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Multiplexing approaches for autoantibody profiling in multiple sclerosis

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ABSTRACT

The preliminary positive effects of B cell depletion therapy in multiple sclerosis (MS) have renewed interest in a potential role of B cells and autoantibodies in the MS disease process. Regardless of a possible pathogenic role of the humoral immune response in MS, the analysis of autoantibodies as disease markers is valuable. Despite intense research, there is no known MS-associated antibody specificity that can individually discriminate between MS patients and controls. Due to the overlap in autoantibody profiles in autoimmune diseases, and due to the complexity of MS, multiplex autoantibody profiling approaches are needed to generate a panel of MS-associated autoantibodies with high combined sensitivity and specificity for MS. In recent years, several multiplexing approaches have been applied in MS autoantibody profiling with promising results regarding the generation of a so-called MS-specific autoantibody fingerprint. We also recently applied a high-throughput autoantibody profiling technique for MS cerebrospinal fluid resulting in the identification of a novel panel of 8 antigenic targets with 45% sensitivity and 86% specificity for the disease.

Identification of MS-specific autoantibody specificities is important for the development of diagnostic and prognostic markers for MS. Moreover, it can provide more knowledge regarding underlying MS disease processes and novel therapeutic targets.

Contents

1. The role of B cells and autoantibodies in multiple sclerosis

In multiple sclerosis (MS) research, the role of B cells and autoantibodies in the pathogenic disease process has long been a point of debate. The evidence for a primary role of an

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abnormal humoral immune response in the MS pathogenesis has up till now been mainly circumstantial (reviewed in [1,2]). The most consistent laboratory abnormality in MS patients is the presence of an intrathecal, antigen-driven immunoglobulin (Ig) production which is demonstrated by oligoclonal immunoglobulin bands in the cerebrospinal fluid (CSF) of more than 90% of MS patients [3]. In addition, B cells, plasma cells and myelin-binding antibodies have been reported in MS lesions [1,2] and ectopic lymphoid follicles containing proliferating B cells and plasmacells have recently been demonstrated in the meninges of a subset of MS patients [4]. Moreover, the presence of a distorted humoral immune response in MS, represented by a higher number of CSF oligoclonal bands and increased CSF Ig levels, has shown to be associated with a worse disease outcome [5]. These findings however, do not fully support a causative role for the humoral autoimmune response in the disease. Recently, more conclusive evidence for a role of the humoral immune response in MS is provided by the efficiency of a novel treatment strategy; the preliminary positive results of B cell targeting therapy in a group of MS patients point towards a causal and pathogenic role of the humoral immune response in MS and has led to a renewed interest in B cells and autoantibodies [6]. Even if B cells and autoantibodies do not have a contributing role in the immunopathogenic processes of the disease itself and even if the described abnormalities in the humoral immune system are epiphenomena of the disease, these distortions can contain information regarding the underlying disease processes and can be used for the development of markers for the disease.

2. Autoantibody markers in MS

In the past, different experimental approaches have been applied for the analysis of autoantibody specificities in the serum and CSF of MS patients (reviewed in [7,8]). Table 1 contains a summary of all previously reported antigenic targets of autoantibodies in MS. As it is thought that in MS, the myelin in the central nervous system (CNS) is the primary target of the aberrant autoimmune response, different myelin components were firstly proposed as autoantigen suspects (Table 1). Numerous reports are available on antibodies directed against myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP) and myelin-associated glycoprotein (MAG) in MS patients [7]. A wide range of anti-myelin antibody sensitivities and specificities for MS have been reported but up till now, none of the myelin-associated candidate antigens definitely discriminates between the humoral immune response in MS patients and healthy controls [7]. The true relevance of anti-myelin antibodies in the MS disease process and in the development of MS disease markers remains inconclusive.

Moreover, other CNS-specific components such as neuronal, axonal and glial compounds have been analyzed as autoantibody targets in MS (Table 1) [8]. There are however, also reports about the presence of antibodies against more ubiquitous *self*-antigens such as heat shock proteins and DNA (Table 1) [7]. Besides antibodies directed against *self* components, antibodies against different viruses and bacteria such as Epstein–Barr virus (EBV) and human herpes virus type 6 have also been detected in higher frequencies in MS patients [7].

Up till now however, the reported sensitivities and specificities of the identified individual autoantibodies for MS are at best only moderate. From a biomarker point of view, this means that none of the reported antigen–antibody systems can be efficiently used as an individual marker for the disease. To overcome the reported relatively low sensitivities of the individual antibody-specificities in MS, different autoantibody markers need to be combined into a biomarker panel, indicating the requirement for the application of multiplex autoantibody profiling techniques.

3. Multiplexing approaches for autoantibody profiling

In recent years, the need for multiplex autoantibody profiling approaches has become evident within the research domain of autoimmunity [9,10], since autoimmune diseases are characterized by broad autoantibody profiles with considerable overlap between the different diseases [11]. For multiple sclerosis, the necessity for a panel of several markers

Table 1
Autoantibody targets in multiple sclerosis.

Myelin antigens (proteins, glycoproteins and lipids)	*Oligodendrocyte antigens*
Myelin oligodendrocyte glycoprotein (MOG)	Surface antigen on oligodendrocyte precursor derived cell line
Myelin basic protein (MBP)	Oligodendrocyte progenitor cell-specific surface glycoprotein (AN2)
Myelin associated glycoprotein (MAG)	Oligodendrocyte-specific protein (OSP)
Proteolipid protein (PLP)	Transaldolase (TAL)
CSF114(Glc)	2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNPase)
Glc(alpha1,4)Glc(alpha)	
Myelin lipids (phosphatidylcholine)	*Heat shock proteins*
Galactocerebroside (GalC)	Alpha B crystalline
Cerebellar soluble lectin (CSL)	Hsp60
	Hsp70
Axonal and neuronal antigens	Hsp90β
Neurofilaments (light and medium chain)	
Gangliosides (GD1a, GM3, sulfatide)	*Ubiquitous and other antigens*
Axolemma-enriched fraction (AEF)	Proteasome
Tubulin	DNA
Nogo-A	Heterogeneous nuclear ribonucleoprotein B1 (hnRNP B1)
Nogo receptor (NGR)	Retinal arrestin
Surface antigen on neuronal cell line	
Neurofascin 186	

Based on [7,8,34–40].

is also explained by the enormous heterogeneity which is characteristic for the disease and is reflected in the diversity of MS disease courses, treatment efficiencies and prevailing pathogenic processes. Different multiplexing approaches have already been used for the identification of a MS-specific autoantibody fingerprint in MS serum and MS CSF (Fig. 1).

3.1. Antigen arrays

In 2006, Kanter et al. were the first to apply antigen microarrays for autoantibody profiling in MS CSF [12]. Microarrays were composed of 50 distinct brain, myelin and microbial lipids and glycolipids that represented potential targets of the MS autoimmune response including ganglioside, sulfatide, cerebroside, and sphingomyelin. Lipid-specific antibody responses in CSF were compared between 16 MS patients and 11 patients with other neurological diseases. Increased lipid-specific antibody reactivities against myelin lipids including sulfatide, sphingomyelin, oxidized lipids and microbial lipids were shown in individuals with MS [12].

Quintana et al. recently reported the use of an array composed of 362 myelin and inflammation-related antigens (proteins and lipids) that encompassed CNS antigens associated with MS, CNS antigens associated with other neurological diseases and heat shock proteins [13]. Unique serum autoantibody patterns that distinguished relapsing–remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS) MS from each other and from healthy controls and other neurological and autoimmune diseases were detected. Moreover, patients with different immunopathologic patterns of MS (based on brain biopsy analysis) could be discerned based on unique autoantibody profiles [13]. Despite the obtained interesting results, the main drawback of antigen array screening is the fact that it is a so-called biased autoantibody profiling approach. This means that in antigen microarrays, only a small number of known and characterized antigens are spotted as candidate antigenic targets which is not fully representative for the heterogeneity present within the *in vivo* target tissue in MS, namely the inflamed central nervous system. Moreover, by applying this approach, the identification of novel antigenic targets is not possible (Fig. 1).

An alternative, less biased protein array system for autoantibody profiling in MS was applied by the research group of Hemmer [14]. Protein arrays were prepared by the spotting of 37,000 different expression clones, obtained from a human fetal brain cDNA library. These arrays were probed with individual CSF samples from MS and control patients (patients with other inflammatory and non-inflammatory neurological diseases) to identify the expression clones with strong reactivity in MS patients but not in controls. The 2 most frequent MS-specific reactivities corresponded to EBV peptide sequences. Immunoreactivities towards the identified EBV proteins were significantly higher in the serum and CSF of MS patients compared to controls [14].

3.2. Immunoblotting

Another high-throughput multiplexing approach for autoantibody profiling is the one performed by Lefranc et al. [15]. This research group applied 1-D immunoblotting to compare serum IgG antibody repertoires against human brain homogenates between 82 MS patients and 46 control subjects. Despite the high degree of heterogeneity with regard to number and nature of recognised protein bands, serum IgG antibody repertoires were identified which could distinguish MS patients from controls. Furthermore, specific IgG reactivity profiles that could discriminate between the three forms of MS (RR, SP and PP) were detected [15].

An analogous 1-D immunoblotting procedure of human brain lysates with purified MS CSF IgG was applied by Kolln et al. [16]. Triosephosphate isomerase (TPI) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were identified as MS CSF antibody targets. ELISA screening of CSF samples from clinically isolated syndrome patients, MS patients and patients with other neurological diseases demonstrated that GAPDH-reactive antibodies and the coexistence of TPI- and GAPDH-reactive antibodies were primarily found in MS CSF. Both antigens were proposed as candidate targets for the MS-associated autoimmune response to neurons and axons [16].

The application of 1-D immunoblotting of brain homogenates for autoantibody profiling allows the identification of novel antigenic targets. In addition, the *in vivo* antigenic composition of the diseased tissue is conserved to a higher extent in this immunoblotting procedure. However, the inherent bias in antigen representation due to use of denaturing polyacrylamide gel electrophoresis (SDS-PAGE) have to be taken into account (Fig. 1).

3.3. Lambda phage expression library screening

Archelos et al. applied an unbiased high-throughput procedure in which a lambda phage cDNA expression library constructed from an oligodendrocyte precursor cell line was screened with pooled CSF from 54 MS patients [17]. By comparison with pooled CSF from patients with other neurological diseases, 6 clones with MS-associated immunoreactivity were identified. For 5 of these clones, a common sequence of 7 amino acids was detected, which was homologous to a translated consensus Alu repeat epitope. By screening sera and CSF from MS patients, it was shown that 44% of the patients reacted with Alu-peptides. Immunostainings with these antibody-positive samples showed strong staining of cytoplasm of oligodendrocyte precursors which indicated the existence of an unknown oligodendrocyte precursor-derived epitope targeted by B cells in a subgroup of MS patients [17].

Owens et al. reported the screening of 2 lambda phage cDNA expression libraries constructed from chronic MS plaques with both crude pooled MS CSF and IgG extracted from pooled MS CSF and MS serum [18]. In this study, no MS-specific target antigens could be discerned. Moreover, screening of a subtracted cDNA library (subtracted with normal human brain), enriched for rare transcripts and sequences over-expressed in MS tissue, with MS CSF did not result in identification of MS-specific autoantibody targets [18].

4. Filamentous phage display for autoantibody profiling

In the 1980s a high-throughput molecular technique, phage display, was developed to identify protein or peptide ligands for a wide range of target molecules [19]. The phage display technique is based on the potential of a filamentous phage virus particle to incorporate foreign DNA into its genome coupled to a

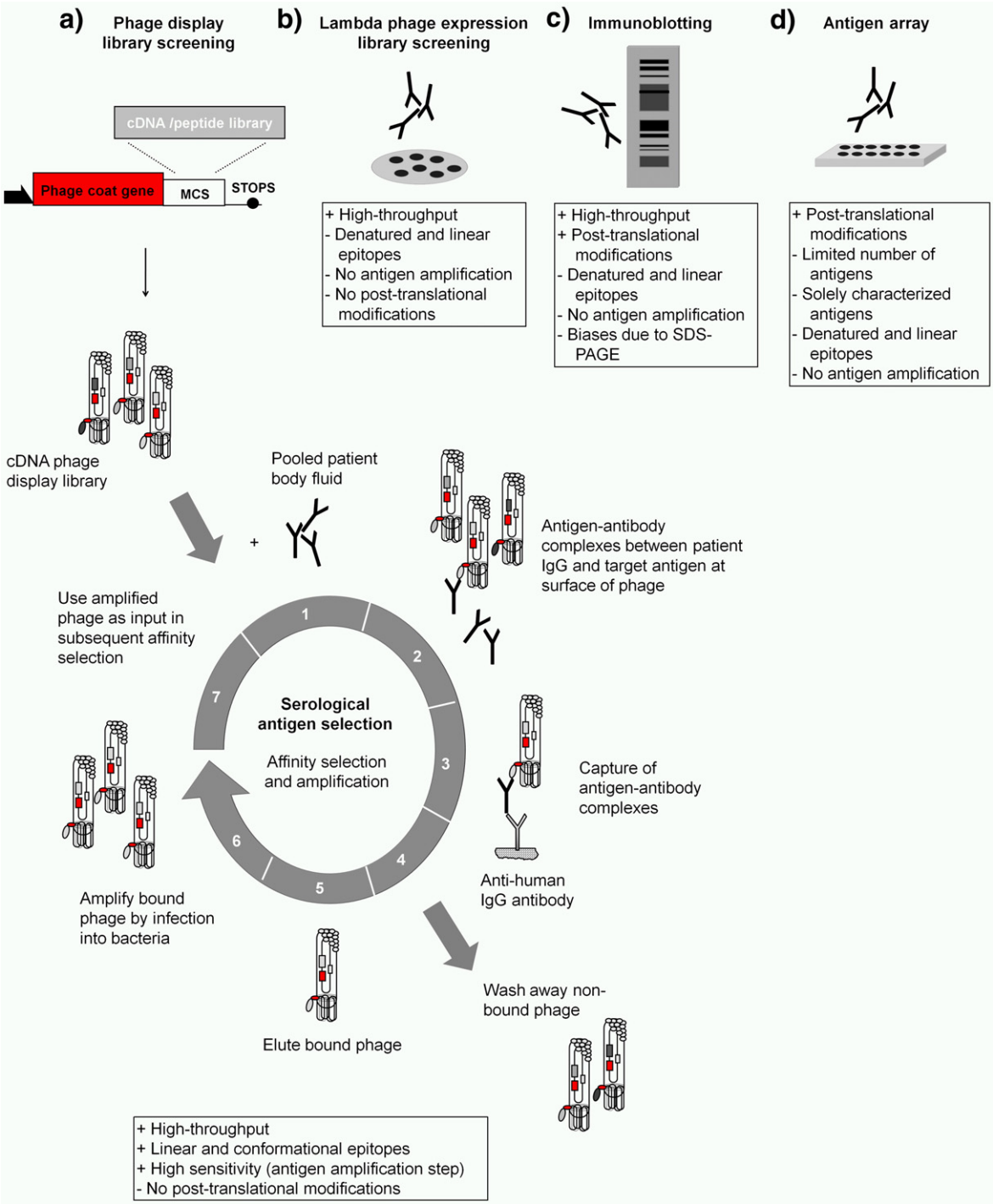

Fig. 1. Multiplexing approaches for autoantibody profiling: a) phage display library screening, b) lambda phage expression library screening, c) immunoblotting, d) antigen arrays. Advantages and disadvantages of the different multiplex autoantibody profiling techniques are summarized. The serological antigen selection procedure entails subsequent rounds of affinity selection of a cDNA phage display library with patient immunoglobulins. An affinity selection round is initiated by incubating phage displaying the cDNA library with pooled patient body fluids (1). During this incubation step, antigen–antibody complexes are formed between the antibodies present in the patient body fluids and their respective target antigens at the surface of the phage (2). These complexes are captured on a solid support by anti-human IgG capture antibody (3) while non-bound phage are washed away (4). Bound phage are eluted (5), amplified through infection of host bacteria (6) and used as input in a subsequent affinity selection procedure (7). The succession of affinity selection and amplification of selected phage results in enrichment of phage-displayed antigens targeted by patient IgG.

gene encoding a phage coat protein. This results in the display of the corresponding DNA product on the phage surface, coupled to the phage coat protein. The strength of the technology lies within the physical link between genotype (DNA) and phenotype (DNA product attached to the viral protein coat), which enables the succession of affinity selection and amplification of selected phage particles resulting in powerful enrichment of selected phage (Fig. 1) [19]. In comparison, by the absence of a physical link between genotype and phenotype in the above-mentioned studies based on lambda phage expression library screening [17,18], an enrichment of selected phage is not achievable (Fig. 1). Phage display is not only applicable for the identification of a ligand for one particular target molecule, it can also be used to identify the epitopes that are recognized by polyclonal mixtures of antibodies in healthy or diseased body fluids. A commonly used phage display procedure encompasses the display of synthetically produced peptide libraries [20]. This peptide phage display technique has been used previously for the elucidation of MS autoantibody profiles.

The technique was applied by Cortese et al. to identify peptides and peptide motifs that were recognized by antibodies in the CSF from MS patients [21,22]. The authors showed that selection with individual MS CSF led to the identification of phage-displayed epitopes that rarely reacted with CSF from other MS patients, suggesting that the repertoire of CSF antibodies in MS patients is patient-specific. Furthermore, antibodies against the selected epitopes were demonstrated with a similar frequency in MS sera as compared to control sera, indicating that the target epitopes mimicked ubiquitous antigens to which many individuals are exposed [21]. In a new attempt to identify MS-specific epitopes, 20 different MS CSF samples were pooled for selection of a random peptide library. However, by performing this experiment, the earlier finding of patient-specific antibody reactivities was confirmed. The authors concluded that common antibody specificities are absent in MS patients, or that they are present in very low concentrations [22]. These findings are however contradictory to the results obtained by other research groups that applied peptide phage display for autoantibody profiling in MS.

Dybwad et al. analyzed the CSF antibody specificities within one oligoclonal band from a MS patient using 6-mer, 9-mer and 15-mer random peptide libraries displayed at the surface of filamentous phage [23]. Different distinct but structurally related peptide motifs were detected, which showed significant linear homology with collagen proteins, the 68 kDa neurofilament protein, versican and other proteins from viruses such as human cytomegalovirus, herpes simplex virus and human papillomavirus [23].

Jolivet-Reynaud et al. reported the use of a decapeptide phage display library to analyze the antibody specificities in the CSF and serum from 4 MS patients [24]. The peptides that were identified by selections with MS serum were not disease-specific. Selections performed with CSF on the other hand, led to the identification of several motifs which were not detected in serum-selected peptides and were recognized more specifically in MS CSF compared to CSF from patients with other neurological diseases. The combination of the selected MS CSF epitopes allowed the detection of antibodies in 21 out of 60 tested MS CSF, in comparison to only 2 out of 27 control CSF. Amino acid similarities were detected between the selected peptides and envelope regions of multiple sclerosis-associated retrovirus and the related endogenous retrovirus (HERV-W) [24].

Rand et al. have used individual CSF from 14 MS patients for the screening of a hexamer peptide phage display library [25]. CSF from 5 out of 14 MS patients selected a common motif which was found in EBV protein EBNA-1 (Epstein–Barr nuclear antigen 1), alpha B crystalline and in the 65 kDa non-structural cytomegalovirus antigen. It was shown that 60–65% of EBV-seropositive MS patients presented with antibodies against the selected motif, and EBNA-1 associated oligoclonal bands were detected in patients with MS [25].

The main disadvantage of the peptide phage display system is the identification of so-called mimotopes. Mimotopes are peptides forming epitopes that structurally mimic the actual *in vivo* antigens. This implies that a mimotope and the corresponding actual antigen are not necessarily identical or similar at amino acid level, although sequence similarity cannot be excluded. A labour-intensive characterization of the identified epitope is required for the identification of the actual *in vivo* target antigen of the detected autoantibody [26]. This drawback of peptide phage display can be overcome by the application of cDNA phage display [27].

5. cDNA phage display for autoantibody profiling in MS

cDNA phage display entails the display of an entire cDNA expression library allowing interaction between cDNA encoded peptides and proteins exposed on the surface of phage particles and antibody selectors. If a cDNA expression library derived from diseased tissue is used for phage library construction, the entire heterogeneity of known and novel expressed antigens present within the affected tissue can be displayed and potentially selected. The antigenic complexity held within the diseased tissue is far greater than that from normal tissue, due to the diversity of processes occurring at this site of autoimmune attack such as inflammatory cell infiltration, activation and migration as well as destruction and possible repair of endogenous brain tissue cells. Moreover, compared to antigen arrays, as yet unknown or uncharacterized expressed sequences are included in the antigenic diversity.

To analyze autoantibody specificities in the CSF of MS patients, we have recently applied a procedure called serological antigen selection (SAS), which is based on cDNA phage display. SAS is a powerful molecular approach which has already been successfully applied for autoantibody profiling in rheumatoid arthritis (Somers K et al., submitted), atherosclerosis [28] and colorectal cancer [29]. Application of the procedure for MS CSF entailed filamentous phage display of a normalized expression library made from three active chronic MS plaques with varying degrees of demyelination and inflammatory activity, obtained from one MS patient [30]. Sequencing of the MS cDNA library confirmed the value of the use of a cDNA expression library made from diseased tissue for the identification of autoantibody targets in MS [30]. The percentage of novel genes and the number of expressed sequences were significantly higher in the MS library compared to normal human brain libraries. The detected sequences were associated with the diversity of processes occurring in and around MS lesions such as inflammatory cell infiltration, activation and migration,

antigen presentation and tissue damage [30]. The MS plaque expression library was cloned C-terminally of filamentous phage coat protein pVI, allowing the expression of full-length cDNA fragments with inherent stop codons [31,32]. Through fusions with minor coat protein pVI, display of cDNA products is monovalent (one cDNA product per phage particle), allowing high-affinity interactions with antibody selectors. The selection of high-affinity interactions is also mediated by the fact that the binding of antibodies to the phage-displayed cDNA products is performed in a solution phase [32].

By application of affinity selection of a MS cDNA phage display library with pooled CSF from 10 RRMS patients (Fig. 1) we recently identified a panel of 4 MS-specific and 4 MS-associated antigenic targets [33]. Immunoreactivity against this panel of 8 novel MS-candidate antigens was analyzed in 73 MS patients, 64 patients with non-inflammatory neurological diseases and 30 patients with other inflammatory neurological diseases. The sensitivity and specificity values of the detection of antibodies against this panel of 8 autoantibody targets for MS are 45% and 86%, respectively. Moreover, antibody reactivity against these 8 targets could be demonstrated in some, but not all of the sera of patients which were antibody-positive based on CSF analysis [33]. Future analysis of the obtained sensitivities and specificities of a combination of these antigen–antibody systems with earlier described autoantibody specificities (such as anti-MBP or anti-MOG antibodies) is warranted to fully elucidate the diagnostic potential of the identified antigenic candidates. Moreover, the identification of these novel targets of the humoral autoimmune response in MS will inevitably result in more valuable knowledge regarding the underlying etiology and disease process of MS.

6. Conclusions

Previously, the search for autoantibody targets in MS has focused on preselected, individual target antigen candidates such as different myelin components. Such a restricted view has not led to the discovery of antigenic targets for which immunoreactivity fully discriminates MS patients from controls. Analysis of the entire MS autoantibody profile however, facilitated by multiplexing approaches, has the potential of generating a panel of different antigenic targets with high combined sensitivity and specificity for the disease. In recent years, different multiplexing approaches have been applied for the analysis of the MS autoantibody profile. Several studies reported the identification of distinct serum autoantibody profiles that were able to distinguish MS patients from controls, indicating the value of multiplexing applications. Not all studies were successful at identifying MS-associated antibodies. An explanation for the difference in procedure efficiency can be found in the different applied methodologies and the well-characterized heterogeneity of MS (and thus also the possible heterogeneity of the autoantibody profiles). The combination of different multiplex autoantibody profiling procedures, each with their inherent biases and advantages, is essential to fully capture the autoantibody complexity in MS body fluids.

The identification of a MS-specific autoantibody profile is valuable from a diagnostic biomarker point of view. In addition, the discovery of novel targets of the humoral autoimmune response in MS patients will generate valuable knowledge regarding underlying disease processes and etiology of MS, and can lead to the identification of needed, novel therapeutic targets.

Take-home messages

- Due to the preliminary positive effect of B cell depletion therapy in MS patients, there is renewed interest in the role of autoreactive B cells and autoantibodies in the disease process of multiple sclerosis.
- Despite intense research into the autoantibody reactivities in MS, there is no known antibody specificity which can definitely distinguish MS patients from controls.
- Due to a great overlap in autoantibody reactivities in different autoimmune diseases, multiplexing is essential to generate a broad panel of different autoantibodies with high combined sensitivity and specificity for multiple sclerosis.
- Autoantibody profiling by affinity selecting a MS cDNA phage display library with pooled MS CSF is an unbiased, high-throughput procedure selecting for high-affinity antigen–antibody interactions.
- The identification of autoantibody specificities which are specific for MS will lead to advances in the development of diagnostic and prognostic markers, and can provide important clues regarding underlying disease processes and possible novel therapeutic targets.

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References

[1] Cross AH, Trotter JL, Lyons J. B cells and antibodies in CNS demyelinating disease. J Neuroimmunol 2001;112(1–2):1–14.
[2] Ziemssen T, Ziemssen F. The role of the humoral immune system in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). Autoimmun Rev 2005;4(7):460–7.
[3] Walsh MJ, Tourtellotte WW. Temporal invariance and clonal uniformity of brain and cerebrospinal IgG, IgA, and IgM in multiple sclerosis. J Exp Med 1986;163(1):41–53.
[4] Corcione A, Aloisi F, Serafini B, Capello E, Mancardi GL, Pistoia V, et al. B-cell differentiation in the CNS of patients with multiple sclerosis. Autoimmun Rev 2005;4(8):549–54.
[5] Izquierdo G, Angulo S, Garcia-Moreno JM, Gamero MA, Navarro G, Gata JM, et al. Intrathecal IgG synthesis: marker of progression in multiple sclerosis patients. Acta Neurol Scand 2002;105(3):158–63.
[6] Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, et al. B-cell depletion with rituximab in relapsing–remitting multiple sclerosis. N Engl J Med 2008;358(7):676–88.
[7] Reindl M, Khalil M, Berger T. Antibodies as biological markers for pathophysiological processes in MS. J Neuroimmunol 2006;180(1–2):50–62.
[8] Huizinga R, Linington C, Amor S. Resistance is futile: antineuronal autoimmunity in multiple sclerosis. Trends Immunol 2008;29(2):54–60.
[9] Tozzoli R. Recent advances in diagnostic technologies and their impact in autoimmune diseases. Autoimmun Rev 2007;6(6):334–40.
[10] Plebani M, Pittoni M, Celadin M, Bernardi D, Mion MM. Recent advances in diagnostic technologies for autoimmune diseases. Autoimmun Rev 2009;8(3):238–43.
[11] Stinton LM, Fritzler MJ. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. Autoimmun Rev 2007;7(1):77–84.
[12] Kanter JL, Narayana S, Ho PP, Catz I, Warren KG, Sobel RA, et al. Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat Med 2006;12(1):138–43.

[13] Quintana FJ, Farez MF, Viglietta V, Iglesias AH, Merbl Y, Izquierdo G, et al. Antigen microarrays identify unique serum autoantibody signatures in clinical and pathologic subtypes of multiple sclerosis. Proc Natl Acad Sci U S A 2008;105(48):18889–94.
[14] Cepok S, Zhou D, Srivastava R, Nessler S, Stei S, Bussow K, et al. Identification of Epstein–Barr virus proteins as putative targets of the immune response in multiple sclerosis. J Clin Invest 2005;115(5):1352–60.
[15] Lefranc D, Almeras L, Dubucquoi S, de Seze J, Vermersch P, Prin L. Distortion of the self-reactive IgG antibody repertoire in multiple sclerosis as a new diagnostic tool. J Immunol 2004;172(1):669–78.
[16] Kolln J, Ren HM, Da RR, Zhang Y, Spillner E, Olek M, et al. Triosephosphate isomerase- and glyceraldehyde-3-phosphate dehydrogenase-reactive autoantibodies in the cerebrospinal fluid of patients with multiple sclerosis. J Immunol 2006;177(8):5652–8.
[17] Archelos JJ, Trotter J, Previtali S, Weissbrich B, Toyka KV, Hartung HP. Isolation and characterization of an oligodendrocyte precursor-derived B-cell epitope in multiple sclerosis. Ann Neurol 1998;43(1):15–24.
[18] Owens GP, Burgoon MP, Devlin ME, Gilden DH. Strategies to identify sequences or antigens unique to multiple sclerosis. Mult Scler 1996;2(4): 184–94.
[19] Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 1985;228(4705): 1315–7.
[20] Scott JK, Smith GP. Searching for peptide ligands with an epitope library. Science 1990;249(4967):386–90.
[21] Cortese I, Tafi R, Grimaldi LM, Martino G, Nicosia A, Cortese R. Identification of peptides specific for cerebrospinal fluid antibodies in multiple sclerosis by using phage libraries. Proc Natl Acad Sci U S A 1996;93(20):11063–7.
[22] Cortese I, Capone S, Luchetti S, Grimaldi LM, Nicosia A, Cortese R. CSF-enriched antibodies do not share specificities among MS patients. Mult Scler 1998;4(3):118–23.
[23] Dybwad A, Flrre O, Sioud M. Probing for cerebrospinal fluid antibody specificities by a panel of random peptide libraries. Autoimmunity 1997;25(2):85–9.
[24] Jolivet-Reynaud C, Perron H, Ferrante P, Becquart L, Dalbon P, Mandrand B. Specificities of multiple sclerosis cerebrospinal fluid and serum antibodies against mimotopes. Clin Immunol 1999;93(3):283–93.
[25] Rand KH, Houck H, Denslow ND, Heilman KM. Epstein–Barr virus nuclear antigen-1 (EBNA-1) associated oligoclonal bands in patients with multiple sclerosis. J Neurol Sci 2000;173(1):32–9.
[26] Cortese I, Capone S, Luchetti S, Cortese R, Nicosia A. Cross-reactive phage-displayed mimotopes lead to the discovery of mimicry between HSV-1 and a brain-specific protein. J Neuroimmunol 2001;113(1): 119–28.
[27] Rhyner C, Kodzius R, Crameri R. Direct selection of cDNAs from filamentous phage surface display libraries: potential and limitations. Curr Pharm Biotechnol 2002;3(1):13–21.
[28] Cleutjens KB, Faber BC, Rousch M, van Doorn R, Hackeng TM, Vink C, et al. Noninvasive diagnosis of ruptured peripheral atherosclerotic lesions and myocardial infarction by antibody profiling. J Clin Invest 2008;118(8): 2979–85.
[29] Somers VA, Brandwijk RJ, Joosten B, Moerkerk PT, Arends JW, Menheere P, et al. A panel of candidate tumor antigens in colorectal cancer revealed by the serological selection of a phage displayed cDNA expression library. J Immunol 2002;169(5):2772–80.
[30] Becker KG, Mattson DH, Powers JM, Gado AM, Biddison WE. Analysis of a sequenced cDNA library from multiple sclerosis lesions. J Neuroimmunol 1997;77(1):27–38.
[31] Jespers LS, Messens JH, De Keyser A, Eeckhout D, Van dB I, Gansemans YG, et al. Surface expression and ligand-based selection of cDNAs fused to filamentous phage gene VI. Biotechnology (N Y) 1995;13(4): 378–82.
[32] Hufton SE, Moerkerk PT, Meulemans EV, de Bruine A, Arends JW, Hoogenboom HR. Phage display of cDNA repertoires: the pVI display system and its applications for the selection of immunogenic ligands. J Immunol Methods 1999;231(1–2):39–51.
[33] Somers V, Govarts C, Somers K, Hupperts R, Medaer R, Stinissen P. Autoantibody profiling in multiple sclerosis reveals novel antigenic candidates. J Immunol 2008;180(6):3957–63.
[34] Zanetta JP, Tranchant C, Kuchler-Bopp S, Lehmann S, Warter JM. Presence of anti-CSL antibodies in the cerebrospinal fluid of patients: a sensitive and specific test in the diagnosis of multiple sclerosis. J Neuroimmunol 1994;52(2):175–82.
[35] Svarcova J, Fialova L, Bartos A, Steinbachova M, Malbohan I. Cerebrospinal fluid antibodies to tubulin are elevated in the patients with multiple sclerosis. Eur J Neurol 2008;15(11):1173–9.
[36] Onoue H, Satoh JI, Ogawa M, Tabunoki H, Yamamura T. Detection of anti-Nogo receptor autoantibody in the serum of multiple sclerosis and controls. Acta Neurol Scand 2007;115(3):153–60.
[37] Lily O, Palace J, Vincent A. Serum autoantibodies to cell surface determinants in multiple sclerosis: a flow cytometric study. Brain 2004;127(Pt 2):269–79.
[38] Chiba S, Yokota S, Yonekura K, Tanaka S, Furuyama H, Kubota H, et al. Autoantibodies against HSP70 family proteins were detected in the cerebrospinal fluid from patients with multiple sclerosis. J Neurol Sci 2006;241(1–2):39–43.
[39] Sueoka E, Yukitake M, Iwanaga K, Sueoka N, Aihara T, Kuroda Y. Autoantibodies against heterogeneous nuclear ribonucleoprotein B1 in CSF of MS patients. Ann Neurol 2004;56(6):778–86.
[40] Gorczyca WA, Ejma M, Witkowska D, Misiuk-Hojlo M, Kuropatwa M, Mulak M, et al. Retinal antigens are recognized by antibodies present in sera of patients with multiple sclerosis. Ophthalmic Res 2004;36(2): 120–3.

Mycophenolate mofetil in juvenile systemic lupus erythematosus

Micophenolate mofetil (MMF) has been largely demonstrated its safety and efficacy in treatment of lupus patients. However, few data in fact exist of the use of this drug in juvenile patients with systemic lupus erythematosus. In a very recent paper, Falcini *et al.* **(Lupus 2009;18:139–43)** have evaluated the role of MMF in 26 children with SLE . Nine out of these 26 patients had a previous nephritis, and 4 developed during MMF treatment . The other children received MMF for other clinical conditions. Twenty-three completed at least 12 months of follow-up. The authors observed that MMF was able to reduce the disease activity or spare glucocorticoid use in 54%, stabilized the lupus disease in 31%, and was ineffective in 15%. Analyzing those patients with renal damage, MMF was effective in 38%, partially effective in 31% and ineffective in 31%. On the other hand, in those patients without kidney lesions, this drug was effective in 69% of the cases. No severe side effect was observed. In summary, this study suggests that MMF use in juvenile SLE seems to have safety and efficacy.

C-reactive protein and anti-CCP in systemic lupus erythematosus

High levels of C-reactive protein have been associated with infections, serositis, and severe arthritis in patients with systemic lupus erythematosus. Amezcua-Guerra *et al.* **(Inflammation research 2008;57:555–7)** have performed a descriptive case-series study with the objective to identify biomarkers for erosive arthritis in lupus patients. The authors found that C-reactive protein levels were higher in erosive in comparison to non-erosive disease. Interestingly, anti-cyclic citrullinated peptide antibodies (anti-CCP) were observed in patients with erosive arthritis and none of them without this joint alteration. Regarding other biomarkers such as interleukin-6, 4 and 10, and interferon gamma, no difference was noted between the two groups. In non-erosive patients, a negative correlation was seen between interleukin-6 and C-reactive protein. In summary, this study demonstrated that high levels of C-reactive protein and positive anti-CCP may identify systemic lupus erythematosus patients with erosive arthritis.