Antigen Discovery in Chronic Human Inflammatory Central Nervous System Disease: Panning Phage-Displayed Antigen Libraries Identifies the Targets of Central Nervous System-Derived IgG in Subacute Sclerosing Panencephalitis

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The presence of increased IgG in the brains of humans with infectious and inflammatory CNS diseases of unknown etiology such as multiple sclerosis may be a clue to the cause of disease. For example, the intrathecally synthesized oligoclonal bands in diseases such as subacute sclerosing panencephalitis (SSPE) or cryptococcal meningitis have been shown to represent Ab directed against the causative agents, measles virus (MV), or Cryptococcus neoformans, respectively. Using SSPE as a model system, we developed a strategy to identify the antigenic targets of the intrathecal disease-relevant IgG in chronic human inflammatory and demyelinating diseases of the CNS. Libraries of cDNA Ags were displayed on the surface of T7Select bacteriophage and biopanned on IgG extracted from the brain of an SSPE patient, or on a monospecific recombinant Fab identified from SSPE brain. After three or six rounds of biopanning on either Ab, positive phage-displayed Ags reacting with IgG were enriched to 35–77% of all panned clones. Sequence analysis of the positive clones identified fragments of the nucleocapsid protein of MV, the cause of SSPE. The sensitivity of the system was determined by diluting the positive clones from this SSPE phage-displayed library at a ratio of 10−6 into another phage-display library that did not contain any detectable MV Ags; after six rounds of phage display, the positive clones comprised 34% of all phage and were also shown to be MV nucleocapsid specific. This strategy will be useful to identify potentially rare Ags in diseases of unknown cause. The Journal of Immunology, 2001, 167: 6009–6014.

The increased IgG and oligoclonal bands (OGBs) found in the brains of humans with infectious CNS diseases have been shown to be Ab directed against the causative agent (reviewed in Ref. 1). For example, OGBs in the cerebrospinal fluid (CSF) of patients with disorders such as cryptococcal meningitis, mumps meningitis, progressive rubella panencephalitis, HSV encephalitis, HTLV-1 myelopathy, subacute sclerosing panencephalitis (SSPE), and Lyme disease are directed against the respective virus, virus, or bacterium (2–8). In SSPE, a chronic progressive measles virus (MV) infection of brain (9), ~95% of the CNS IgG is synthesized locally (10), and 25–75% of the CNS Ab is directed against MV (7, 11). Additional IgG reactivities in the CSF of SSPE patients have been detected against other viruses, as well as myelin basic protein and various oligodendrocyte proteins (12–15). However, the pathogenic role of these autoantibodies is not clear, since Abs to myelin basic protein and myelin oligodendrocyte glycoprotein are frequently found in patients with various nonnondemyelinating neurologic conditions (16). It is also not known whether such minor reactivities contribute to the robust and persistent humoral immune response present in many chronic CNS inflammatory diseases. The complexity of these immune responses may indicate a link between persisting viruses and autoimmunity through mechanisms such as molecular mimicry or the unveiling of cryptic self epitopes (17–19). Nevertheless, the dominant humoral response of intrathecal IgG in chronic inflammatory diseases of the CNS appears to be directed against the cause of disease. OGBs are also present in the brains of several inflammatory CNS diseases of unknown cause, such as multiple sclerosis (MS) and CNS sarcoidosis. Although OGBs are found in 88–100% of CSF from MS patients, their corresponding Ags are unknown (1, 20). We predict that identification of the antigenic targets of the OGBs will help to understand the pathogenesis and even the cause of these diseases.

In this study, we describe a strategy to identify the Ag targets of the IgGs present in the CNS of a patient with SSPE, a chronic inflammatory encephalitis caused by MV. We constructed phage-display Ag libraries in the T7Select vector from mRNA expressed in the brain of an SSPE patient. The libraries were biopanned on IgG extracted from SSPE brain, as well as on a monoclonal Fab specific for the MV nucleocapsid, to determine whether such IgG could capture their specific Ags.

Materials and Methods

Brain tissue, cDNA library construction, and IgG isolation

Pathologically verified SSPE brain of a 22-year-old man (CNL) was removed at death, frozen quickly, and stored at −70°C. Total RNA was extracted with guanidinium isothiocyanate and acid phenol (21). Poly(A) RNA was isolated and used previously to synthesize and directionally clone cDNA into a ZAP (Stratagene, La Jolla, CA) cDNA expression library (22). The cDNA was size selected for inserts containing >400 nt before insertion into the vector, resulting in a directionally cloned library with an average insert size of 1.5 kb and a complexity of 9 × 106 PFU. A
second cDNA expression library was also constructed in λ ZAP from the postmortem brain of a 55-year-old woman with MS (23).

IgG was purified from SSPE brain tissue. Briefly, frozen brain was homogenized in TBS and centrifuged, and the insoluble pellet was resuspended in TBS. Soluble fractions were combined, and IgG was extracted by protein A-affinity chromatography, as described elsewhere (24). The MV nucleocapsid-specific Fab 4, purified from an SSPE brain by panning a Fab phage-display library on MV-infected Vero cells (25), was also used as Ab. After digestion of the selected pCOMB3H bacteriophage clones with SpeI/ NcoI restriction endonucleases, soluble Fabs were released into the culture media and purified by affinity chromatography on protein A-Sepharose.

**Phage-display library construction**

Ag phage-display libraries were constructed in the T7Select10-3b vector (Novagen, Madison, WI). cDNA from the SSPE λ ZAP library (described above) were first isolated as excised Bluescript plasmid DNA (Stratagene), and then purified from the Bluescript backbone by restriction with BamHI/ XhoI enzymes and agarose gel extraction using the QuickExtract Gel Extrac- tion kit (Qiagen, Valencia, CA). Inserts were subcloned into the T7Select10-3b vector by three separate strategies to maximize in-frame expression of the cDNAs on the T7 phage surface (see Table I): A, cDNA was partially restricted with Alul, purified from agarose gels, and 240 ng was ligated with 500 ng of T7Select10-3b digested with Smal (3:1 molar ratio), resulting in a library with a complexity of $1.5 \times 10^6$ PFU; B, cDNA was partially restricted with Sau3AI and 1 μg was ligated with the BamHI site of T7Select10-3b vector; and C, cDNA inserts from the λ ZAP library were directly subcloned into the BamHI/XhoI sites of T7Select10-3b. The libraries were packaged using T7 packaging kits (Novagen) and amplified by infection of log-phase BLT5615 bacteria ($A_{600} = 0.6$) in 50 ml of Luria-Bertani broth according to the manufacturer’s instructions.

**Selection of IgG-binding clones**

The three SSPE T7 libraries (Table I) were mixed proportionate to their specific activities. The combined library was amplified on the day before panning by infection of 50 ml of log-phase BLT5615 cells ($A_{600} = 0.6$) with 50 μl of mixed phage library in the presence of 1 mM isopropyl β-D-thiogalactoside, with continued incubation at 37°C until lysis was visible and centrifugation of the lysate at 8000 × g for 10 min. The phage-containing supernatant was biopanned on IgG. A total of 1 μg of brain IgG or 300 ng of Fab in 100 μl was incubated overnight at 4°C in each of two wells of a 96-well microtiter plate (Costar 3590; Costar, Cambridge, MA). Wells were rinsed twice with distilled water and blocked with 5% nonfat milk in TBS for 1 h at 37°C. The SSPE phage library supernatant was added directly to the wells for panning. In separate experiments, the SSPE library was diluted into a phage-display library constructed from a MS brain and devoid of any MV Ags. The proportion of SSPE IgG-reactive clones in the mixture with MS-derived clones was $1 \times 10^{-6}$ to $1 \times 10^{-6}$. After addition of 100 μl of phage supernatant to each well, the plate was sealed and incubated at 37°C for 1 h and washed five times with TBS/0.05% Tween 20 (TBST), and bound phage was eluted by incubation of 200 μl of 1% SDS for 10 min, followed by scraping the well surface with a pipette tip. The elution buffer from both wells was combined, and phage was tethered by serial dilution and infection of 250 μl of BLT5615 cells for 15 min, followed by growth overnight at room temperature on 100-mm Luria-Bertani agar plates containing 50 μg/ml carbenicillin. The remainder of the diluted phage was amplified by infection of 50 ml of log-phase BLT5615 cells as above, and phage lysate supernatants were used in immunoblotting with the IgG used for panning.

**Analysis of biopans**

After each pan, aliquoted lysate of the eluted phage was plated at limiting dilution by infection of 250 μl of BLT5615 cells, as described above. Replica nitrocellulose filters were lifted from plates containing 300–500 PFU, blocked in TBS/3% BSA for 1 h at room temperature, and incubated with 5 μg/ml panning substrate IgG or Fab in blocking buffer overnight at room temperature with agitation. Filters were washed five times for 5 min each with TBST and incubated for 1 h with 1/300 dilution of goat anti-human IgG Ab conjugated to alkaline phosphatase (Vector Laborato- ries, Burlingame, CA). Filters were washed five times for 5 min each with TBST, and Ab-positive plaques were visualized with nitroblue tetra- zolium. The total number of PFU was counted on each plate, as well as the number of PFU reacting with Ab. After overlaying the culture plate on the filter, positive plaques were randomly picked from the final pan in each experiment with sterile pipet tips. cDNA inserts from those clones were PCR amplified using the primers T7Up2 (5'-gggagacgattgtaactg-3') and T7Down2 (5'-ggtgagtccgagcgcagc-3'). Table I. SSPE Ag libraries

<table>
<thead>
<tr>
<th>SSPE T7 Library</th>
<th>Restriction of Inserts</th>
<th>Complexity (PFU)</th>
<th>% Inserts</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Partial Alul</td>
<td>1.5 × 10^6</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>B Partial Sau3AI</td>
<td>11 × 10^6</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>C BamHI/XhoI (full length)</td>
<td>2 × 10^6</td>
<td>70</td>
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</table>

Results

Phage-displayed Ag libraries were constructed from the postmortem brain of a 22-year-old SSPE patient in three subcloning strategies, using the repertoire of cDNA inserts contained in a previous expression library in the λ ZAP vector (22). cDNA was excised by BamHI/XhoI restriction digestion and directionally cloned into the corresponding sites of the T7Select10-3b phage display vector (library C in Table I). In addition, the cDNA was partially digested with Alul or Sau3AI and cloned into the Smal or BamHI site of T7Select10-3b, respectively (libraries A and B). These partially restricted libraries contain many smaller inserts than the BamHI/XhoI library, and are likely to contain expression products from cDNAs too large to easily clone into the T7 vector. Furthermore, each of the two partially restricted libraries is likely to express epitopes that may be interrupted in the other libraries by the re- striction digestion. Although the full-length cDNA library (C) contained only 200,000 distinct clones, the partially restricted libraries (A and B) contained 1.5 × 10^6 and 11 × 10^6 clones, respectively, several times the complexity of the original CNL library in λ ZAP.

Before panning, the three T7 libraries were combined according to their relative complexities, so that the mixture contained phage from each library proportional to its size. Approximately $3 \times 10^{10}$ of the resulting mixture was incubated in two wells of a microtiter plate preadsorbed with SSPE IgG from CNS tissue (Fig. 1). After washing the plate, bound phage were eluted with 1% SDS and amplified by infection of bacterial cultures. The amplified lysates were then reapplied to additional IgG in subsequent pans for further enrichment of Ab-specific clones. In parallel, the same phage-displayed Ag libraries were biopanned on wells adsorbed with a nonspecific recombinant Fab directed against the MV nucleocapsid protein (25). After three to six pans on either Ab, an equivalent number of PFU was plated from each pan. Replica filters were lifted from the plates and immunoblotted with the IgG used as the panning substrate (Fig. 2). This blot provided a convenient assay to measure the proportion of Ab-specific clones and to determine whether additional rounds of panning were necessary. The number of positive plaques was determined for each pan and expressed as a proportion of the phage eluted during that pan. Immunoblotting revealed ~1% positive clones in the initial SSPE phage-display libraries (Table II). However, after three rounds of panning on the IgG purified from SSPE brain, positive clones comprised 77% of all eluted phage. In similar pans on the MV nucleocapsid-specific Fab, 35% of all eluted phage were positive after phage display.

**Table I. SSPE Ag libraries**

- Phage-displayed Ag libraries were constructed by cloning cDNA derived from SSPE brain into the phage-display vector T7Select10-3b. Three libraries were produced by differential restriction digestion and insertion into different cloning sites (Materials and Methods) to maximize the number of cDNAs cloned and expressed in the correct reading frame. The complexity of each library and the percentage of phage containing inserts are given.
six rounds. The total number of eluted phage at each pan typically increased with increasing proportions of positive phage, providing an additional measure to monitor clone enrichment.

The sensitivity of the panning procedure for selecting specific Ags was extended by diluting the Ag libraries before panning. The three SSPE phage-display libraries were mixed with a separate T7 phage-display library constructed from the postmortem brain of an MS patient, a library that contained no PCR-amplified fragments of any MV components and no positive phage by immunoblotting with SSPE Abs. Although we have detected anti-MV Abs in other MS cases, the IgG from this MS brain failed to immunoblot MV lysates or stain SSPE brain sections (data not shown). The resulting diluted libraries contained positive clones for SSPE Ab at a dilution of 10^{-6} or 10^{-7}. The libraries were then panned on the SSPE IgG, as described above. After only three rounds of selection, the positive clones initially diluted to 10^{-4} enriched to 37% of all eluted phage (Table III). In parallel panning experiments, the SSPE library was initially diluted to 10^{-6} positive clones in the background of the MS library. This panning required six rounds of selection to enrich the positives to similar levels of 34% (Table III). Replica filters lifted from each panning round were immunoreacted on replica filters for the detection of positive clones.

![Figure 1](image1.png)

**FIGURE 1.** Panning strategy. Phage-displayed Ag libraries were incubated in wells containing IgG substrates and washed, and bound phages were eluted with 1% SDS. An aliquot of the eluted phage was titered on bacterial plates, and the remainder of the eluted phage was amplified by infection of BLT5615 bacterial cultures and reapplied to a fresh microtiter plate adsorbed with additional IgG for subsequent biopanning. Phage that was titered on bacterial plates was immunoreacted on replica filters for the detection of positive clones.

![Figure 2](image2.png)

**FIGURE 2.** Detection of positive clones by immunodetection. Eluted phage from each pan were plated at limiting dilution to densities of 300–500 PFU/dish. Replica nitrocellulose filters were lifted from each dish and reacted with the panning substrate IgG. Positive clones were detected by reaction with alkaline phosphatase-conjugated secondary Ab and nitroblue tetrazolium. Positive clones were enriched on substrates of either SSPE IgG (SSPE 214) or monospecific MV nucleocapsid Ab (anti-N Fab 4), from very low numbers in the initial pans to a large percentage of the total PFU in later rounds.

<table>
<thead>
<tr>
<th>Table II. Panning undiluted Ag libraries on SSPE Absa</th>
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<tr>
<td>Undiluted Ag Library on SSPE IgG (214)</td>
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<tr>
<td>Pan 0</td>
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<td>Pan 1</td>
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<tr>
<td>Pan 2</td>
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<td>Pan 3</td>
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<tr>
<td>Monoclonal Fab (4)</td>
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<tr>
<td>Pan 0</td>
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<td>Pan 1</td>
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<td>Pan 6</td>
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a After each pan, aliquots of eluted phage were titrated on bacterial culture plates and the number of eluted phage in 1 μl of elution buffer was calculated. Replica filters were lifted from the plates, and positive clones were detected by reaction to the Ab used as the panning substrate. The number of IgG-reactive PFU as a percentage of the total PFU was calculated for each pan.

b n/a, Not applicable.

The sensitivity of the panning procedure for selecting specific Ags was extended by diluting the Ag libraries before panning. The three SSPE phage-display libraries were mixed with a separate T7 phage-display library constructed from the postmortem brain of an MS patient, a library that contained no PCR-amplified fragments of any MV components and no positive phage by immunoblotting with SSPE Abs. Although we have detected anti-MV Abs in other MS cases, the IgG from this MS brain failed to immunoblot MV lysates or stain SSPE brain sections (data not shown). The resulting diluted libraries contained positive clones for SSPE Ab at a dilution of 10^{-4} or 10^{-5}. The libraries were then panned on the SSPE IgG, as described above. After only three rounds of selection, the positive clones initially diluted to 10^{-4} enriched to 37% of all eluted phage (Table III). In parallel panning experiments, the SSPE library was initially diluted to 10^{-6} positive clones in the background of the MS library. This panning required six rounds of selection to enrich the positives to similar levels of 34% (Table III). Replica filters lifted from each panning round were immunoreacted on replica filters for the detection of positive clones from the early rounds, they rapidly accumulated in later rounds (Fig. 3).

After high enrichment of IgG-reactive phage, a random sample of the positive plaques was picked from the culture plate after alignment to the immunoblotted filter, and the displayed Ags in the phage were identified. The clones selected on the SSPE IgG were identified by sequence analysis of the respective cDNA inserts. Initially, five positive plaques were picked after the last round of panning with the undiluted library and each of the diluted libraries. The cDNA insert from each clone was PCR amplified using adjacent primers in the T7Select10-3b vector, and the PCR fragment was sequenced at the University of Colorado Cancer Center Core sequencing facility. Fourteen of these 15 sequences revealed distinct, but overlapping clones, representing a small region of the MV nucleocapsid gene. All of the 14 clones terminated at a natural Sau3A1 site found in the nucleocapsid sequence (Fig. 4). The last sequence was a nonoverlapping clone of the same nucleocapsid gene. For the clones selected on the recombinant anti-nucleocapsid Fab, 10 plaques were randomly picked, PCR amplified, and analyzed by gel electrophoresis. Four distinct sets of similarly sized cDNA fragments were revealed. Hybridization of these fragments

<table>
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<tr>
<th>Table III. Panning of diluted Ag libraries on SSPE IgGa</th>
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<td>10^{-4} diluted library on SSPE IgG (214)</td>
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<tr>
<td>Pan 0</td>
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<td>Pan 1</td>
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<td>10^{-5} diluted library on SSPE IgG (214)</td>
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a SSPE Ag libraries were diluted into a phage-display library constructed from a MS brain, so that the positive clones for SSPE IgG were present at 10^{-4} and 10^{-5}. The diluted libraries were panned sequentially on SSPE IgG, and positive clones were enriched to detectable levels after three to six rounds of panning.
b n/a, Not applicable.
to a $^{32}$P-labeled probe representing the MV nucleocapsid cDNA revealed 9 positive clones of the 10 examined (data not shown).

To search for selected phage-displayed clones representing different MV proteins or clones that may represent autoimmune Ags other than MV, 30 additional phage that reacted with SSPE IgG were picked from early pans in which the proportion of positives was only 2–3% (in Table III, Pan 2 for the $10^{-4}$ panning or Pan 4 in the $10^{-6}$ panning). Many of these additional clones were also chosen for their weaker reactivity with the SSPE IgG, and therefore may have represented secondary immune responses in the SSPE brain. Most of the cDNAs identified in these additional clones encoded the same regions of the MV nucleocapsid gene, or different regions of that gene (Fig. 4). One clone encoded the 5′ untranslated region of a human small ribonucleoprotein (accession number XM018380). However, the peptide that is expressed in T7Select phage at the 5′ end of this clone (RPRRGGAAGDA) did not demonstrate homology to any measles sequence, or any other sequence in the Swissprot or translated GenBank databases. The SSPE IgG reacts weakly with this phage, but it is not known what this epitope represents.

**Discussion**

In other chronic inflammatory CNS diseases of humans in which oligoclonal bands (OGBs) of IgG are present, the immunologic targets of the OGBs have been shown to be the causative agent of the disease (reviewed in Ref. 1). However, in other human inflammatory and demyelinating CNS diseases that contain OGBs, such as MS, CNS sarcoidosis, Behcet’s disease, and many of the vasculitides, the cause is unknown. We have developed a strategy to identify the antigenic targets of the OGBs present in the CNS, particularly when the Ag is present at low abundance. Rare Ags displayed on the surfaces of a phage library can be enriched and amplified to enhance detection and identification. Using SSPE as an experimental paradigm, we have demonstrated that Ags in the brain, including those from pathogens, can be displayed on phage surfaces and biopanned on CNS-derived IgG. The enrichment and identification of the corresponding Ags, in this case MV, identified the target of the immunologic response and the etiologic agent of the disease.

To maximize the probability of expressing all transcripts in brain, we used three separate strategies to construct Ag-display libraries from an SSPE brain in the T7Select vector. In addition to full-length cDNAs from the original λ library, we subcloned smaller fragments of the cDNAs from partial digests with two distinct restriction endonucleases, Alul and Sau3A1. In this way, cDNAs subcloned from one internal site (e.g., Alul) that do not allow in-frame expression in T7 may be properly expressed from a different site (e.g., Sau3A1). Partially restricted cDNA inserts also increased the probability that each epitope is expressed, either from its nearest upstream restriction site or from a more distant site.

The biopanning of phage-displayed Ag libraries on IgG substrates allows the selection and enrichment of specific Ags. All of the positive clones identified from the last pans in our experiments were cloned into the BamHI site of the T7Select vector, and thus were derived from the largest library B. Positive clones picked from earlier pans were also derived from library B, except for one positive clone from the $10^{-4}$ panning experiment that was derived from the Alul library (A). The selection of the majority of clones from the largest library may simply reflect the relative contribution of the libraries to the original phage population.

The rate of enrichment, i.e., the number of pans necessary before an Ag is clearly distinguished and readily picked from a plate of only several hundred clones, depends in part on the selecting Ab. As shown in Table II, the SSPE library panned on IgG from the brain yielded 77% positive clones in the population eluted from the third pan, whereas the same library panned on a monospecific Fab required six rounds to recover 35% positive clones. The faster enrichment of clones with brain IgG may be expected if such IgG selects multiple epitopes from a single Ag (Fig. 4).

The vast majority of the clones selected on SSPE IgG encoded the nucleocapsid protein (Fig. 4). Although many of the clones overlapped the same region of the nucleocapsid sequence, distinct sets of clones would have translated from distinct sites in the nucleocapsid transcript, indicating that they represented different clones in the original library that were probably coselected due to the common epitopes, rather than a population derived from one predominant or fast-growing clone.

Although from other SSPE brains we have identified IgG reactivities to other MV components, including high affinity reactivities to the phosphoprotein (25, 27), the IgG from the SSPE brain used in the current study selected almost exclusively nucleocapsid clones, and is consistent with other studies that demonstrate a high reactivity for the MV nucleocapsid (28, 29). Indeed, intrathecally synthesized IgG from SSPE patients has detected multiple MV components (24, 25, 27, 30–32). However, this Ag-panning technique may prove more useful for determining the major reactivities exhibited by the IgG. The detection of minor or multiple reactivities of IgG in other chronic inflammatory CNS diseases using this panning strategy may be hindered by the very rapid enrichment of high affinity phage-bound Ags. However, the selection of clones at early panning stages may reveal different Ags with widely differing reactivities. In this study, clones selected from early pans revealed a larger set of MV nucleocapsid clones than those selected in the last pans, as well as one clone unrelated to MV. Although the SSPE IgG used in this study has only immunostained SSPE brain containing MV, and not control brains from MS patients (data not shown), the identification of this additional clone as a
non-MV Ag also demonstrates the potential for selecting self Ags as well as exogenous ones with IgG extracted from the CNS. This may be possible even in the presence of an overwhelming response to a single Ag, as seen in this study.

In addition to the MV clones, one clone was selected with SSPE IgG that expressed a peptide from the S' untranslated region of the human ribonucleoprotein. Although surprising, the peptide’s antigenicity is more likely to represent a related reactivity for a different non-MV Ag in the SSPE brain, which was not suggested by homology searches of various protein databases. This non-MV reactivity in SSPE may reflect the autoimmune response seen in several SSPE studies (12–15).

Our panning strategy requires that the Ag persist in the tissue used to produce the Ag library, and is most useful to select and amplify low-abundance Ags that may otherwise be undetectable. This is likely to occur in chronic CNS conditions that demonstrate persistent OGB profiles, a condition that probably requires continued antigenic stimulation to maintain OGB production. However, the initial IgG-stimulating Ag might not be present when the Ag library is constructed, or its transcripts might be degraded during the cDNA synthesis. Alternative strategies have been developed for the panning of phage-displayed libraries of synthetic peptides to identify IgG-binding motifs (33, 34). However, the panning of short oligomeric peptides works best on substrates of single or highly restricted IgGs, since the antigenic correlates of the bound phage are best discerned from the presence of common sequences or motifs in the eluted phage-bound peptides and from their subsequent alignment to sequence databases. Multiple IgG reactivities may select populations of diverse peptide motifs, complicating the identification of shared sequences. Furthermore, the phage-displayed cDNA libraries described in this work typically provide larger sequences in the selected clones to accurately identify the endogenous Ag.

Our phage-display Ag library strategy provides several advantages over previous techniques used for Ag identification, such as expression library screening (35). First, the phage-display panning of Ags requires far less IgG to detect and isolate the positive clones than the hundreds of micrograms typically required in expression libraries. Second, the panning technique enables more rapid screening of a cDNA library displayed on phage, with the potential to examine a complex library containing $10^7$ clones in 1–2 wk and limited only by the complexity of the constructed library. Phage-display panning of cDNAs has been successfully used to identify Ab-binding epitopes in tobacco mosaic virus and hepatitis C core proteins, as well as ligands for transcription factors, lipocalin, and type 1 plasminogen activator inhibitor (36–40).

Our data demonstrate that phage-displayed Ag libraries constructed from the brains of chronic human inflammatory CNS diseases can be used to select and identify the appropriate Ags targeted by the humoral response, even to enrich relatively rare clones present in a complex library. This method will identify persistent Ags without bias to their pathogenic or autoimmune nature, and will be useful in identifying disease-relevant Ags in other CNS inflammatory diseases of unknown cause.

Acknowledgments

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