Exploring cDNA phage display for autoantibody profiling in serum of multiple sclerosis patients: Optimization of the selection procedure

C. Govarts[a], *K. Somers*[a], *R. Hupperts*[b], *P. Stinissen*[a], *V. Somers*[a, c]

[a]Hasselt University, Biomedical Research Institute, and Transnationale Universiteit Limburg, School of Life Sciences, Agoralaan, Building A, B-3590 Diepenbeek, Belgium.

[b]Department of Neurology, Academical Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands

[c] Corresponding author: veerle.somers@uhasselt.be, 0032(0)11269202, fax: 0032(0)11269209

Abstract

We applied a cDNA phage display method called serological antigen selection (SAS) to identify immunogenic targets that evoke an autoantibody response in serum of multiple sclerosis (MS) patients. This method involves the display of a cDNA expression library, in this study a MS brain library, on filamentous phage and subsequent selection using patient immunoglobulin G (IgG). To apply the SAS technology for autoantibodies in serum of MS patients, an optimization was necessary to deplete for cDNA products that encode IgG-fragments derived from B cells present in the MS brain plaques. We describe a differential screening procedure in which positive selection rounds on MS serum and negative selection rounds on healthy control serum were alternated to optimize the selection procedure. As a result, a substantial decrease of IgG-displaying phage clones was observed after each negative selection round, thereby preventing an overgrowth of IgG-displaying phage clones. Our depletion strategy was therefore successful in preventing enrichment of IgG-displaying phage clones. This approach will facilitate the identification of possible MS-related antigens.

Keywords: serological antigen selection, filamentous phage, cDNA display, multiple sclerosis, autoantibody repertoire, autoimmune disease

1. Introduction

Multiple sclerosis is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). It is the most common disabling neurological disease affecting mostly young adults with a predominance for females and a prevalence in the USA and Northern Europe of ~100 per 100,000 people.[1] The etiology of MS is still unclear but most evidence supports an autoimmune pathogenesis of the disease in which both the innate and the adaptive part of the immune system are involved. According to this autoimmune hypothesis, autoreactive T cells are considered to be the most important contributors to the inflammation in MS. However, it is now clear that B cells and antibodies also contribute in many ways to the pathology of MS by being involved in several processes like antigen capture and presentation, tissue damage and even remyelination and tissue repair.[2]

The most compelling evidence for a role of the humoral immune response is the intrathecal antibody production by plasma cells in the cerebrospinal fluid (CSF), resulting in oligoclonal bands (OCBs) in more than 95 % of MS patients.[3] The oligoclonal bands differ between patients but remain constant over time in each individual patient, indicating a long-lived chronic intrathecal immune response.[4-6] OCB antibodies are not specific for MS but are also found in other inflammatory neurological diseases like subacute sclerosing panencephalitis (SSPE).[7] In SSPE, the majority of the OCB antibodies are directed against the measles virus that is causing the disease whereas for MS there is still no general consensus on the nature of the antigens that react with the OCB present in the CSF.[8,9]

Several groups showed the oligoclonal expansion of B cells in the CSF and lesions of MS patients. When studying the immunoglobulin (Ig) heavy chain variable regions (VH) repertoire, it is clear that a preferential usage of VH1 and VH4 chains occurs. Also numerous somatic hypermutations were reported with a high replacement-to-silent ratio in the CDR3 regions.[10-12] Together these data indicate an antigen-driven B cell response in the CNS of MS patients. This hypothesis is further supported by the identification of chronically activated B-lineage cells and short-lived plasma blasts within the meninges and CSF of MS patients.[13,14] The high proportion of these cells indicates an ongoing local exposure of

disease-relevant antigens to the immune system. The important role of the humoral immune response in MS has also been demonstrated in pathological studies. Luchinetti et al. have shown that 4 distinct lesion patterns exist in MS patients. The most common pattern (pattern II) of MS lesion pathology is characterized by the presence of antibodies and complement in addition to T cells and macrophages.[15] This study indicated the existence of a subset of MS patients in which the humoral immune response plays a key role.

Irrespective of their exact role in the disease, antibodies may also serve as valuable biomarkers in MS. Biomarkers can be used as indicators of disease activity and/or prognosis of MS. Subtyping patients according to their immunopathogenic profile and subsequent adjustment of therapy is another potential application of such markers. Because of the complexity of the MS pathogenesis, it is highly unlikely that a single biomarker will predict the outcome and prognosis of MS. Rather, a panel of biomarkers must be used to give a good indication of the different disease processes. Several studies have used antibodies in MS serum as a means to detect biomarkers or predict disease course. The group of Prin et al. used the IgG-antibody response against brain antigens to analyse antibody profiles of MS patients and controls. Based on these antibody profiles, they were able to discriminate controls from MS patients. Moreover, specific IgG-patterns allowed them to distinguish between the three subtypes of MS.[16] Other groups reported the identification of possible disease related antibodies in serum of MS patients.[17-19] Together, these results clearly indicate the importance of serum as a source of valuable biomarkers for MS.

One way to analyse the antibody profile in serum of MS patients is by using phage display. Phage display was introduced by Smith et al. in 1985[20] and has since then successfully been used to identify any type of ligand. Display of proteins on the surface of filamentous phage is possible by fusion of the gene of interest to one of the phage coat genes like gIII, gVIII or gVI. Expression of the fusion product and its subsequent incorporation into the mature phage coat results in the ligand being expressed on the phage surface while its genetic material resides within the phage particle, thereby providing a physical link

between genotype and phenotype. This physical link makes it possible to select rare phage carrying the desired gene from large phage populations carrying unwanted sequences. After several consecutive rounds of affinity selection, target-specific phage populations are obtained and the selected gene products can be analysed and identified.

Phage display technology has already been used in the search for biomarkers in MS research. Most research until now focused on random peptide libraries (RPL) displayed on phage. However, mostly candidate antigens with patient-specific immunoreactivity were identified[21,22] due to the use of individual CSF for the screening of RPL. Other studies using RPL have identified some candidate markers that need further evaluation.[23,24] An important disadvantage when using random peptide libraries is the recovery of antigen mimotopes which require further elaborate analysis to identify the actual antigen.

To search for novel biomarkers, we used a cDNA phage display-based method named serological antigen selection (SAS) that makes use of antibody profiles present in serum or CSF of MS patients to identify antigens that evoke an antibody response. In a high-throughput selection procedure, antibodies present in serum or CSF are used to screen a MS cDNA display library derived from MS brain plaques with varying degrees of inflammation and demyelination. The successive cycles of selection and amplification of selected antigens lead to the identification of candidate MS antigens. Previously, we successfully applied the SAS procedure to identify immunogenic markers with CSF of MS patients (Somers et al., in preparation). To apply this technology in serum of MS patients an optimization procedure was necessary due to the broad antibody profile present in serum. Furthermore, our previous experiments showed that screening of selected antigens was hampered by the occurrence of cDNA products that encoded for IgG-transcripts derived from B cells present in the MS brain plaques when constructing the MS cDNA library. In this paper, we describe the optimization of a differential screening procedure in which positive selection rounds on MS serum and negative selection rounds on healthy control (HC) serum were alternated. Using this approach the aspecific enrichment of IgG-encoding cDNA fragments was highly reduced.

2. Materials and methods

2.1. Patient material

Serum samples were obtained from MS patients, patients with other inflammatory (meningitis, polyneuropathy,…) and non-inflammatory neurological disorders (hernia, epilepsy, dementia,…) and healthy volunteers. After collection, serum samples were stored at –80°C.

For the affinity selection, pooled serum from 10 untreated RR MS patients was used (8 women, 2 men, mean age 47, mean Expanded Disability Status Scale (EDSS): 2, range: 1-4). Pooled serum was first adsorbed against *Escherichia coli* (*E. coli)* and phage components by repeated passage through columns of Sepharose 6MB (Pharmacia, Uppsala, Sweden) coupled to lysates of *E. coli* Y1090 and bacteriophage-infected *E. coli* XL1Blue. After adsorption steps, aliquots of sera were prepared in 0,2 % (w/v) skimmed milk powder in 1 x tris-buffered saline (TBS) (50mM Tris-HCl (pH 7.9) and 150 mM NaCl) and stored at –20°C.

2.2. *Construction of cDNA phage display library*

A cDNA library (1.0 x 10^6 primary recombinants), derived from active chronic MS plaques with varying degrees of demyelination and inflammatory activity was kindly provided by Dr. Soares (University of Iowa). This library was normalized by a procedure based on reassociation kinetics.[25] The cDNA library was transferred into our cDNA phage display vectors, named pSPVIA, pSPVIB and pSPVIC,[26] each representing one of three different reading frames. Cloning procedure was performed as described previously.[27]

2.3. Serological antigen selection of phage cDNA repertoires

Pooled serum samples of 10 RR MS patients were used for affinity selections. Selections were performed as described previously.[27] Briefly, immunotubes (Nunc, Roskilde, Denmark) were coated with 10 μg/ml rabbit anti-human (RαH) IgG (Dako, Glostrup, Denmark) in coating buffer (0.1 M sodium hydrogen carbonate pH 9.6), overnight at 4°C. After washing the immunotubes with phosphate-buffered saline/Tween 20 (PBSTween: 50 mM Tris, 150 mM NaCl, pH 7.5, 0.1 % Tween 20 (v/v)) and PBS, the tubes were blocked for 2h at room temperature (RT) with 2 % MPBS (2 % w/v milk powder in PBS). Phage were purified from each library (MS-pSPVIA, MS-pSPVIB and MS-pSPVIC) as described previously.[28] Approximately equal numbers of phage derived from each library (ca. 2 x 10^{12} pfu) were added to pooled serum (1:100 diluted in 4 % MPBS) and incubated in a glass tube for 1.5 h at RT on a rotating platform. After washing the coated immunotube with 0.1 % PBSTween and PBS, the preincubated serum and phage mix was transferred to the coated immunotube and incubated for 30 min on a rotating platform and for 2h standing at RT. Tubes were washed extensively with 0.1 % PBSTween and PBS to remove non-bound phage. Bound phage were eluted with 1 ml of 100 mM triethylamine for 10 min on a rotating platform and neutralized with 0.5 ml 1 M Tris-HCl, pH 7.4. Input and output phage were amplified by infecting *E. coli* TG1 cells and were plated on 2 x YT agar plates containing ampicillin and glucose (31 g/l Difco™ yeast extract tryptone (BD, Erembodegem, Belgium), 15 g/l bacto™ agar (BD, Erembodegem, Belgium), 100 μg/ml ampicillin and 2 % (w/v) glucose) at each round of

selection. Resulting colonies were scraped and phage were rescued for further rounds of affinity selections. Input and output phage from each round of selection were titrated and the ratio of output/input phage was determined to monitor enrichment of specific clones.

2.4. Depletion of aspecific phage

In previous experiments using MS serum, specific enrichment of phage displaying candidate MS antigens occurred after each selection cycle. However, after a few selection cycles, cDNA products encoding for IgG transcripts were identified. For that reason, an alternative selection strategy, called negative selection, was introduced to deplete these antigens. After each positive selection round phage were prepared and used for a subsequent negative selection cycle on healthy control (HC) serum (1:100 diluted in 4 % MPBS). After a preincubation for 1.5h at RT on a rotating platform, the preincubated HC serum and phage mix was transferred to the coated immunotube for 30 min on a rotating platform and 2h standing at RT. Non-bound phage were then recovered and used for infection of *E. coli* TG1 cells. Phage were rescued again and the selection process of alternating selections on MS patient and normal sera was repeated. After each selection round, output phage were used for amplification and titration.

2.5. Screening for enriched clones with PCR and fingerprinting

After a few selection rounds, individual colonies were picked and the insert size was determined by PCR. Primers (Eurogentec, Ougree, Belgium) flanking the *Eco*RI and *Not*I cloning sites of the vector were used (forward: 5'-CTC TCT GTA AAG GCT GC-3' and reverse: 5'- CGC CAG GGT TTT CCC AGT CAC GAC-3'). Briefly, after an initial denaturation step for 5' at 95°C, colonies were subjected to 35 cycles (30" 94°C, 30" 55°C, 1' 72°C) of PCR amplification in an ICycler (Biorad, Nazareth Eke, Belgium). Amplification products were analysed by gel electrophoresis on a 1.0 % agarose gel to confirm the presence and size of the cDNA inserts. PCR products were used for fingerprinting analysis by incubating 10 µl of the amplification product with 5U of the restriction enzyme *Bst*NI (Roche Diagnostics, Vilvoorde, Belgium) for 2h on 37°C. After enzyme digestion, restriction products were analysed on a 2 % agarose gel.

2.6. ELISA of ligand-displaying phage

Ninety-six-well flat-bottomed microtiter plates (Falcon/BD, Erembodegem, Belgium) were coated overnight at 4°C with 200 µl of RαH IgG (Dako, Glostrup, Denmark), 10 µg/ml in coating buffer (0.1 M sodium hydrogen carbonate pH 9.6) and blocked with 200µl of 2 % MPBS for 1h at RT. Following preincubation of 50 µl diluted serum (1:100 in 2 % MPBS) with 100 µl of PEG-purified phage (10^{10} phage/well) in a 96-well round-bottomed plate (Nunc, Roskilde, Denmark) for 1h at 37°C, the plates were shaken for 30 min at RT. After washing three times with 0.1 % PBSTween and PBS, the preincubation mixture was transferred to the RαH IgG-coated plate and incubated for 1h at 37°C and 30 min shaking at RT. After washing the plate with 0.1 % PBSTween and PBS, 150 µl of a peroxidase conjugated anti-M13 monoclonal antibody (Amersham/ Pharmacia/ Biotech, Diegem, Belgium), diluted 1:5,000 in 2 % MPBS was incubated for 1h shaking at RT. After washing the plate with

0.1 % PBSTween en PBS, 130 µl of a 3,3',5,5' tetramethyl-benzidine dihydrochloride (TMB) chromogen solution (10mg/ml) was added. Colour development was stopped with 65 µl/well 2 M H_2SO_4. The plates were read at 450nm in a Bio-Rad Benchmark microplate reader (Bio-Rad, Nazareth Eke, Belgium).

For identification of plastic-binders, plastic-binding capacity was assessed with the above described standardized ELISA-protocol (in presence of serum) and several experimental conditions (in absence of serum): i) non-coated microtiter plates blocked with 2 % MPBS, ii) non-coated microtiter plates blocked with 2 % BSA (in PBS) and iii) non-coated microtiter plates blocked with PBS.

3. **Results**

3.1 Serological antigen selection of a MS cDNA library with patient serum

Pooled serum samples of 10 untreated RR MS patients were used for selections of a MS phage display library against a polyclonal anti-human IgG antibody. We decided to pool serum samples to diminish patient-specific reactivity and maximize the identification of MS-related antigens. The SAS procedure is outlined in figure 1a. For a first, positive selection round, equal numbers of phage purified from our three cDNA phage display libraries MS-pSPVIA, MS-pSPVIB and MS-pSPVIC are incubated with pooled serum from MS patients. IgGs present in serum are able to bind to specific antigens that are displayed on phage. Selected phage clones are amplified and used for a subsequent selection cycle. After each selection round, input and output titers are determined. Table 1 shows a rise in output/input ratio with increasing rounds of selection which is a reflection of enrichment of specific phage clones. From the 4th positive selection round, a stabilization in the output/input ratio was obtained. Individual colonies from each round of selection were picked and the insert size was determined by colony PCR. Insert sizes ranged from 600 to 2100 bp.

3.2 Depletion of aspecific phage clones

To reduce the number of phage clones that express IgG in our expression library, a depletion strategy was developed that entailed the use of negative selection rounds in which pooled serum of HC was used. After each positive selection round (using MS serum), a negative selection procedure was performed as depicted in figure 1b. In total, 10 alternating selection rounds were performed (5 positive and 5 negative

selection rounds). PCR and fingerprinting analysis were used to determine the percentage of IgG-displaying phage clones. Table 2 shows the percentage of IgG transcripts from selection rounds 1-4. The percentage of IgG-displaying phage clones substantially decreased after each negative selection cycle.

To analyse enrichment of phage clones, fingerprinting analysis was performed after each selection. For the first 3 selection rounds, no enrichment was found while for the 4th selection round 3 phage clones, UHMSs-1, UHMSs-2 and UHMSs-3 (short for UHasselt MS serum pool-number of the clone) were enriched 3, 2 and 5 times, respectively. Enrichment was also determined in later rounds of selection but ELISA experiments revealed that this enrichment was aspecific.

3.3 *Phage ELISA screening of antigens selected with serological antigen selection*

The efficiency of selection was determined by testing the number of phage clones reactive in ELISA with the MS serum pool used in the selection procedure. Positive clones were identified after 4 rounds of selection. Empty phage was used as a negative control. Reactivity of selected antigens was further determined in the individual sera comprising the MS serum pool used for the SAS procedure. Figure 2a illustrates the results of phage clones UHMSs-1 and UHMSs-2 derived from the 4th selection round. Both phage clones show immunoreactivity in two individual sera of the MS serum pool. The threshold line of the background values was set at 1.5 times the mean of the background values. Strikingly, more aspecific phage clones were identified as the number of selection cycles increased. From the 7th selection round on, immunoreactivity of 6 enriched phage clones was tested in the individual sera of the pool. From these phage clones, 4 showed an aspecific binding capacity (plastic-binding) while the others did not show immunoreactivity with the individual serum samples. Figure 2b illustrates the results of phage clones UHMSs-4 (derived from the 7th selection round) and UHMSs-5 (derived from the 8th selection round). Empty phage was used as control. Reactivity of UHMSs-4 and UHMSs-5 is very high in all tested conditions which indicates that the reactivity is directed against the plastic surface of the microtiter plates. This is in contrast to reactivity of empty phage which was comparable with normal background signals when tested under standard conditions and when the microtiter plate was blocked with 2 % MPBS (in absence of serum). When different blocking conditions were applied (2 % BSA or PBS) reactivity of

empty phage was higher than standard background signals (using 2 % BSA) and comparable (using PBS) to the aspecific signal of the plastic-binders.

Despite the fact that in later rounds aspecific phage clones were selected, we identified immunoreactive clones in earlier selection rounds which displayed specific reactivity in individual sera of the pool. Immunoreactivity and MS-specificity of these phage clones will be tested on a larger panel of serum samples derived from both MS patients and controls.

4. Discussion

In our study, we used a cDNA phage display method called serological antigen selection (SAS) as a strategy to identify new antigenic markers in serum of MS patients. Previously, our group successfully applied this technology in CSF of MS patients. A panel of 8 novel antigenic MS markers was identified and these candidates are currently under further investigation (Somers et al., in preparation). In this and our previous CSF study a cDNA expression library derived from active chronic MS plaques with varying degrees of demyelination and inflammatory activity was transferred into our pVI-cDNA phage display vectors resulting in high diversity libraries (total library size of 1.1×10^7 cfu). The use of a cDNA library displayed as a C-terminal fusion of minor coat protein VI has already been proven successful in several studies.[29,30] Fusion of cDNA to minor coat protein VI results in functional monovalent display of foreign proteins.[26,29] In that way, high affinity antigen-antibody interactions are preferably selected and weak background interactions are reduced. When cDNA is used, both small gene fragments and whole genes can be displayed, depending on the targets that need to be identified. Small fragments have the advantage of more efficient expression and less toxicity to bacteria and are excellent for mapping binding domains. It should however be kept in mind that fragmented libraries need to be larger in order to include a great diversity. Expression of larger fragments (up to 3 kbp) is also possible but toxicity to bacteria can be a problem regarding the efficient expression of the corresponding proteins.[31]

To apply SAS in serum, an optimization of the procedure was needed to deplete cDNA products encoding for IgG-transcripts and to diminish the broad antibody profile existing in serum. In serum a natural antibody repertoire exists of which the majority is non-disease related. By alternating positive selection rounds, using MS serum, with negative selection rounds, using HC serum, a depletion of IgG–encoding phage clones and non-disease related phage clones occurs, thereby facilitating the identification of MS-related cDNA phage clones. For a first, positive round, the cDNA phage display library is incubated with pooled serum of MS patients. Serum antibodies interact with their specific antigen displayed on phage. Phage-antibody complexes are isolated and selected phage used for a subsequent negative selection round using pooled serum of HC. HC antibodies will bind non-MS related phage clones and IgG-displaying clones. Therefore, phage not interacting with HC serum antibodies are isolated and used for amplification. Throughout the repeated cycles of selection and amplification a specific enrichment of possible MS-related phage clones occurred which was reflected by a rise in the output/input ratio. In doing so, even a low titer immune response would be expected to recover the specific antigen. Furthermore, by alternating positive and negative selection rounds, a significant decrease of IgG-displaying phage clones was observed after each negative selection round, thereby preventing an overgrowth of IgG-displaying phage clones.

Efficiency of selection was monitored by testing immunoreactivity of selected phage clones in individual sera of the MS serum pool. Reactivity is especially found in phage clones derived from the first 4 selection rounds. Phage clones derived from later selection rounds showed either no reactivity or aspecific polyreactivity against individual sera of the pool. Possibly when selection cycles are repeated, antigens that display a high affinity for antibodies are selected. These high-affinity antibodies could be very relevant regarding pathogenesis and diagnosis of MS, but probably their concentration in serum is too low to detect with phage-ELISA. This can explain the lack of reactivity for some of the selected phage clones in later rounds of selection. Another option is that throughout the elaborate selection cycles there is a preferable selection of the best growing phage clones instead of the most relevant ones. Hence, aspecific

phage clones, for example plastic-binders, can be enriched, explaining the polyreactivity of some clones. This phenomenon of plastic-binders has also been described by others.[32] Also, a stabilization in the output/input ratio was obtained at round 4. Further rounds of selection did not result in a additional specific enrichment indicating that the limit of enrichment has been reached. Together, these data show that in following experiments possible MS-related antigens have to be determined in phage output from the first 4 selection rounds, before the presence of aspecific phage clones could hamper the screening process.

After determining immunoreactivity in the individual sera of the MS pool, MS-specificity will be determined by evaluating immunoreactivity of selected phage clones in the serum of several control patients that will be divided into 2 groups consisting of non-inflammatory neurological diseases and other inflammatory neurological diseases. The final aim is to compose a panel of markers in serum of MS patients.

In conclusion, as phage display is amenable to high-throughput analysis, SAS is a very powerful technology to generate broad disease stage serological antigen profiles in autoimmune diseases like MS and rheumatoid arthritis. These antigen profiles can serve as surrogate markers and aid in the diagnosis and prognosis of several autoimmune diseases.

Acknowledgments

C.G. is supported by a grant from the Bijzonder Onderzoeksfonds Limburg (BOF) of Hasselt University and by the transnational University Limburg (tUL). K.S. is supported by the “Fonds voor Wetenschappelijk onderzoek” (FWO Vlaanderen). We thank Igna Rutten for technical assistance, Dr. Soares for providing the MS cDNA library and Dr. Medaer for collection of the MS serum samples.

References

1. Noseworthy, J.H., C. Lucchinetti, M. Rodriguez et al. 2000. Multiple sclerosis N. Engl. J. Med. **343:** 938-952.

2. Duddy, M. & A. Bar-Or. 2006. B-cells in multiple sclerosis Int. MS J. **13:** 84-90.

3. Freedman, M.S., E.J. Thompson, F. Deisenhammer et al. 2005. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement Arch. Neurol. **62:** 865-870.

4. Olsson, J.E. & H. Link. 1973. Immunoglobulin abnormalities in multiple sclerosis. Relation to clinical parameters: exacerbations and remissions Arch. Neurol. **28:** 392-399.

5. Hershey, L.A. & J.L. Trotter. 1980. The use and abuse of the cerebrospinal fluid IgG profile in the adult: a practical evaluation Ann. Neurol. **8:** 426-434.

6. Ebers, G.C. 1984. Oligoclonal banding in MS Ann. N. Y. Acad. Sci. **436:** 206-212.

7. Dyken, P.R. 2001. Neuroprogressive disease of post-infectious origin: a review of a resurging subacute sclerosing panencephalitis (SSPE) Ment. Retard. Dev. Disabil. Res. Rev. **7:** 217-225.

8. Correale, J. & de los Milagros Bassani Molinas. 2002. Oligoclonal bands and antibody responses in multiple sclerosis J. Neurol. **249:** 375-389.

9. Reiber, H., S. Ungefehr & C. Jacobi. 1998. The intrathecal, polyspecific and oligoclonal immune response in multiple sclerosis Mult. Scler. **4:** 111-117.

10. Owens, G.P., H. Kraus, M.P. Burgoon et al. 1998. Restricted use of VH4 germline segments in an acute multiple sclerosis brain Ann. Neurol. **43:** 236-243.

11. Baranzini, S.E., M.C. Jeong, C. Butunoi et al. 1999. B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions J. Immunol. **163:** 5133-5144.

12. Qin, Y., P. Duquette, Y. Zhang et al. 2003. Intrathecal B-cell clonal expansion, an early sign of humoral immunity, in the cerebrospinal fluid of patients with clinically isolated syndrome suggestive of multiple sclerosis Lab Invest **83:** 1081-1088.

13. Corcione, A., F. Aloisi, B. Serafini et al. 2005. B-cell differentiation in the CNS of patients with multiple sclerosis Autoimmun. Rev. **4:** 549-554.

14. Cepok, S., B. Rosche, V. Grummel et al. 2005. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis Brain.

15. Lucchinetti, C., W. Bruck, J. Parisi et al. 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination Ann. Neurol. **47:** 707-717.

16. Lefranc, D., L. Almeras, S. Dubucquoi et al. 2004. Distortion of the self-reactive IgG antibody repertoire in multiple sclerosis as a new diagnostic tool J. Immunol. **172:** 669-678.

17. Archelos, J.J., J.T rotter, S. Previtali et al. 1998. Isolation and characterization of an oligodendrocyte precursor-derived B-cell epitope in multiple sclerosis Ann. Neurol. **43:** 15-24.

18. Lennon, V.A., T.J. Kryzer, S.J. Pittock et al. 2005. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel J. Exp. Med. **202:** 473-477.

19. Zhou, D., R. Srivastava, S. Nessler et al. 2006. Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis Proc. Natl. Acad. Sci. U. S A **103:** 19057-19062.

20. Smith, G.P. 1985. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface Science **228:** 1315-1317.

21. Cortese, I., S. Capone, S. Luchetti et al. 1998. CSF-enriched antibodies do not share specificities among MS patients Mult. Scler. **4:** 118-123.

22. Cortese, I., S. Capone, R. Tafi et al. 1998. Identification of peptides binding to IgG in the CSF of multiple sclerosis patients Mult. Scler. **4:** 31-36.

23. Dybwad, A., O. Flrre & M. Sioud. 1997. Probing for cerebrospinal fluid antibody specificities by a panel of random peptide libraries Autoimmunity **25:** 85-89.

24. Rand, K.H. & H. Houck. 2000. Improved methods for the application of random peptide phage libraries to the study of the oligoclonal bands in cerebrospinal fluid of patients with multiple sclerosis J. Neurosci. Methods **101:** 131-139.

25. Soares, M.B., M.F. Bonaldo, P. Jelene et al. 1994. Construction and characterization of a normalized cDNA library Proc. Natl. Acad. Sci. U. S. A **91:** 9228-9232.

26. Hufton, S.E., P.T. Moerkerk, E.V. Meulemans et al. 1999. Phage display of cDNA repertoires: the pVI display system and its applications for the selection of immunogenic ligands J. Immunol. Methods **231:** 39-51.

27. Somers, V., C. Govarts, N. Hellings et al. 2005. Profiling the autoantibody repertoire by serological antigen selection J. Autoimmun. **25:** 223-228.

28. Marks, J.D., H.R. Hoogenboom, T.P. Bonnert et al. 1991. By-passing immunization. Human antibodies from V-gene libraries displayed on phage J. Mol. Biol. **222:** 581-597.

29. Jespers, L.S., J.H. Messens, A. De Keyser et al. 1995. Surface expression and ligand-based selection of cDNAs fused to filamentous phage gene VI Biotechnology (N. Y.) **13:** 378-382.

30. Somers, V.A., R.J. Brandwijk, B. Joosten et al. 2002. A panel of candidate tumor antigens in colorectal cancer revealed by the serological selection of a phage displayed cDNA expression library J. Immunol. **169:** 2772-2780.

31. Jacobsson, K., A. Rosander, J. Bjerketorp et al. 2003. Shotgun Phage Display - Selection for Bacterial Receptins or other Exported Proteins Biol. Proced. Online. **5:** 123-135.

32. Adey, N.B., A.H. Mataragnon, J.E. Rider et al. 1995. Characterization of phage that bind plastic from phage-displayed random peptide libraries Gene **156:** 27-31.