector (Promega, La Jolla, CA) and transfected into *Escherichia coli* DH5a according to Hanahan (Hanahan, 1985). Power sample size estimation with nQuery software

Table 1
Summary of patients

Cases	Gender	Age at death	Age at onset	Course of MS	Duration of disease (years)	Cause of death	Autolysis (h)
1	F	57	43	RR (7Y) SP (7Y)	14	Sudden unexpected death, severe MS	19.75
2	F	47	27	RR (9Y) SP (11Y)	20	Unable to determine from available clinical information	11.5
3	M	59	47	PP (14Y)	14	Pneumonia	15
4	M	50	21	PP (29Y)	29	Respiratory failure, pneumonia	13.75