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Measles virus–specific plasma cells are prominent in subacute sclerosing panencephalitis CSF

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Abstract

Objective—To demonstrate the specificity of expanded CD138⁺ plasma cell clones recovered from the CSF of a patient with subacute sclerosing panencephalitis (SSPE) for measles virus (MV).

Methods—IgG variable region sequences of single-antibody-secreting CD138⁺ cells sorted from SSPE CSF were amplified by single-cell PCR and analyzed. Human IgG1 recombinant antibodies (rAbs) were produced from four expanded CD138⁺ clones and assayed for immunoreactivity against MV proteins.

Results—Clonal expansion was a prominent feature of the SSPE plasma cell repertoire, and each of the four rAbs assayed was specific for either the MV fusion or the MV nucleocapsid protein.

Conclusions—Expanded plasma cell clones in the CSF of patients with subacute sclerosing panencephalitis produce disease-relevant antibodies. Recombinant antibodies derived from CSF B cells could provide a tool to identify target antigens in idiopathic inflammatory disorders.

Features of chronic inflammatory and infectious CNS diseases include increased intrathecal immunoglobulin (Ig) G synthesis, the presence of oligoclonal bands, and recruitment and expansion of B lymphocytes and CD138⁺ plasma cells within the CNS.^{1–4} When the specificity of these antibody responses has been studied, the oligoclonal IgG has been shown to be directed against the causative agent.⁵ To demonstrate whether clonally expanded and differentiated plasma cells in subacute sclerosing panencephalitis (SSPE) CSF are disease relevant, we produced recombinant antibodies (rAbs) from variable (V) region sequences of expanded CD138⁺ clones and assayed their specificity for measles virus (MV).

CASE REPORT

A 12-year-old Caucasian boy born in Albania had a 1-year history of decline in school grades and increasingly disruptive and aggressive behavior. Two weeks before hospitalization, he became anorexic, lethargic, and drowsy. Two days before presentation, he began to exhibit bilateral jerking of his limbs, mostly in his arms. He had experienced various childhood exanthems before age 5, but a definitive diagnosis of measles was not made. At age 5, he moved to the United States and received vaccinations including measles vaccine. The neurologic examination on admission revealed drowsiness and orientation only

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to person. Although regarded as a good English speaker, he could follow commands only in his native language. Rhythmic whole-body flexion movements were observed every 5 to 7 seconds. Muscle tone and strength were normal. Coordination was impaired in all extremities, and his gait was wide-based. Deep tendon reflexes were normal, and there were no pathologic reflexes. Brain MRI revealed multiple foci of increased T2-weighted signal in the subcortical white matter and adjacent cortex. There was no enhancement or restricted diffusion. An EEG revealed generalized periodic discharges, typically lasting less than 1 second, every 5 to 7 seconds. The CSF was clear and colorless and contained seven cells, all mononuclear; CSF glucose was 62 mg%, protein was 40 mg%, IgG was 23.7 mg%, and there were 17 oligoclonal bands as determined by isoelectric focusing and immunodetection with anti-human IgG. The IgG/protein ratio in CSF was 59%. The diagnosis of SSPE was confirmed by the detection, on two separate occasions, of high titers of antibody to MV: ELISA value >7.00 (Arup Laboratories, Salt Lake City, UT) and >8.00 (University of Colorado Hospital, Denver, CO) in both serum and CSF.

CD19⁺ B lymphocytes and CD138⁺ antibody-secreting cells were sorted from CSF as described.⁶ After gating for cells in the approximate size range of lymphocytes and plasma cells, CD19⁺ or CD138⁺ cells were separated from CD3⁺ T cells and individually sorted into each well of a 96-well PCR plate. The CD19⁺ and CD138⁺ populations accounted for 3.9 and 1.25% of the mononuclear cell fraction, respectively, values comparable with those found in CSF of patients with multiple sclerosis (MS),^{2,4,6} optic neuritis,⁷ and other infectious CNS diseases.⁴ Although levels of intrathecal IgG synthesis in SSPE can be an order of magnitude greater than those found in MS, this difference probably reflects an overall increase in the number of CSF lymphocytes rather than a shift in mononuclear cell populations toward the B cell lineage. Co-expression of CD19 by CD138⁺ cells was variable and represented a continuum from CD19⁺-expressing plasmablasts to CD19⁻, CD138⁺ plasma cells (data not shown). Like CSF from MS and other CNS inflammatory diseases,⁴ most CD138⁺ cells expressed an intermediate level of CD19, indicating that plasmablasts were the prominent antibody-secreting B cell in SSPE CSF.

Immediately after cell sorting, cDNA was synthesized from single CD138⁺ cells, heavy (H) and light (L) chain V regions were amplified using established PCR primers and a nested PCR protocol, and PCR products were purified and sequenced with appropriate constant region antisense primers.^{6,8} The features of H and L chain V regions recovered from 71 different CD138⁺ cells categorized according to their H and L chain CDR3 amino acid sequence (table) were similar to those found in B lymphocytes and plasma cells from MS and optic neuritis CSF.⁶⁻⁹ Clonal expansion was prominent with 50 of 71 IgG-expressing CD138⁺ cells found within clonal populations. Seven different plasma cell clones were identified in which each cell within a clone expressed the same V_H and V_L germline rearrangements. The only exception was clone 5, where cells expressing the same H chain sequence expressed one of two different functional L chain rearrangements. Clone 1 was large and accounted for almost one-half of CD138⁺ cells analyzed. Alignment of each V region to human germline sequences in VBASE identified the most homologous germline segment and the extent of somatic mutation. Although germline segments from most V_H families were observed in this repertoire, those from the V_{H1} family were utilized most often and represented 12 of 28 (43%) of the unique V_{DJ} rearrangements. In normal peripheral blood, V_{H1} H chains are found in about 20% of naive B cells, reflecting their genomic prevalence. Skewing of the SSPE repertoire toward V_{H1} germlines most likely reflects antigenic selection by MV epitopes. All H chains demonstrated varying degrees of somatic mutation (87 to 98.3%) from their respective germline segment. L chains were about equally distributed between kappa and lambda chains and also exhibited some degree of somatic mutation, although usually to a lesser extent than the paired H chain sequence.

We constructed bivalent human IgG1 monoclonal rAbs from the three most prominent plasma cell clones (clones 1, 2, 3) and from less abundant sequences (clone 6). Cloning of H and L chain V region sequences into the pIgG1-Flag and pCEP4 expression vectors, respectively, transfection of HEK 293 cells, and rIgG purification from tissue culture supernatants have been described.^{10,11} Production and purification of disulfide-bonded rIgG was verified by sodium dodecyl sulfate polyacryl-amide gel electrophoresis and immunoblotting under nonreducing conditions (data not shown).

RESULTS

IgG purified from the patient's CSF and each CSF-derived rAb was assayed for MV immunoreactivity by staining acetone-fixed MV-infected and -uninfected Vero cells (figure 1).¹⁰ rIgGs representing clones 1 to 3 intensely stained MV-infected multinucleated giant cells, but not uninfected Vero cells. Clone 6 rIgG did not stain MV-infected cells (data not shown). To identify the specific proteins recognized by the MV-positive rIgGs, Vero or HEK 293 cells transfected with the MV nucleocapsid (N), fusion (F), hemagglutinin (H), and phosphoprotein (P) genes were stained with the individual rIgGs. Clone 1 rIgG stained transfected cells expressing the MV-F protein, but not control cells or cells expressing other MV genes, whereas clones 2 and 3 stained only cells transfected with the MV-N gene (shown in figure 1 for MV-N- or MV-F- transfected cells).

Immunoblot analysis (figure 2) showed that clone 3 rIgG recognized a 60-kd protein in MV-infected cell lysates corresponding in size to the MV-N protein. Clone 1 and clone 2 rIgG did not react with their target MV proteins under denaturing conditions. Interestingly, clone 6 rIgG, which failed to recognize MV by immunostaining, showed weak reactivity to a approximately 60-kd protein in the MV lysate that comigrated with MV-N protein recognized by clone 3 (figure 2). This suggests that clone 6 rIgG is reactive against a MV-N protein epitope that is better exposed under denaturing conditions. None of the rIgGs or SSPE brain IgG reacted to proteins in the uninfected Vero cell lysate.

DISCUSSION

SSPE is a chronic encephalitis caused by persistent MV infection of neurons and glia. Our patient's neurologic features, mildly inflammatory CSF, oligoclonal bands, and high titers of antibody to MV in both serum and CSF, are characteristic of SSPE. Most cases occur in children and teenagers with a history of measles infection, often before age 2; rarely, SSPE occurs in a child after vaccination.¹² In our patient, it is unknown whether vaccination represented his initial exposure to MV.

High titers of MV-specific antibody are present in the SSPE brain and CSF¹³ as a result of the diffuse infiltration of lymphocytes and plasma cells into the brain parenchyma.¹⁴ Sequence analysis of rearranged Ig genes amplified from SSPE brain cDNA libraries¹ and single microdissected CD38⁺ cells¹⁰ reveals a limited H chain repertoire with prominent clonal expansion and somatic hypermutation indicative of a CNS-targeted humoral response to MV. In this study, we demonstrate that a similar MV-targeted humoral immune response is identifiable in CSF of SSPE patients and that the specificity of this response can be characterized from a small amount of CSF using recombinant antibody technology.

CSF is more readily accessible than brain tissue and thus is routinely used to monitor ongoing immunity in CNS inflammatory diseases. In this study, we demonstrate that three of four rAbs derived from expanded CD138⁺ clones in SSPE CSF react to MV proteins, providing a link between intrathecal synthesis of MV-specific IgG and the expansion and migration of antibody-secreting cells in brain. Although the precise relationship between

expanded B and plasma cell clones and oligoclonal bands requires further comparison, it is clear that B cells found in both brain parenchyma and CSF are components of the same antigen-targeted humoral response. The recent discovery of ectopic germinal centers in the meninges of some MS patients¹⁵ and the enrichment of B cell subsets with phenotypes characteristic of germinal center reactions³ in MS and non-MS inflammatory CSF suggest that B cell differentiation into antibody-secreting plasma cells may be sustained within the CNS in chronic inflammatory disorders. In conclusion, our current data show that CD138⁺ cells recovered from SSPE CSF are primarily targeted against disease-relevant antigens. Equivalent techniques and strategies can be used to identify unknown antigens or to monitor ongoing immunity in other CNS inflammatory diseases.

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Figure 1.

Recombinant antibodies derived from expanded CD138⁺ clones in SSPE CSF immunostain MV-infected cells

Purified recombinant IgG (10 μ g/mL) from CD138⁺ clones 1 to 3 and subacute sclerosing panencephalitis (SSPE) CSF IgG stained measles virus (MV)-infected Vero cells, but not uninfected cells. Further immunostaining of Vero or HEK 293 cells transfected with cDNAs of individual MV proteins revealed reactivity of clone 1 rIgG with the MV fusion (MV-F) protein and reactivity of clones 2 and 3 rIgG with the MV nucleocapsid (MV-N) protein. CSF IgG from the patient with SSPE also stained both MV-N and MV-F transfected cells. Immunostaining was detected using a 1:500 dilution of goat anti-human IgG conjugated to alkaline phosphatase (Vector Laboratories, Burlingame, CA) with Vector new fuschin as substrate. Coverslips were counterstained with hematoxylin.

Figure 2.

Immunoblot detection of MV-specific proteins with SSPE CSF-derived rAbs

Measles virus (MV)-infected or -uninfected Vero cell lysates ($15\ \mu\text{g}/\text{lane}$) were separated in a 10% sodium dodecyl sulfate polyacrylamide gel, transferred to nitrocellulose, and probed with each subacute sclerosing panencephalitis (SSPE) recombinant antibodies (rAb) ($5\ \mu\text{g}/\text{mL}$) or with IgG eluted from SSPE brain. Antibody binding to MV proteins was detected using a 1:500 dilution of goat anti-human IgG conjugated to alkaline phosphatase. Under denaturing conditions, clones 1 and 2 rIgG did not recognize the MV-F or MV-N protein, whereas clone 3 rIgG did recognize a 60-kd MV protein. Although clone 6 rIgG did not immunostain MV-infected cells, it was weakly reactive with a 60-kd protein found only in the infected cell lysate, suggesting that clone 6 rIgG may also be directed against the MV-N protein. SSPE brain IgG also identified several MV-specific proteins.

Table

Features of V region sequences amplified from single CD138⁺ plasma cells sorted from subacute sclerosing panencephalitis IC06-1 CSF*

CDR3 - V _H	No.	Family	V _H	Homol, %	CDR3 - V _L	Family	V _L	Homol, %
Clone 1								
GTSEWLRSFYFDS	31	VH1	1-69	92.9	QQYNNYTPA	VK1	L12a	94.3
Clone 2								
DRAAGLGRFGMDV	6	VH4	4-59	89.3	QQSHTEPRT	VK1	L5	92.3
Clone 3								
ESPAKKSFVVLRHGMVDV	3	VH3	3-48	93.2	QTWDSGRV	VL4	4b	94.4
Clone 4								
DDGNTRDGYKLDG	3	VH3	3-21	93.2	GADHNDGNKFFVWW	VL9	9A	98
Clone 5								
GASEQQLSHSFYYGYGLDV	2	VH4	4-31	92.3	QQYDNLPLFT	VK1	O18	94.3
GASEQQLSHSFYYGYGLDV	1			92.3	MQRIEFPWT	VK2	O1	98.3
Clone 6								
HVWDDSSGYRWAKIDY	2	VH4	4-59	95.9	MQVLQTPLS	VK2	A3	98.3
Clone 7								
VARFDFNI	2	VH3	3-72	96.3	SSWDDSLSGRV	VL1	1g	97.9
Single Sequences								
DVVAKDKAAAGAFGMVDV		VH1	1-69	92.9	QQRSNWREYT	VK3	ND	
HEGAGLSSGYPTTHFEY		VH5	5-51	98.3	WSYAGRNTYWW	VL2	2b2	96.2
CSKSRNWNDGRIFGLDV		VH1	ND		QDLKSYPSFT	VK1	L8	93.5
RSGQWLARSYQYYYGMDV		VH3	3-30	93.9	MQALQTPPA	VK2	A3	98.6
QYDILTGGY		VH3	3-15	97				
DQAYDTIGYYGYDL		VH1	1-18	92.5	QMSGTSPWT	VK3	A27	98.2
FVLMVYDDLHSYYGYGMDV		VH3	3-48	95.2	SSYTDSPTPL	VL2	2a2	96.9
ARYTSGMSFDS		VH3	3-23	94.2	GTWDDSSLAPWV	VL1	1b	98.6
SSLSVAGTRGYFDL		VH1	1-69	94.2				
ALYDEYIRGVWFDS		VH1	1-69	86.9	QQYNSYSVT	VK1	L12a	94.3
NRAYTYGADAFDV		VH3	3-30	90.1				

CDR3 - V _H	No.	Family	V _H	Homol, %	CDR3 - V _L	Family	V _L	Homol, %
DRGIMPLPIYQYFGLDV		VH1	1-02	90.5	SSYTTSGTPIV	VL2	2a2	90
GPSSPTPIQTDD		VH3	3-23	87	SAWDDSLSGVV	VL1	1b	91
ALCKGNAYYYHYGMDV		VH3	3-23	97.6	AAWDDSLNVYYV	VL1		99
VKTGLITMVRGDIENAFDI		VH1	1-02	99	GTWDDSSLAPWV	VL1		99.6
NPDYGGGGLTLDY		VH5	5-51	95.9				
VRSAYRGVPLA		VH1	1-69	89.6	HQYATTATWT	VK3		93.4
DRAGKYYGSGSYMFD		VH1	ND					
SHHRGTQFLEWLLHD		VH1	1-e	89.8	QQVNSFPPLT	VK1		95.4
EGAGFDWSSSSSNYYGLDV		VH1	1-e	90.1	QQYYASPNT	VK4		97.3
VAHSFEPFRFGVVMGWFD		VH5	5-51	97.6	QQYYNT PFT	VK4		99

* Each row indicates the heavy and light chain CDR3 amino acid sequence, the number of CD138⁺ cells expressing that rearrangement, the most homologous human V region family and germline segment (VH or VL indicated by locus), and the homology (Homol) of each V region sequence to that germline.

ND = not done.