Myelin-associated serological targets as applicable to diagnostic tools to be used at the preclinical and transient stages of multiple sclerosis progression

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ABSTRACT

MS is a severe progressive autoimmune disease with slight short-term relapses in its course. Autoagression against vulnerable myelin-associated Ags results in multiple lesions throughout the CNS. Abnormal responses against nervous issues are mainly affected by cell-mediated and humoral immunity. The first one plays a key role in the restructuring of myelin, while the last one is a biomarker of MS and does not participate in the gradation of the disease. Wide-scale autoimmune attack towards nervous tissues leads to a stepwise demyelination with concomitant release of myelin Ags (epitope spreading), formation of Abs and, consequently, systematization of pro-inflammatory responses. Monitoring of antimyelin-antibodies (Abs: OSP, MOBP, BMP, MOG, PLP) in peripheral blood and cerebrospinal fluid (CSF) is just a brick for making the preclinical diagnosis of MS and timely implementation of predictive measures and preventive treatment. Major autoAbs and their target antigens are discussed in this chapter with a special emphasize on the possibility of their impact for identification of pre-morbid stages and differential diagnosis of MS.

Keywords: Multiple Sclerosis; Antibodies; Autoimmunity; Pre-Clinical Diagnosis; Diagnosis; Prediction; Proteomics

1. INTRODUCTION

Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the CNS resulting in axon loss and development of disability. Autoantibodies (autoAbs) are one of the major features and crucial mechanisms in MS pathogenesis known to illustrate this autoagression. The major feature in the pathogenesis of MS is a primary myelin damage, which is mediated by autoAbs and triggers a process of releasing pathogenically valuable myelin-associated epitopes into the bloodstream to form a set of the principal sensitizing factors to provoke the immune system and then to maintain the progression of the disease.

The worldwide median estimated incidence of MS is 2.5 per 100,000 and prevalence is estimated at approximately 1.5 million cases. Usually onset of MS is between age 20 years and 40 years. Men are affected approximately twice as rare as women.

There are three groups of autoAbs to be specific for MS: anti-myelin autoAbs (*e.g.*, anti-MBP, anti-MOG and anti-neurofilament autoAbs); non-myelin autoAbs (*e.g.*, anti-HSP autoAbs, among others); and autoAbs demonstrating different levels of specificity and functionality (*e.g.*, catalytic autoAbs [*i.e.*, antibody proteases]). Clonal expansion of B cells and T cells as hallmarks of inflammation in the CNS are found in MS. The viral mimicry hypothesis was formulated to explain the initiation of this disorder. But a poor understanding of the etiology of MS has complicated the development of effective therapeutics.

During the last 10 years, it has been found that Abs contributed to the degradation of a number of autoantigens. These and related "antibody-enzymes", also termed abzymes, were shown to be able to cleave DNA, RNA, carbohydrates, peptides, and proteins. Recently, abzyme-dependent catalytic degradation of an autoantigen, MBP, was associated with the clinical course of the neurodegenerative disease MS and its rodent model, experimental autoimmune encephalomyelitis. Autoantibody-mediated degradation of MBP was shown to be site-specific with cleavage sites localized within the immunodominant epitopes of the protein molecule. Ample data indicate that a significant portion of MS cases is characterized

by the presence in the blood of autoantibodies against myelin protein components. Moreover, high-resolution microscopic analysis detected myelin-specific autoantibodies in the areas of demyelination (plaques) in human MS and a MS-like disease of marmosets, suggesting their direct contribution to the myelin destruction. Nonetheless, the mechanisms responsible for the induction of autoantibodies and their possible contributions to MS progression are still unknown and are somewhat contro- versial.

2. CONSTITUENTS OF MYELIN: INDUCERS OF MS AND PROVOKING FACTORS

2.1. Anti-Myelin Basic Protein Antibodies

Diagnosis of multiple sclerosis without using immunodiagnostics methods requires long-term observation and magnetic resonance imaging. Analysis of anti-MOG and anti-MBP antibodies is an inexpensive, rapid and fairly accurate method for the early prediction of multiple sclerosis. This can be extremely useful for assessing the clinical state and proper selection of the treatment strategy to be used in patients at the initial stages of demyelination [1]. At the same time there is no correlation between the status of multiple sclerosis based on either the McDonald or Poser and antibody status of patient blood serum in the sub-clinical stage of disease [2,3]. Studies with using part of MBP as an epitope showed selective reactivity of antibodies to the two MBP fragments 43 - 68 and 146 - 170 distinguished the other neuronal disorders and multiple sclerosis patients. That, due to the author's opinion, let use anti-MBP antibody as an additional marker to monitor the disease progression [4]. Anti-MBP antibodies can be observed both in patient with clinically confirmed diagnosis and some healthy people. In this case, mononuclear cells obtained from the healthy people produce in response to MBP such substances as tumor-necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10), whereas in MS patient, in addiction to increasing expressing proteins, γ-interferon, interleukins 4 and 5 is excreted [5].

So comments on the role of antibodies in the pathogenesis and diagnosis is still quite controversial. And, because of this, now can be effective only to the individual approach to each patient's immune responses.

2.2. Antiganglioside Antibodies

Antiganglioside antibodies detected in serum in many neurological diseases. It just appears at the immunological analysis of patients with multiple sclerosis. The most studied are antibodies to GM-1 ganglioside (anti-GM1).

It is not sufficiently reliable marker for the accurate diagnosis of "multiple sclerosis", but it is a good tool for determining the current phase of the disease in the back-and-remitting type of disease [6]. At the same time, the presence of different antibodies against gangliosides in patient serum may be used as a criterion for classifying MS for types. Thus, for the relapsing-remitting MS is more typical anti-GM1-antibodies, and for progressive MS—the more common group of AGA-antibodies [7]. In addition, we must note, that anti-GM3 antibodies are typical for primary-progressive MS unlike to secondary-progressive MS and other neuronal disorders. Also activating T-cells these antibodies increase damage to the myeline sheath [8].

2.3. Anti-Myelin Oligodendrocyte Glycoprotein Antibodies

MOG (myelin oligodendrocyte glycoprotein) is a small 218 amino acid transmembrane glycoprotein of Ig superfamily, mainly expressed by oligodendrocytes (OD) and external lammels and located at the surface of myelin. The abundance of MOG is significantly lower compared with the other components of myelin (≈0.01% - 0.05%). The centrifugal location at the surface of myelin fibers make it particularly vulnerable   both to the action of cell and humoral immune responses, including MOG-specific autoAbs, which induce the destruction of myelin (demyelination) in animal models. However, such Abs are poorly known in humans. Located at the surface of myelin sheath, native glycosylated MOG can be a strategic target for the autoimmune attack by anti-MOG autoAbs at the early time points. The usage of such specific biomarkers in the diagnosis of pre-clinical stages of MS seems to be very perspective, e.g. these Abs possibly play one of the major roles in initiation and progression of pathogenic reactions, typical for MS. In addition, cell-based assay provides a detection of valuable serological markers in the primate model of MS at the early stages of the disease [8-11].

As mentioned above, the preferential position of MOG in the nerve fibers is the surface of myelin sheath. Nevertheless, it is rather intriguing that target sites of autoimmune attack also can be located in the deep bottom of the CNS. Subsequently, circulating T and B cells are ready for the specific autoaggression against key epitopes of MOG. There are several mostly discussed mechanisms whose participation is likely to induce MOG-specific expansion of B cells. MOG-specific T cells are also able to infiltrate the naïve CNS and to create conditions for immune responsiveness and to provoke the activation and further expansion of B cells in T cells-enriched areas. These processes are the crucial elements of the "epitope spreading", the phenomenon, which con-

sists in autoAgs and pseudo-autoAgs widespread allocation over nervous tissues, later induction of autoimmune attack by immunocompetent cells and cyclic events of degenerative-destructive lesions along with restorative reinstitution of myelin sheath [12] (**Figure 1**).

Furthermore, another phenomenon can explain the autoaggression against sets of MOG epitopes. Despite the overwhelming expression of MOG gene in the CNS, its mRNA is also detected in other non-CNS organs (e.g. thymus, liver, spleen), as well as small concentrations of protein in peripheral blood. As it may be inferred from above, local imbalances resulted in the collapse of immunological priorities (target Ags) in peripheral organs, is able to provoke generation of new autoreactive immunocompetent populations. In its turn, they affect the restructuring of the myelin sheath directly in the CNS [13-16].

Application of anti-MOG autoAbs in diagnosis of primary—demylination and secondary—MS requires precise differentiation and complete description of the next criteria:

Time intervals of reliable and diagnostically significant peak in titers of anti-MOG

- autoAbs, which could be applied as serological tool for the diagnosis of initial disease;
- stages or its transition to the complicated course;
- pathogenic role of anti-MOG autoAbs, as this type of Abs can be found both in healthy individuals and in patients with MS;
- capability of reliable prognosis. Such prognosis must predict the events, which stimulate accelerated demyelination and aggravation of the disease within concrete intervals of serum anti-MOG autoAbs levels [17-20].
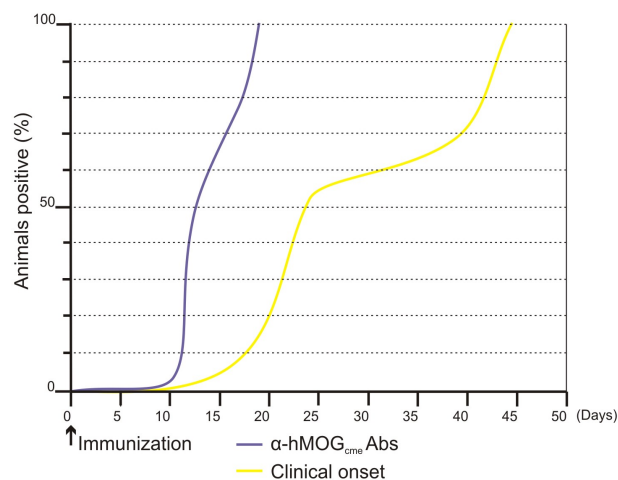

Figure 1. This graph shows the period between immunization, appearance of antibodies and clinical manifestation. As it can be seen from above, the detection of antibodies and early clinical manifestation starts at 10 days. However, positive tests for antibodies in all animals have been recorded already at day 18, whereas clinical manifestation is usually observed after 40 day from the beginning of the assay.

Development of economically beneficial, reliable and precise tests for detection of autoAbs/changes in auto Abs titers is necessary for pre-clinical diagnosis of MS and possible evolution of the disease. Conventional methods to count titers of anti-MOG autoAbs and, besides, identification of autoAbs to MOG analogues (recombinant MOG (rMOG)) can be used for these purposes. In the study on the association of autoAbs against rMOG in patients with MS, a significant enlargement of autoAbs has been detected. Moreover, individuals with progressive type of MS showed higher rMOG indexes (marker for intratecal production of anti-MOG Abs) compared with relapse-remitting one [21].

Some data suggest that circulating anti-MOG autoAbs are exclusive markers of demyelination, and their use for differentiation and prediction of different clinical or pre-clinical stages is impossible [22].

Collectively, application of anti-MOG Abs is constrained because of the incomplete information about the role of these Abs (as direct inducers of pathological processes or only as epiphenomenon) in etiology and progression of the disease, controversial data about their role in MS and in healthy individuals.

2.4. Myelin-Associated Oligodendrocytic Basic Protein

One of the most significant constituents of myelin sheath in etiopathogenesis of MS is myelin-associated oligodendrocytic basic protein (MOBP), a potential target for the autoreactive T cell clones. In murine models, MOBP-reactive T cells can trigger MS-like disease, caused by perivascular and parenchymal infiltration, destruction and degeneration of axons, optic neuritis and large-scale demyelinating processes in the CNS. The leading role of MOBP in autoimmune responses and complex pathology of the CNS is still unclear. Nevertheless, analysis of T cells, autoreactive towards MOBP, suggests MOBP15-36 to be the major target of autoimmune attack in SJL/L mice by virtue of its encephalitogenic potentialities (encephalitogenic epitopes). Autoreactivity of cell immunity towards MOBP is supposed to be a qualitatively new biomarker of MS [23,24].

2.5. Oligodendrocyte-Specific Protein (OSP)/Claudin-11

Oligodendrocyte-specific protein (OSP)/claudin-11 is a 207 amino acid hydrophobic protein of myelin sheath with 4 transmembrane domains. The prevalence of OSP in the myelin sheath is the third among other proteins in the CNS and accounts approximately 7% of total. OSP is one of the candidate autoAgs, involved in the pathophysiology of MS. It is known that demyelination is

primary affected by the reactivity of CD4+ T cells, encephalitogenic towards OSP55-80 in the SJL/L mice model of optic neuritis. The major target of humoral immunity (autoreactive autoAbs) is OSP114-120 [25,26]. Investigation of mice with the knockout of OSP gene showed that the main function of OSP is the formation of tight junctions between myelin components and the integrity maintenance of its structure. Presumably, OSP is an analogue of the protein, contained in peripheral nerve tissues, so-called peripheral myelin protein—PMP-22) [27].

Abnormal structure of myelin together with degeneration of axons, reduction in the activity of metabolic processes in cells and decrease of axon diameter is observed in mice with insufficient/lack of OSP [28]. When exposed to the agents of autoimmune system, intra-lamellar contacts (tight junctions) are exposed to severe degradation. By virtue of this action, the integrity of myelin sheath is violated and restructuration of inter-cellular matrix with consequent disruption of basic myelin constituents occurs [29,30].

OSP is a relatively new protein, whose encephalitogenic properties as well as its pathogenic role in MS are still unclear. Nevertheless, chronic OSP-induced EAE in C57BI/6J mice has a strong association with pathogenic T cells, aimed at minor encephalitogenic regions OSP 199-207 and OSP22-46. Major OSP epitopes 55-80 and 179-207 are the leading pathogenic target for auto-reactive T cells in SJL/L mice. In general, there are a number of epitopes with strong encephalitogenic potential: 52-71, 82-101, 102-121, 142-161, 182-201, and 192-207. In vast majority of experimental data these sequences caused severe relapse-remitting EAE and formation of mononuclear cell infiltrates. Infiltration of parenchymal organs is a characteristic feature of every encephalitogenic OSP except OSP142-161 and OSP182-201, including that ones, which don't lead to the clinical form of MS: OSP72-91 and OSP132-151 [31-34] (**Figure 2**).

2.6. Proteolipid Protein

Proteolipid protein (PLP), also known as lipophilin, is the most abundant protein in the structure of myelin in the CNS ($\approx$50%). It is a hydrophobic and highly conservative molecule, represented in the human body with 2 basic transcripts: 276 full-length amino acid and DM-20. The last isoform differs from the full-length form by the absence of 35 amino acids, expressed mainly in cerebrum and spinal cord prior to myelination and in non-CNS tissues. Interestingly, the major encephalittogenic and immunodominant PLP peptide (139-154) is contained in full-length PLP but not in DM-20. This observation is thought to account for the encephalitogenicity and immunodominance of the PLP (139-154) peptide, since it is essentially not available for thy-

mus-related negative selection and consequently a high pre-cursor frequency of PLP(139-154)-specific T cells has been observed even in naive unprimed animals. The autoimmune attack against such a conventional target as PLP190-209 leads to multiple lesions of brainstem and cerebellum [35].

At the same time, there is a significant correlation between the enhanced activity of humoral and cell-mediated immunity towards PLP 184-209 with HLA-DR4, HLA-DR7 or HLA-DR13 and progression of cerebral lesions of demyelination. Additionally, more than a half of MS patients with primary spinal cord and brain damage show an enlarged reactivity of T cells against PLP 184-209. According to Greer JM et al., the presence of HLA-DR4, DR7 or DR13 does not mean that they have an increased propensity to the developing of brainstem or cerebral lesions.

However, if the autoimmune attack really occurs, the density of lesions in the myelin sheath will be much higher in that case [36].

Ultimately, these data prove the theory that an enlarged reactivity of T cell immunity towards PLP184-209 results in the enhanced attack against brainstem and/or cerebellum. It is important to point out that during the first attacks of demyelination, autoreactive T cell clones can be detected both in peripheral blood, and in CSF. The phase of remission reveals the reduced reactivity of T cells, but prior to the first clinical manifestation a substantial expansion of T cells, directed against PLP184-190 and/or PLP190-209, is observed [37,38].

2.7. Tubulin Polymerization Promoting Protein (TPPP/P25)

Tubulin polymerization promoting protein (TPPP/p25), localized in adult oligodendrocytes, takes part in aggregation of cytoplasmic oligodendrocyte inclusions in case of systemic atrophy of nerve tissues and can be a valuable diagnostic and prognostic marker of MS. The loss of TPPP/p25-positive OD in demyelinated lesions of the CNS is a prospective biomarker for quantification and quality characterization with a special view to estimate physiological state of OD in different types and stages of the disease. It is also possible to use this method for testing the efficacy of applied therapy and both allows to predict the very disease and to count the possibilities for its transition into the complicated form. When conducting a monitoring of average TPPP/p25 levels in CSF, Vinze *et al.* found that individuals with clinically isolated syndrome and relapse-remitting type of MS show a significant increase in concentrations of TPPP/p25 (62.8 and 64.7 μg/l, correspondingly) compared with the control group (27.9 μg/l) [39,40].
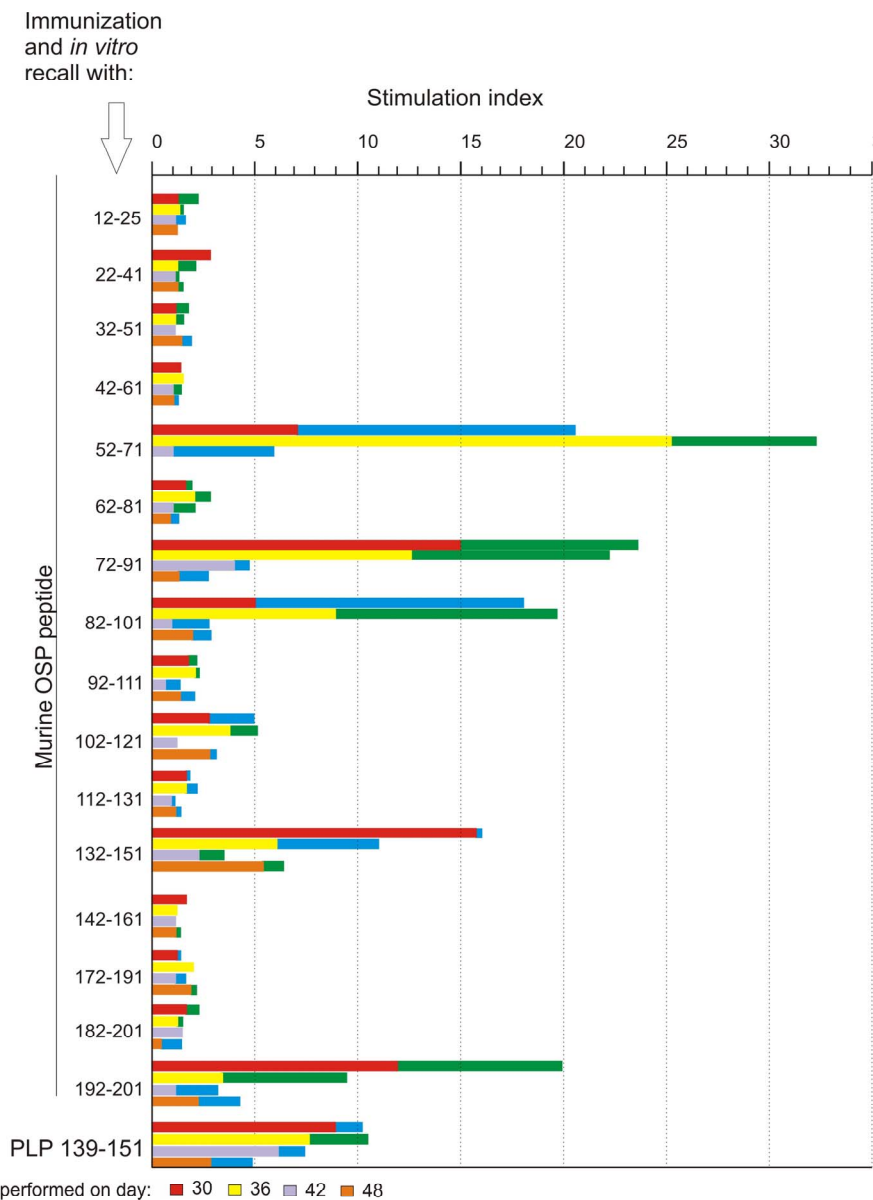
Figure 2. T cell proliferative recall responses to OSP peptides in SJL mice immunized with individual OSP peptides. Spleen cell proliferation assays were performed on one mouse from each group on days 30, 36, 42, and 48 post-injection. OSP peptides were added to a final concentration in culture of 5, 20, and 40 μg/ml. The best SIs were obtained at 20 or 40 μg/ml. The PLP139-151 peptide was used at approximate molar equivalences. The highest SI obtained for each individual mouse's spleen cells cultured at 4 × 106 and 2 × 106 cells/ml is plotted of adjacent like-patterned bars. The red/yellow/grey/orange part of each bar represents the smallest SI obtained from the OSP peptide titration at 4 × 106 cells/ml or 2 × 106 cells/ml. The green part of bar submit how much SI at 4 × 106 more than 2 × 106, or blue part when SI at 2 × 106 more than 4 × 106.

3. CONCLUSIONS

On the basis of the ample data in conventional Abs-proteases and Abs with functional reserve in diagnosis of minor lesions occurring in MS patients as well as differentiated diagnosis, we discussed the crucial antigenic targets for autoimmune attack and their prospects as a valuable tool for detecting of dormant pathological processes in the myelin sheath. Nonetheless, auto-Abs are the key agents involved in the destruction and degeneration of myelin in the CNS and expansion of autoimmune responses, e.g. pro-inflammotary agression and epitope spreading. As it can be inferred from above, Abs by virtue of its peculiar properties, circulate in pe-

ripheral blood and CSF for years before clinical manifestation. Subsequently, using these biomarkers, we can define prospectives for further progression of current pre-morbid state to the stages of profound clinical sympthoms and blockade it by time-lapse introducing of modern predictive and preventive therapeutic protocols. In addition, determination of specific epitopes in different constituents of myelin (BMP, MOG, PLP, MOBP, OSP) in some cases allows to find predominant sites of autoagression (e.g. cerebrum, spinal cord).

Serological assay based on the identification of specific immune biomarkers and monitoring of their spectra are fundamental principles of Predictive and Preventive Medicine. Further search for prospective biomolecules and description of their potential role regarding the principles of pre-clinical diagnosis is especially important for their capable application and impact in clinical and pre-clinical practice.

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