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Recombinant antibodies generated from both clonal and less abundant plasma cell immunoglobulin G sequences in subacute sclerosing panencephalitis brain are directed against measles virus

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Abstract

Increased immunoglobulin G (IgG) and intrathecally produced oligoclonal bands (OGBs) are characteristic of a limited number of inflammatory central nervous system (CNS) diseases and are often directed against the cause of disease. In subacute sclerosing panencephalitis (SSPE), the cause of disease and the target of the oligoclonal response is measles virus (MV). The authors previously showed that clonally expanded populations of CD38+ plasma cells in SSPE brain, the likely source of OGBs, are directed against MV. In characterizing the breadth of the plasma cell reactivities, the authors found that a large proportion of the less abundant plasma cells are also directed against MV. The intrathecal response may be useful in determining the causes of other inflammatory CNS diseases, such as multiple sclerosis, Behcet's disease, and neurosarcoidosis.

Keywords

antibodies; antigen specificity; inflammation; plasma cells; SSPE

Increased immunoglobulin G (IgG) and oligoclonal bands (OGBs) are found in the cerebrospinal fluid (CSF) of humans with chronic infectious central nervous system (CNS) diseases such as neurosyphilis, cryptococcal and tuberculous meningitis, Lyme disease, some viral meningitides, varicella-zoster virus vasculopathy, and subacute sclerosing panencephalitis (SSPE). Analysis of the specificity of CSF OGBs has revealed that the oligoclonal IgG in SSPE (Vandvik *et al*, 1976), neurosyphilis (Vartdal *et al*, 1981), mumps meningitis (Vandvik *et al*, 1978), cryptococcal meningitis (Porter *et al*, 1977), varicellazoster virus vasculopathy (Burgoon *et al*, 2003), and other disorders is directed against the agent that causes disease (reviewed in Gilden *et al*, 2001). This led to the hypothesis that the oligoclonal IgG in the brain and CSF of patients with chronic inflammatory CNS disease of unknown etiology such as multiple sclerosis, sarcoidosis, and Behcet's disease is anti-body directed against the agent that causes disease. Better strategies

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and techniques to identify disease-relevant antibodies and their cognate antigens may identify the causes of inflammatory diseases of unknown etiologies. We have used SSPE as a model to study the complexity of the intrathecal response to disease-relevant or ancillary antigens.

We previously used laser capture microdissection to isolate individual CD38+ plasma cells from the brain of a patient with SSPE followed by single-cell reverse transcriptase—polymerase chain reaction (RT-PCR) to amplify individual IgG heavy (H) and light (L) chain sequences expressed by each cell (Burgoon *et al*, 2005). Analysis of a repertoire of the expressed IgGs in brain (Table 1) showed that 55 of the 65 plasma cells were in clonally expanded groups (clones 1 to 11), whereas 10 plasma cells were encountered only once. Analysis of functional recombinant antibodies (rIgGs) constructed from 8 of the clonally expanded Ig sequences, which were most likely to represent the intrathecally synthesized OGBs, showed that most of these rIgGs recognized measles virus (MV), the cause of SSPE (Burgoon *et al*, 2005).

The question remains whether additional antibody reactivities are present, particularly toward autoantigens that might confound the disease-relevant response. For example, in multiple sclerosis, antibodies directed against various self or novel antigens have been found in both blood and CSF, but have not been shown to be part of the oligoclonal IgG in most patients (reviewed in Burgoon *et al*, 2004). Furthermore, antibody to components of myelin have been detected in the serum and CSF in SSPE patients, but the contribution of these minor reactivities to the oligoclonal response has not been determined (Panitch *et al*, 1980; Ruutianen *et al*, 1981; Gorny *et al*, 1983; Mathiesen *et al*, 1989). Thus, we studied the specificity of antibodies produced by less abundant plasma cells in SSPE brain whose sequences were only seen once during repertoire analysis.

Functional rIgGs were constructed from 8 of the 10 less abundant plasma cell IgG sequences (bold in Table 1). H chain variable regions were cloned into the modified expression vector pIgG Flag, which contains the remaining constant domains to express a full-length IgG1 H chain (Yu *et al*, 2006). The entire L chains from plasma cells (kappa or lambda) were cloned into the expression vector pCEP4. The H/L chain constructs representing each plasma cell were cotransfected into HEK293 cells, and the culture supernatants containing secreted rIgG were collected for analysis. After confirmation of size and H/L chain conformation for the rIgGs by electrophoresis in nonreducing gels and immunodetection by antihuman IgG antibody (H+L), the rIgG concentration in the supernatants was determined by capture enzyme-linked immunosorbent assay (ELISA) as described (Burgoon *et al*, 2005). All rIgGs were used at 3 to 7 μg/ml.

Immunostaining assays revealed that four of the eight rIgGs specifically stained MV-infected cells, but not uninfected Vero cells (Figure 1). rIgGs were also tested on Vero cells transfected with cDNAs encoding each of six major MV proteins. Although native IgG eluted from SSPE brain stained cells trans-fected with the MV nucleocapsid, hemagglutinin, or fusion proteins, only rIgG G5 specifically stained nucleocapsid-transfected cells, but nothing else (Figure 2). None of the antibodies detected the MV phosphoprotein, DNA polymerase, or matrix proteins (data not shown), consistent with the low immunogenicity of these proteins in acute measles infection and in SSPE (Hall *et al*, 1979; Graves *et al*, 1984; Dhib-Jalbut *et al*, 1988). By comparison, the rIgGs prepared from five clonally expanded plasma cell sequences in the same SSPE brain stained MV-infected cells, and all five recognized the MV nucleocapsid (Burgoon *et al*, 2005).

In Western blot analysis of MV-infected and uninfected cell lysates, none of the less abundant rIgGs recognized MV-specific proteins (Figure 3a). Similarly, none of the rIgGs

immunoprecipitated MV-specific proteins in infected cell lysates (Figure 3b). These results contrast with our previous findings that all five rIgGs prepared from clonally expanded plasma cell sequences that reacted with MV-infected cells recognized a 60-kDa species in MV-infected cells corresponding to the nucleocapsid protein, and that three of these rIgGs precipitated the nucleocapsid protein (Burgoon *et al*, 2005). The less abundant rIgGs that failed to recognize antigens in the present study were also unsuccessful in immunoblotting any antigens in SSPE brain, and none of the rIgGs or native IgG eluted from SSPE brain recognized specific antigens in normal brain (data not shown).

These analyses demonstrate that a significant proportion of the rIgGs from the less abundant plasma cell sequences encountered in SSPE brain are still disease relevant. Like those derived from the more abundant, clonally expanded plasma cell sequences, four of the eight rIgGs produced in SSPE brain were directed against MV. However, compared to rIgGs prepared from clonally expanded plasma cells, less abundant rIgGs were generally less successful in identifying specific MV proteins in the assays used here. This could be due to a lower abundance of certain MV proteins in transfected cells compared to productively infected cells in tissue culture. For example, each rIgG prepared from clonally expanded plasma cells recognized the MV nucleocapsid protein (Burgoon *et al*, 2005), which is the most abundant and immunologically reactive component of MV (Graves *et al*, 1984; Griffin, 2001). Alternatively, the conformation of MV-specific epitopes may have been changed by the immunoblotting or immunoprecipitation conditions, or some antigens might represent epitopes formed by interactions of MV components with cellular proteins, which fail to be recognized by immunoprecipitation analysis. The MV expressed in these assays may also contain epitopes that differ from those of the MV strain that developed in this SSPE brain. Finally, the less abundant rIgGs reactive to MV-infected cells could be lower-affinity antibodies. Identifying specific reactivities of rIgGs to these MV components will require additional assays.

Our findings were based on an analysis of 65 total plasma cells, 55 of which were clonally expanded. Although the 10 plasma cell sequences characterized here were encountered only once, it is possible that these sequences might be clonally expanded if a larger repertoire were examined. Nevertheless, these plasma cell sequences were less abundant than the large clonally expanded groups, and are less likely to represent the major oligoclonal immune response characteristic of the disease.

It is remarkable that when specific reactivity was detected for these minor clones, they were also disease-relevant, i.e., specific for MV. Although diverse immune specificities have been found in inflammatory CNS disease such as multiple sclerosis and acute disseminated encephalomyelitis, rIgGs prepared from either clonally expanded or less abundant plasma cells resident in chronic SSPE brain did not react with any ancillary antigens. Overall, functional analysis of rIgGs prepared from minor plasma cell sequences in SSPE brain revealed only disease-relevant antigens. In other inflammatory CNS conditions, molecular immunological analysis of plasma cells and B lymphocytes resident in brain may be use-ful to identify their cognate antigens and the inciting agents in disease.

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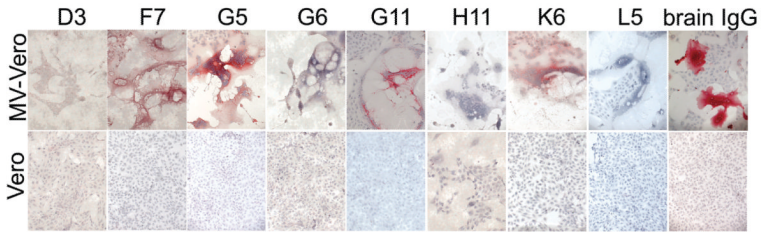

Figure 1.

rIgGs immunostain measles virus. Recombinant IgGs from eight of the less abundant CD38 plasma cells (7 μg/ml) and IgG eluted from SSPE brain (5 μg/ml) were used to immunostain MV-infected or uninfected Vero cells. Four of the eight rIgGs (F7, G5, G11, K6) and the brain-eluted IgG specifically stained MV-infected cells. Positive staining (*red*) was visualized by New Fuschin and counterstaining with hematoxylin.

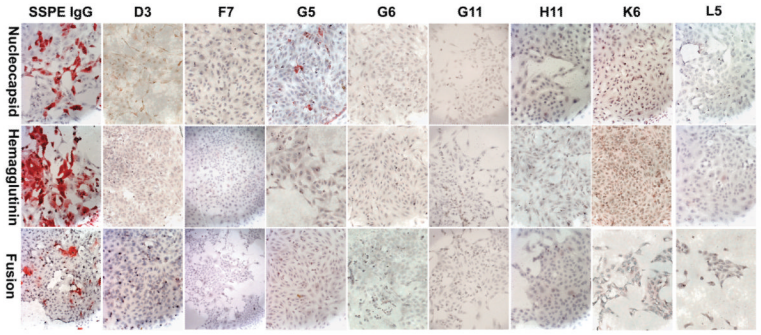

Figure 2.

Immunostaining of individual MV components. The four rIgGs that reacted with MV-infected cells, or IgG eluted from SSPE brain, were used to immunostain Vero cells transfected with the MV nucleocapsid, hemagglutinin, or fusion protein. IgG eluted from SSPE brain recognized each MV component. One rIgG (G5) stained the MV nucleocapsid protein.

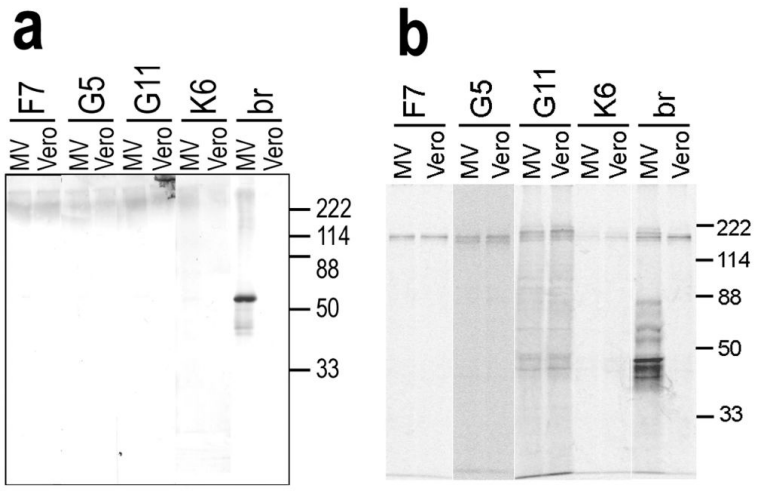

Figure 3.

Immunoblotting and immunoprecipitation of MV by rIgG. (**a**) Each positive rIgG (3 μg/ml) was applied to lysates of MV-infected or uninfected Vero cells. None of the rIgG recognized specific proteins, although IgG (3 μg/ml) extracted from SSPE brain (br) recognized multiple MV proteins in MV-infected cells. (**b**) Positive rIgGs or IgG extracted from SSPE brain (br) (2 μg) were used to immunoprecipitate ^{35}S-labeled lysates of MV-infected or uninfected Vero cells. The rIgGs did not precipitate MV proteins, but SSPE brain-extracted IgG precipitated multiple bands from the MV-infected lysates. Molecular mass standards are indicated on the right.

Table 1

IgG sequence analysis of CD38+ plasma cells in an SSPE brain

Clone	*CD38+ V_H CDR3*	*Abundance*	*Germline*	*Family*	*% ID*	*V_K CDR3*	*V_A CDR3*	*Germline*	*Family*	*% ID*
1	ALKKGEGGLRFLELYYFD	10	DP47	V_H3	92.1		QTWGSGMGV	Loc4b	Vλ4a	93.7
	ALKKGEGGLRFLELYYFD	3	DP47	V_H3	92.1					
	ALKKGEGGLRFLELYYLT	1	DP47	V_H3	92.1	QQNYSSPQT		DPK24	Vκ4	(t)
2	LPAAGPRSFFETYNWGMD	8	DP79	V_H4	93.6		AAWDDSLNAVWV	DPL2	Vλ1	97
	LPADGPRSFFETYNNGMD	1	DP79	V_H4	93.6		AAWDDSLNGWV	DPL2	Vλ1	97
	LPAAGPRSFFETYNWGMD	1	DP79	V_H4	93.6					
3a	IRAGAFD	2	DP31	V_H3	95.2	MQALQTFTF		DPK15	Vκ2	99.7
3b	IRAGAFD	4	DP31	V_H3	95.2	MQATQSWTF		DPK16	Vκ2	98.2
	IRAGAFD	1	DP31	V_H3	95.2					
	IRAGAFD	1	DP31	V_H3	95.2	(m)				
4	DFTSDSRGPLGWFD	4	DP79	V_H4	93.9		YSTDSSGDHRV	Loc3p	Vλ3	98.1
	DFTSDSRGPLGWFD	1	DP79	V_H4	93.9					
5	GGLAARARLVLARMD	3	DP63	V_H4	93.8	QQSYNTPITF		DPK9	Vκ1	95.1
	GGLAARARLVLARMD	1	DP63	V_H4	93.8					
6	VRATVLTGTSMD	2	DP58	V_H3	91.8		GADHGSGSNFVWV	DPL22	Vλ9	97.6
	VRATVLTGTSMD	1	DP58	V_H3	91.8					
7	DTGGSGSNYYHYGMD	2	DP10	V_H1	93.2					
	DTGGSGSNYYHYGMD	1	DP10	V_H1	93.2	QQYNAWPPALT		DPK21	Vκ3	(t)
8	DRGGESDYDVGRGYSDHYGMD	2	DP71	V_H4	86.9	QQCGFSPKT		DPK22	Vκ3	92.5
9	DQERGTILTYSDMD	2	DP47	V_H3	95.9	LQHNSYPHFRRR*		DPK3	Vκ1	95.5
10	DQVPVNNWFD	2	DP14	V_H1	95.2	(m)				
11	SLTMIRGVMAFFD	2	DP25	V_H1	87.6	QQTYSSPSTF		DPK9	Vκ1	90.9
D3	*DQVIYTGWSD*	1	DP47	V_H3	91.2		CLYAGSTTWV	DPL10	Vλ2	96.3
B11	*GYYDSTGYKSAND*	1	DP14	V_H1	94.0	QQTYSSPSTF		DPK9	Vκ1	90.9
D10	*LKSRIARGSYYQYFMD*	1	DP27	V_H2	93.1					
F7	*SADTSTAYYGLD*	1	DP47	V_H3	96.6	LQDYNYPLTF		DPK3	Vκ1	99.2

Clone	*CD38+ V_H CDR3*	*Abundance*	*Germline*	*Family*	*% ID*	*V_K CDR3*	*V_A CDR3*	*Germline*	*Family*	*% ID*
G5	*STGTDYYSYYMD*	1	DP73	V_H5	86.6		YSTDTSGNFRV	Loc3p	Vλ3	99.2
G6	*EGQLALDQYYYYYMD*	1	DP50	V_H3	96.3		NSYTSISTVV	DPL11	Vλ2	93.6
G11	*DRTGYTSFLFD*	1	DP31	V_H3	90.0		SSYAGRNKGYV	DPL12	Vλ2	96
H11	*DPEEQWLADYFD*	1	DP47	V_H3	97.6		GTWDSSLSARV	DPL5	Vλ1	98.9
LS	*VEVGPNEDFYMD*	1	DP88	V_H1	90.1	QQSYSFPWTF		DPK9	Vκ1	89.4
K6	*EVAGGADIEVVPAAIGVDYHYGI*	1	DP79	V_H4	97.3		QSADSSGSYKV			(t)

Note. Each line identifies the CDR3 amino acid sequence and prevalence of each distinct H chain clone, the germline family, most homologous germline segment, percent identity to the closest germline, and the associated L chain amplification for that clone. Less abundant clones are highlighted in italics. rIgGs were constructed from clones in bold. ∗an in-frame stop codon; (m), mixed sequence that could not be analyzed further; (t), truncated sequence.