

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Antibody-Proteases in the Pathogenesis of Autoimmune Demyelination and Monitoring Patients with Multiple Sclerosis

Dmitry Kostyushev<sup>1</sup>, Dmitry Gnatenko<sup>1</sup>, Mikhail Paltsev<sup>3</sup>,  
Aleksandr Gabibov<sup>4</sup> and Sergey Suchkov<sup>1,2</sup>

<sup>1</sup>*I.M. Sechenov First Moscow Medical State University*

<sup>2</sup>*Moscow State University of Medicine and Dentistry*

<sup>3</sup>*National Research Center "Kurchatov Institute"*

<sup>4</sup>*M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic  
Chemistry, Russian Academy of Sciences  
Russia*

## 1. Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system (CNS) resulting in axon loss and development of disability. (Gabibov et al., 2011) Autoantibodies (autoAbs) are one of the major features and crucial mechanisms in MS pathogenesis known to illustrate this autoaggression. The major component in the pathogenesis of MS is primary myelin damage, which is mediated by autoAbs, which trigger the release of separate and pathogenically valuable myelin-associated epitopes into the bloodstream. These molecules acting as a group of sensitizing factors may provoke the immune system and drive disease progression. Being identified at the pre- or early stages or the demyelination, such autoAbs dominate during the whole course of the disease.

Natural catalytic antibodies (*catAbs*) or natural *abzymes* today are one of the principal effectors of the adaptive immune system. In constructive sense, *catAbs* are multivalent immunoglobulins (Igs), presumably, of IgG and IgM isotypes, endowed with a capacity to hydrolyze an antigenic substrate.

Traditionally, the basic structure of the Ab molecule is essentially Y-shaped, with the two tips (*Fab*-fragments) designed to recognize and bind non-self agents or cells. Moreover, the catalytic capacity is also present in *Fab*-fragments of the molecule. In general, the mechanisms of Ab-mediated catalytic action include nucleophilic catalysis, induction of conformational strain, coordination with ions, and stabilization of transition states (TS). Ag-specific or targeted *catAbs* are preferentially found in the Ig repertoire of patients with a broad scope of diseases to act as pathogenically valuable tools. Since the discovery of *catAbs*, a wide spectrum of the disease-related *abzymes* regardless to their natural history or engineering protocols has been described. Moreover, the immune system was shown to express an intrinsic drive to generate natural *abzymes* in different pathological states in humans. Among them, proteolytic (*Ab-proteases*) and DNA-hydrolyzing (*DNA-abzymes*) autoAbs are of a special practical value.

And today catAbs can be used as either therapeutic tools or as vehicles for delivering therapeutic agents to damaged cells in the human body. Recent applications of abzymes have included a broad scope of medical and allied areas, i.e.,

1. the conversion of drugs to their inactive (non-toxic) forms;
2. the degradation of drugs and harmful substances;
3. the activation of pro-drugs for targeted chemotherapy;
4. the inhibition of infectivity;
5. others.

The aim of this review is to compare data on the medical applications and implementations of disease-associated and engineered abzymes as new tools for treatment of MS, and to specifically focus on their potential value for clinical research and for clinical utility and public health as well.

The mechanisms of catAb action include nucleophilic catalysis, induction of conformational strain, coordination with metal ions, and stabilization of TS. Only Abs that stabilize the Ag TS more than the GS can be catalytic, and such Abs are thus usually identified among those that bind tightly to an analogue of the TS of the relevant reaction. Abs would thus provide a unique opportunity to combine specific Ag recognition with enzymatic turnover.

For Ab-proteases, for instance, a typical mechanism of nucleophilic catalysis has been established. Since the catalytic efficiency of Ab-proteases derives substantially from the ability to recognize the GS with high affinity, proteolytic Abs while demonstrating an exclusive targeted specificity can be used to selectively address a wide range of metabolically and pathogenically valuable protein targets.

In case of DNA-abzymes, Ab-induced conformational strain is proposed to activate phosphodiester bonds and result in DNA hydrolysis, aided by coordination of bivalent cations. As being similar to proteolytic sites, the nuclease activity of DNA-hydrolyzing Abs is described to be encoded by germline variable region genes (V-genes). In general, the specific nature of the Ab catalysis was demonstrated by the adherence of those reactions to the well-known Michaelis-Menten equation, the complete inhibition by a proper hapten analogue, and the failure of the Ab to catalyze the hydrolysis of the substrate antagonists.

During the last 10 years, it has been found that Abs contribute 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 (myelin basic protein), was associated with the course of the neurodegenerative disease MS and its rodent model, experimental autoimmune encephalomyelitis (EAE). Autoantibody-mediated degradation of MBP was shown to be site specific, with cleavage sites localized to the immunodominant epitopes of the protein. These findings were supported by studies from others. Interestingly, this reaction was inhibited *in vitro* by glatiramer acetate (Copaxone), an established treatment for MS. (Belogurov et al., 2008)

There are three groups of autoAbs that are specific for MS: anti-myelin autoAbs (e.g., anti-MBP, anti-MOG (MOG - myelin oligodendrocyte glycoprotein) and anti-neurofilament autoAbs); nonmyelin 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]). The latter group of anti-myelin antibody proteases is of particular interest in terms of disease monitoring, prognosis and preclinical (pre-early) diagnostics of MS. Catalytic antibodies are endowed with a capacity to hydrolyze an antigenic substrate. This has moved antibodies to the level of physiological functionality by providing such

antibodies with the ability to mediate direct catalytic and indirect cytotoxic effects on the target. This property is buried in the Fab fragment of the immunoglobulin molecule. Antibody protease were found in most autoimmune conditions, particularly in MS, accomplishing sequence-specific proteolytic cleavage of myelin antigens and controlling the degradation of myelin sheath. (Gabibov et al., 2011)

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 and 40 years. Men are affected approximately twice as rare as women. (Stuve & Oksenberg, 2006 )

Autoantibody mediated tissue destruction is among the main features of organ-specific autoimmunity. 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, myelin-specific autoantibodies are detected by high-resolution microscopic analysis in the regions of demyelination plaques in human MS and a MS-like disease of marmosets, suggesting their direct contribution to myelin destruction. (Ponomarenko et al., 2006)

Nonetheless, the mechanisms responsible for the induction of autoantibodies and their possible contributions to MS progression are still unknown and are somewhat controversial. Also clonal expansion of B cells and T cells, hallmarks of inflammation in the CNS, are found in MS. The viral mimicry hypothesis was formulated to explain the initiation of this pathology. (Belogurov et al., 2008) But a poor understanding of the etiology of MS has complicated the development of effective therapeutics. (Hafler, 2004)

Despite strong evidence for the contribution of T cell responses to manifestations of autoimmunity in the CNS of patients with MS, recent findings encouraged investigators to search also for B cell-mediated contributions to the MS pathogenesis. (Klawiter & Cross, 2007; Nikbin et al., 2007)

Development of new diagnostics and treating tactics can improve patient life quality and decrease MS treatment cost, as in other autoimmune diseases such as type 1 diabetes. (Hahl et al., 1998)

## 2. Antibodies-mediated demyelination in the pathogenesis of MS

Antibodies can cause demyelination by several effector mechanisms. One of these is the opsonization of myelin for subsequent phagocytosis by macrophages, which has been observed in MS and EAE. Serum anti-myelin antibodies raise macrophage phagocytosis, and the uptake by macrophages of CNS myelin increases after opsonization with complement. Another mechanism of demyelination that involves autoantibodies is through activation of the entire complement cascade leading to membrane attack complex (MAC) deposition and complement-mediated cytotoxicity.

Antibodies with specificity against minor myelin components have also been detected in MS patients. MOG is the most interesting candidate B-cell autoantigen in MS. Because of its location it is an ideal target for antibody-mediated demyelination. Anti-MOG antibodies are indeed able to cause myelin destruction in EAE models, while other antibodies against major myelin proteins such as MBP or PLP, which are both not located on the myelin surface, do not cause myelin destruction on their own. Anti-MOG Abs mediate a characteristic vesicular transformation of compact myelin in acutely demyelinated lesions that also has been documented in human MS lesions strongly suggesting a role of anti-MOG Abs in MS. The B-cell response to MOG is enhanced in MS also supporting the pathogenic importance of anti-MOG Abs. Interestingly, the presence of serum anti-MOG antibodies,

with or without anti-MBP antibodies, in patients presenting with an initial clinical event suggestive of central nervous system demyelination and evidence of multifocal lesions on MRI studies, is predictive of subsequent clinical events that establish the diagnosis of clinically definitive MS. However, the presence of anti-MOG antibodies in patients with nondemyelinating diseases of the CNS, as well as in a substantial number of healthy individuals, has raised important questions about the role of these antibodies in MS, and these need to be addressed in future studies.

## 2.1 Catalysis

The neurodegenerative model of MS is based on the proposition of a primary lesion in myelin followed by myelin breakdown and the release of myelin-compartmentalized proteins, particularly MBP. This is followed by the generation of MBP-derived peptides that become the main sensitizers of T cells. (Hafler, 2004) The hypothesis that interactions between MBP and T cells must occur at sites other than the myelin membrane has been challenged by recent physicochemical studies demonstrating that myelin can become structurally unstable secondary to specific posttranslational modifications of MBP structure. This was shown to result in the increased surface exposure and susceptibility to proteolysis of MBP 83–92. (Husted, 2006) The close association between the proteolytic sensitivity of MBP and the pattern of posttranslational modifications of the molecule may represent one of the key regulatory mechanisms in epitope generation (Lolli et al., 2005). Proteolysis may proceed by any of four distinct pathways that may exert a concerted attack on the MBP molecule, although the mechanisms responsible for the activation and regulation of these potential activities are not known. Thus, epitope generation may occur via:

1. autocatalytic cleavage of the MBP molecule (D'Souza et al., 2005);
2. protease digestion (D'Souza et al., 2006);
3. autoantibody-mediated site-specific cleavage (Ponomarenko et al., 2006);
4. Abdependent oxidative pathway (Wentworth et al., 2001).

The last mechanism, which has been demonstrated in a number of autoimmune pathologies, remains unproven for MS (Wentworth et al., 2001). These pathways are characterized by dramatic differences in reaction velocities and in cleavage site specificity. Obviously the rates of enzymatic reactions are several times higher than those for autocatalysis and Abenzymes (abzymes), perhaps making this pathway the major player in epitope generation. In the case of abzymes, the large excess of the biocatalyst, its high specificity, and its close compartmentalization with the MBP substrate shown in demyelinating lesions are suggestive of its likely effectiveness in vivo. (Ponomarenko et al., 2006; Genain et al., 1999)

Today, the advent of antibody catalysis has demonstrated that antibodies can be programmed to perform complex cell biochemistry, and thus a logical question arises: does the original (nature-gifted) potential for catalysis relate to antibody function? In the sense of the question, it is very interesting to consider the evolution of antibodies, while generally accepting that natural enzymes are primitive molecules compared with antibodies, and that antibodies arose just after the birth of enzymes.

A great deal of evidence has been also adduced to support the medical concept of the *living soul* of abzymes and their significance for utilizing broader autoantibodies properties in the formation of pathogenic patterns and clinical settings at different autoimmune and other conditions. Moreover, the medical concept of abzymes was tested in two ways, clinically and experimentally, i.e., on human and animal models. The progress achieved earlier in



designing artificial abzymes initiated the study of biological activities of natural catalytic autoantibodies and their involvement in pathogenesis of major clinical disorders, i.e., evoked the onset of the era of medical abzymology. (Suchkov et al., 2001; Zhou et al., 2002; Tellier, 2002; Nathan, 2002; Kozyr et al., 1998)

Medical abzymology has made a great contribution to the development of general autoimmunity theory: it has put the antibody as a *functional entity* and as the key brick of the theory to the level of *physiological functionality* by providing such antibody with the ability to catalyze and mediate direct and independent cytotoxic effect on cellular and molecular targets.

### 3. Major antigenic targets of the anti-myelin autoAbs

There are extensive information on a significant portion of MS cases, characterized by the presence of autoantibodies against myelin protein components in serum of patients with MS. (Chamczuk et al., 2002; Reindl et al., 1999) Although the mechanism of the autoantibody role in MS pathogenesis is unknown, autoantibodies to MBP and MOG were proposed as biomarkers for clinical prognosis of MS. (Berger et al., 2003) Similar immunoglobulins were also found in mice with induced EAE, which is an animal model of MS. (Fritz et al., 1983)

#### 3.1 Myelin basic protein

Myelin basic protein is by far the best studied myelin component in MS. It is the second most abundant myelin protein (approximately 30-40%) after PLP. There are five MBP isoforms with 14-21.5 kDa molecular weights in mammals that result from differential splicing of 11 axons within the Golli-MBP locus. The highly basic MBP is positioned at the intracellular surface of myelin membranes and via interactions with acidic lipid moieties is involved in maintaining the structure of compact myelin. The most abundant 18.5 kDa isoform (170 amino acid length) has been used in most immunological studies.

Different from MOG, and PLP (proteolipid protein), MBP is found in both central and peripheral myelin, and MBP transcripts have also been demonstrated in peripheral lymphoid organs such as lymph nodes and thymus.



Fig. 1. MBP sites of proteolysis by different enzymes-proteases and Abs-proteases and as a result of spontaneous autocatalytic hydrolysis. The sequence of the encephalitogenic peptide 81-103 is shown in red. Abbreviations: GelB (Gelatinase B), MMP-3 (matrix metalloproteinase-3), CatD (Cathepsin D), ACat (Autocatalytic cleavage), Abz (Abzyme), LP (Lysosomal Proteases), Tryp4 (Trypsin 4).

### 3.2 Proteolipid protein

Proteolipid protein (PLP) is the most abundant protein in CNS compact myelin (about 50%), highly hydrophobic and evolutionarily conserved across species. There are two main transcripts, the full-length 276 amino acid isoform and DM-20, an isoform that lacks 35 amino acids and is mainly expressed in brain and spinal cord prior to myelination, but also in peripheral lymphoid organs such as the thymus, where full-length PLP is barely found. Interestingly, the major encephalitogenic 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 thymic negative selection and consequently a high precursor frequency of PLP (139-154)-specific T cells has been observed even in naive unprimed animals.

### 3.3 Myelin oligodendrocyte glycoprotein

Myelin oligodendrocyte glycoprotein, a 218 amino acid transmembrane glycoprotein of the IG superfamily, is much less abundant (0.01 - 0.05%) than MBP and PLP, and also different from the two major myelin proteins in being located not in compact myelin but exposed on the outermost surface of the oligodendrocyte membrane. Because of this “strategic” location, it is directly accessible to antibodies and believed to be particularly relevant as a target for both cellular and humoral immune responses in MS. MOG, is expressed relatively late during myelination and is only found in the brain/spinal cord and the retina but not in peripheral nerve. Furthermore, MOG expression is either completely or almost completely lacking in peripheral lymphoid tissues, although MOG transcripts have been seen in nonhuman primate peripheral nerve and a few samples of human tonsils and thymus.

## 4. Nonmyelin autoAbs

### 4.1 DNA abzymes and antibody proteases

Catalytic antibodies are endowed with a capacity to hydrolyze an antigenic substrate. (Paul S. 1998; Paul et al., 2005; Friboulet et al., 1999) This has moved antibodies to the level of physiological functionality by providing such antibodies with the ability to mediate direct catalytic and indirect cytotoxic effects on the targets. This property is buried in the Fab fragment of the immunoglobulin molecule. Antibody proteases (antibodies with proteolytic activity) were found in most autoimmune conditions, particularly in MS, accomplishing sequence-specific proteolytic cleavage of the myelin antigens and controlling the degradation of the myelin sheath. The levels of proteolytic activity of the antibody proteases revealed significant correlations with the clinical activity of the course over the disease, and thus with the disability of the patients. The most attractive point is that, in contrast to canonical proteases, for antibody proteases, there is an extra set of cleavage sites in the targeted autoantigens (for sequence-specific proteolytic cleavage) focused predominantly at the immunodominant sites. (Gabibov et al., 2002)

Physiological interaction of autoAb with a living cell is mediated by Fc fragment (Wilkinson et al., 2001). DNA-abzymes are able to realize both cytotoxicity mechanisms-complement-dependent cell lysis and K-cell-mediated lysis. (Fishelson et al., 2001) Fab fragment is not involved in these reactions. This fundamentally distinguishes Ab-mediated classical and DNA-mediated mechanisms of cytotoxicity due to direct involvement of Fab fragment into the catalytic attack on the target cell genome. Catalytic and cytotoxic activities of DNA-

abzymes are closely related. This allows to assume on a hypothesis on a new mechanism of the contribution of autoAbs into the pathogenesis of autoimmune disorders. Such mechanism acts independently of complement and cytotoxic T cells; it requires a catalytically active Fab fragment but ignores Fc fragment whose structure is deprived of even buried resources providing direct cytotoxic effect. (Ponomarenko et al., 2000)

Two different mechanisms of DNA-abzymes cytotoxic potential utilization are established by means of direct cytotoxic effect on a target cell involving the catalytically active Fab fragment and by means of apoptosis due to high affinity of DNA-binding autoAbs for membrane receptors providing the cell with features of possible target. Cross-reactive with such cells, DNA-abzymes can provoke their degradation resulting in the development of different syndromal manifestations: lupus nephritis in glomerule endothelium cross-reactivity, articular syndrome in synovial cross-reactivity, extracardial manifestations and MCS progression in CM cross-reactivity. (Rekvig et al., February 2004; Raz et al., 1993)

Cross-reactivity of DNA-binding autoAbs with glutamate receptors accumulating at neural cells is of a special interest. Such autoAbs with catalytic and cytotoxic activities can initiate neural cells apoptosis providing the possibility for the development of CNS autoimmune degenerative disorders (Kotzin et al., 2001). The penetration of DNA-binding autoAbs (and DNA-abzymes) into a cell results in activation of cytotoxicity mechanisms, apoptosis induction, etc.

#### 4.2 Molecular mechanisms of the involvement of DNA-abzymes in the development of different autoimmunity conditions and pathogenesis of autoimmune disorders

One antigen may generate up to  $10^2$ - $10^4$  different antibody molecules, a number that may further increase by somatic mutagenesis. Therefore, it seems feasible that different DNA-binding and other non-catalytic antibodies, as well as antibodies with catalytic activities can be synthesized in the course of immune response, either directed against the substrate or as antiidiotypic antibodies to enzymes hydrolyzing nucleic substrates. It is certainly difficult to predict the clinical significance of these catalytic activities, but it is likely that they modify the pathogenesis or clinical process of these autoimmune diseases.

The interest to catalytic (i.e., DNA-hydrolyzing activity) of the autoantibodies is kept on growing up and is strongly supported by the new data including those illustrating cytotoxic activities of the biocatalysts and evidence that abzyme-mediated cytotoxic effects observed in human SLE (system lupus erythematosus) and mouse SLE-like syndromes are caspase-dependent and thus apoptosis-related.

### 5. Prediction of MS

The use of assays for Abs to MBP and MOG for diagnostic and prognostic purposes in patients with a clinically isolated syndrome (CIS), a frequent precursor to clinically definite MS (CDMS), has yielded conflicting results. One study showed that the presence of myelin Abs in the sera of CIS patients was predictive of a shorter time course to the development of CDMS. (Tomassini et al., 2007) Others, however, indicated that positive tests had no prognostic value for progression to CDMS. (Kuhle et al., 2007)

Symbols indicate OD450 values for Abs from individual subjects within each EDSS category for specific MBP peptides or intact MBP and MOG proteins. Lines indicate the relation between EDSS value and the binding activity of specific peptides/proteins: MBP 81-103 ( $R^2$  0.332;  $p$  0.005), MBP 130-156 ( $R^2$  0.381;  $p$  0.011), MBP 146-170 ( $R^2$  0.310;  $p$  0.033), intact MBP



( $R^2$  0.458;  $p$  0.005), intact MOG ( $R^2$  0.052;  $p$  0.001), and control Trx carrier protein ( $R^2$  0.055;  $p$  0.376)

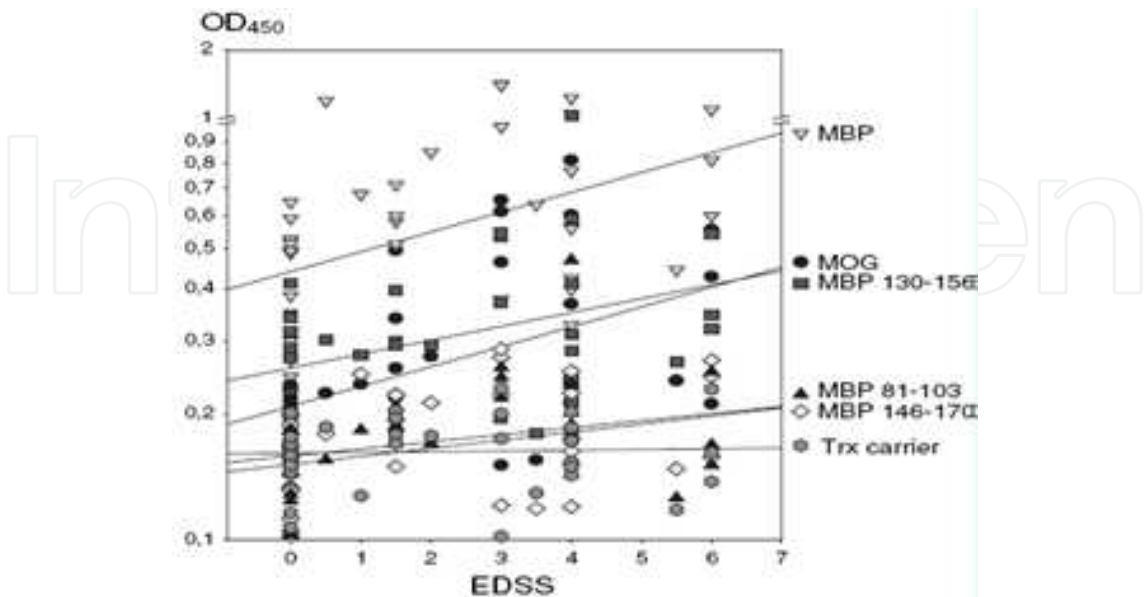


Fig. 2. Correlation of the EDSS of MS patients with the levels of autoantibodies to MBP fragments.

The latter results are in keeping with our demonstration that the binding activities of Abs to intact MBP and MOG proteins were indistinguishable for patients with MS and other neurological diseases (OND). In addition, a screen of MBP peptides showed that the reactivity of Abs from patients with MS and OND could be distinguished only by their differential binding to MBP 43–68 and MBP 146–170. Follow-up studies of patients with MS using assays based on the use of these fragments will be needed to determine whether it provides an improved prognostic tool for patients with CIS.

Interestingly, the parameters of Ab binding associated with disease progression were different from those with potential prognostic value. Disease severity, as determined by Expanded disability status scale (EDSS), correlated with level of autoantibodies to intact MBP and MOG proteins as well as MBP fragments 81–103, 130–156 and 146–170. These findings are in keeping with the demonstrated correlations between levels of anti-MOG and anti-MBP Abs and inflammatory signs revealed by MRI and cerebrospinal fluid analyses. (Kuhle et al., 2007)

### 5.1 Diagnostic protocol

The appearance of Ab-proteases at the pre-early (*preclinical*) stages of demyelination to illustrate myelin degradation has been documented.

The results we had obtained would allow for approaching to a novel generation of diagnostic technologies based on the protocols of clinical and preclinical diagnostics to exploit Ab-proteases and their sequence-specificity.

The protocols would include:

1. isolation of individual Ab-proteases from sera of MS patients or persons to be at risks for suspicious MS (e.g., the relatives);
2. quantification of the proteolytic activity of Ab-proteases;

3. determination of sequence-specificity of the individual Ab-proteases towards MBP as the whole molecule and separate peptides (epitope-bearing) fragmented from the molecule as well;
4. verification of the diagnosis and prediction for the future.

A phenomenon of the *pre-clinical* formation of the abzyme-based armamentarium and Ab-proteases, in particular, would be interpreted as a portion of the proper immune response of the body to the environmental and other shifts. So, a selection of the mode of action for proteolytic antibodies would depend on the metabolic motivation occurring at a particular stage of the disease. Of great interest would be driving motivation to control the mode of action at the *preclinical* stages.

The occurrence of antibody proteases among healthy individuals is due to the development of the pre-early immune imbalances and formation of the preclinical conditions. So, when bursts of the antibody-associated proteolytic activity or the tendency for the latter were evident, the pre-early stages preceding formation of CIS and the exacerbation of the disease could be predicted, even with no observable clinical manifestations. There was definite correlation between the interval before CIS and autoAb (including antibody proteases) status. The occurrence of antibody proteases and the degree of their activity were significantly associated with symptomatology/severity of CIS and clinical course of the disease.

## 5.2 Sequence specificity and clinical implementation

Sequence specificity of Ab-proteases is the capacity to distinguish or not distinguish between particular epitopes in the MBP molecule. Serum anti-MBP auto-Abs obtained from MS patients exhibited sequence-specific proteolytic cleavage of the MBP molecule. Such antibody proteases revealed significant correlations with different manifestations of MS (i.e., progressive and/or remittance phases of the disease), with Expanded Disability Status Scale scores and thus with scales of demyelination and, moreover, with the degree of disability of MS patients. For instance, anti-MBP antibody proteases that are able to recognize 43-68 and 146-170 amino acid sequences within the MBP molecule are predominantly more typical of MS patients, but not of patients with neurodegenerative diseases other than MS. In MS patients with low disease activity (in remission), a family of antibody proteases has been detected with low proteolytic activity that predominantly targets 43-68 and 146-170 amino acid sequences within MBP. Progression of disability in MS patients is accompanied by the bursts of anti-MBP antibody-associated sequence-specific proteolytic activity. Moreover, signs of a trend in the specificity of the activity were found (i.e., specificity of the sequence recognition from 43-68 and 146-170 amino acid sites with low affinity to 81-103 and 82-98 amino acid sites, demonstrating that high-affinity indices are recognized by anti-MBP antibody proteases). (Ponomarenko et al., 2006)

## 5.3 Perspectives in the application of catalytic antibodies for clinical medicine

More than a hundred of abzyme-catalyzed reactions have been described. Catalytic efficacy of some abzymes is comparable with those of enzymes, but the specificity of abzymes is even higher. The practical relevance of abzymes is due to the unique feature drastically distinguishing them from other biocatalysts, i.e., abzymes could be produced for the catalysis of not only all the reactions occurring in the living systems but also be designed for the development of principally new catalysts with no natural counterparts. (Gabibov et al., 2002; Zhou et al., 2002) In this sense, we can anticipate that manmade catalytic antibodies

will have considerable practical potential in many different medical applications, i.e., would form the frame of medical abzymology.

Medical abzymology as a novel trend in medical immunology and enzymology, and a new avenue in the clinical practice appears to demonstrate a revolutionary growth today. The antibody catalysis as itself and regardless to a defined field of medical application appears to stress a new area of medical research and a novel field of medical application that provoke considerable interest for medical investigators and clinical practitioners. (Suchkov et al., 2006; Suchkov et al., 2001)

Autoimmune diseases actually represent a challenging frontier in contemporary medical research and clinical practice, and, thus, in areas overlapped with medical abzymology. The proper relation of clinical autoimmunity to the generation of natural catalytic antibody response is absolutely evident, and, in general terms, the phenomenon of autoantibody catalysis can potentially be applied to isolate efficient catalytic domains directed against autoimmune epitopes pathogenically and clinically relevant. This can be done by exposing the autoimmune repertoire to identify autoantigens or their mimicking counterparts capable of recruiting the germ line genes encoding the catalytic site.

## 6. Conclusion

The prospects for progress in developing novel approaches for the diagnosis and treatment of MS have been greatly encouraged by several observations. These include:

1. the identification of a major immunogenic region in MBP, peptide 82–98;
2. the demonstration that both B and T cell responses to MBP are focused on this region, particular for patients who express HLA-DR2;
3. clinical studies suggesting that patients treated with MBP peptide 82–98 can be made tolerant to the protein in association with delayed disease progression or reduced disease activity over time. Autoantibody mediated tissue destruction is among the main features of organ-specific autoimmunity. Since the original discovery of catalytic antibodies, ample data established their contribution to pathological effects in disease as well as their possible biomedical applications. The practical relevance of abzymes is due to the unique characteristic distinguishing them from other biocatalysts: abzymes could be produced for the catalysis of almost all reactions occurring in living systems and catalyzed by appropriate enzymes. Abzymes may also be designed for the development of fundamentally new catalysts without natural analogues. The proved possibility to set a cell on secretion of engineering biocatalysts is intensively discussed. Serum level of proteolytic Abs may provide a clinically important predictive biomarker for demyelination in MS patients and formulating prognosis of the disease. The serum levels of the Ab-mediated catalytic activity as prognostic criteria could also differentiate MS patients with probably favorable or severe disease course or outcome, and outcome criteria are now being designed to assess the overall impact of MS as dependent variables for clinical studies. Ultimately, analysis of autoAb-mediated MBP degradation may provide a supplementary clinical and laboratory tool for assessing the disease progression and disability of MS patients. The possibility of forced stimulation of B-cells to produce proteolytic Abs with a given design is also intensively discussed. Such technology could be widely applied to treat and prevent socially relevant autoimmune disorders.

Achievements in medical abzymology could be a promising basis to design new medicines. The most dynamic trend is related to the synthesis of catalytic Abs that are able to destroy

circulating drugs before initiating toxic effects on nervous system and other tissues. Catalytic Abs directly affecting the physiologic reconstruction of tissues and organ systems with complex architectonics including neuroglia are of special value. Neurology is estimated as an interesting area for abzyme application since Abs can be used for the induction of remyelination and the restoration of previously lost glia functions in MS.

List of abbreviations:

MS - Multiple sclerosis  
CNS - central nervous system  
autoAbs - Autoantibodies  
catAbs - catalytic antibodies  
Igs - immunoglobulins  
TS - transition states  
MBP - myelin basic protein  
EAE - experimental autoimmune encephalomyelitis  
MOG - myelin oligodendrocyte glycoprotein  
HSP - heat shock protein  
MAC - membrane attack complex  
PLP - Proteolipid protein  
SLE - system lupus erythematosus  
CIS - clinically isolated syndrome  
CDMS - clinically definite multiple sclerosis  
EDSS - Expanded disability status scale  
MRI - magnetic resonance imaging

## 7. References

- Abraham S., Guo F., Li L.S., Rader C., Liu C., Barbas C.F. 3rd., Lerner R.A., Sinha S.C. Synthesis of the next-generation therapeutic antibodies that combine cell targeting and antibody-catalyzed prodrug activation. *Proc. Natl. Acad. Sci. USA* 2007, 104(13):5584-5589
- Altmann D. (2005) Evaluating the evidence for multiple sclerosis as an autoimmune disease. *Archives of neurology*. Vol.62, No.4 (April 2005) ISSN 688-9
- Anglucchi F, Mirabella M, Frisullo G, Caggiula M, Tonali PA & Batocchi AP, (2005) Scrum levels of anti-myelin antibodies in relapsing – remitting multiple sclerosis patients during different phases of disease activity and immunomodulatory therapy. *Disease markers*. Vol.21, No.2 (2005) ISSN 49-55.]
- Belogurov AA, Kurkova IN, Friboulct A et al.' (2008). Recognition and degradation of myelin basic protein peptides by serum autoantibodies: novel biomarker for multiple sclerosis. *Journal of immunology*. Vol.180, No.2 (January 2008) ISSN 1258-67]
- Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F. & Reindl, M. (2003) Anti-myelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *The New England journal of medicine* Vol.349, No.2 (July 2003) ISSN 139-45

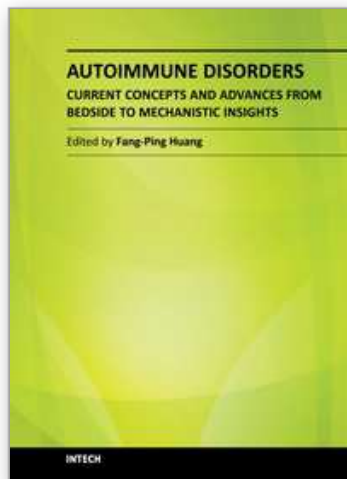


- Chamczuk, A. J., Ursell, M., O'Connor, P., Jackowski, G. & Moscarello, M. A. (2002) A rapid ELISA-based serum assay for myelin basic protein in multiple sclerosis. *Journal of immunological methods*. Vol.262, No.1-2 (April 2002) ISSN 21-7.,
- D'Souza, C. A., & M. A. Moscarello. (2006). Differences in susceptibility of MBP charge isomers to digestion by stromelysin-1 (MMP-3) and release of an immunodominant epitope. *Neurochemical research*.. Vol.31, No.8 (August 2006) ISSN 1045-54.
- D'Souza, C. A., D. D. Wood, Y. M. She, & M. A. Moscarello. (2005). Autocatalytic cleavage of myelin basic protein: an alternative to molecular mimicry. *Biochemistry* Vol.44, No.38 (September 2005) ISSN 12905-13.
- Fishelson Z, Attali G, Mevorach D. (2001) Complement and apoptosis. *Molecular immunology* Vol.38, No.2-3 (August 2001) ISSN 207-19
- Friboulet A, Avalle B, Debat H, Thomas D (1999) A possible role of catalytic antibodies in metabolism. *Immunology today*. Vol.20, No.10 (October 1999) ISSN 474-5
- Friboulet A., Avalle B., Debat H., Thomas D. A possible role of catalytic antibodies in metabolism. *Immunol. Today* 1999, 20(10):474-478
- Fritz, R. B., Chou, C. H. & McFarlin, D. E. (1983) Induction of experimental allergic encephalomyelitis in PL/J and (SJL/J x PL/J)F1 mice by myelin basic protein and its peptides: localization of a second encephalitogenic determinant. *Journal of immunology* Vol.130, No.1 (January 1983) ISSN 191-4
- Gabibov A.G., Gololobov G.V., Makarevich O.I., Schourov D.V., Chernova E.A., Yadav R.P. DNA-hydrolyzing autoantibodies. *Appl. Biochem. Biotechnol.* 1994, 47(2-3):293-302
- Gabibov AG, Ponomarenko NA, Kozyr AV et al (2002) Catalytic antibodies and pathology: human and mice models. *Journal of immunological methods*. Vol.269, No.1-2 (November 2002) ISSN 197-211
- Genain, C. P., Cannella, B., Hauser, S. L. & Raine, C. S. (1999) Identification of autoantibodies associated with myelin damage in multiple sclerosis., *Nature medicine* Vol.5, No.2 (February 1999) ISSN 170-5.
- Hafler, D. A. (2004). Multiple sclerosis. *The Journal of clinical investigation*. Vol.113, No.6 (April 2004) ISSN 788-94.
- Husted, C. (2006). Structural insight into the role of myelin basic protein in multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*. Vol.103, No.12 (March 2006) ISSN 4339-40.
- Jones LH, Wentworth Jr P. The therapeutic potential for catalytic antibodies: from a concept to a promise. *Mini Rev Med Chem* 2001;1:125-32
- Kotzin BL, Kozora E. (2001) Anti-DNA meets NMDA in neuropsychiatric lupus. *Nature medicine* Vol.7, No.11 (November 2001) ISSN 1175-6
- Kozyr A.V., Kolesnikov A.V., Aleksandrova E.S., Sashchenko L.P., Gnuchev N.V., Favorov P.V., Kotelnikov M.A., Iakhnina E.I., Astasurov I.A., Prokaeva T.B., Alekberova Z.S., Suchkov S.V., Gabibov A.G. Novel functional activities of anti-DNA autoantibodies from sera of patients with lymphoproliferative and autoimmune diseases. *Appl. Biochem. Biotechnol.* 1998, 75(1):45-61
- Kozyr A.V., Kolesnikov A.V., Zelenova N.A., Sashchenko L.P., Mikhlap S.V., Bulina M.E., Ignatova A.N., Favorov P.V., Gabibov A.G. Autoantibodies to nuclear antigens: correlation between cytotoxicity and DNA-hydrolyzing activity. *Appl. Biochem. Biotechnol.* 2000, 83(1-3):255-262



- Kozyr A.V., Sashchenko L.P., Kolesnikov A.V., Zelenova N.A., Khaidukov S.V., Ignatova A.N., Bobik T.V., Gabibov A.G., Alekberova Z.S., Suchkov S.V., Gnuchev N.V. Anti-DNA autoantibodies reveal toxicity to tumor cell lines. *Immunol. Lett.* 2002, 80(1):41-49
- Kuhle, J., C. Pohl, M. Mehling, G. Edan, M. S. Freedman, H. P. Hartung, C. H. Polman, D. H. Miller, X. Montalban, F. Barkhof, et al. (2007). Lack of association between anti-myelin antibodies and progression to multiple sclerosis. *The New England journal of medicine*. Vol.356, No.4 (January 2007) ISSN 371-8
- Kuhle, J., R. L. Lindberg, A. Regeniter, M. Mehling, F. Hoffmann, M. Reindl, T. Berger, E. W. Radue, D. Leppert, and L. Kappos. (2007). Anti-myelin antibodies in clinically isolated syndromes correlate with inflammation in MRI and CSF. *Journal of neurology*. Vol.254, No.2 (February 2007) ISSN 160-8.
- Lacroix-Desmazes, S., Bayry, J., Misra, N., Horn, M. P., Villard, S., Pashov, A., Stieltjes, N., d'Oiron, R., Saint-Remy, J. M., Hoebeke, J., et al. (2002) *N. Engl. J. Med.* 346, 662–667.
- Lerner R.A. Catalytic antibodies: the concept and the promise. *Hosp. Pract. (Off. Ed.)* 1993, 28(7):53-59
- Lolli, F., B. Mulinacci, A. Carotenuto, B. Bonetti, G. Sabatino, B. Mazzanti, A. M. D'Ursi, E. Novellino, M. Pazzagli, L. Lovato, et al. (2005). An N-glucosylated peptide detecting disease-specific autoantibodies, biomarkers of multiple
- Matsuura K., Ohara K., Munakata H., Hifumi E., Uda T. Pathogenicity of catalytic antibodies: catalytic activity of Bence Jones proteins from myeloma patients with renal impairment can elicit cytotoxic effects. *Biol. Chem.* 2006, 387(5):543-548
- Mets, B., Winger, G., Cabrera, C., Seo, S., Jamdar, S., Yang, G., Zhao, K., Briscoe, R. J., Almonte, R., Woods, J. H. & Landry, D. W. (1998) *Proc. Natl. Acad. Sci. USA* 95, 10176–10181.
- Nathan C. Immunology. Catalytic antibody bridges innate and adaptive immunity. *Science* 2002, 298(5601):2143-2144
- Paul S, Li L, Kalaga R, Wilkins-Stevens P, Stevens FJ, Solomon A. Natural catalytic antibodies: peptide-hydrolyzing activities of Bence Jones proteins and VL fragment. *J Biol Chem* 1995;270:15257–61.
- Paul S, Nishiyama Y, Planque S, Karle S, Taguchi H, Hanson C & Weksler ME. (2005) Antibodies as defensive enzymes. *Springer seminars in immunopathology* Vol.26, No.4 (March 2005) ISSN 485-503. ;
- Paul S. (1998) Mechanism and functional role of antibody catalysis. *Applied biochemistry and biotechnology* Vol.75, No.1 (October 1998) ISSN 13-24
- Pillet D, PaonM, Vorobiev II, Gabibov AG, Thomas D, Friboulet A. Idiotypic network mimicry and antibody catalysis: lessons for the elicitation of efficient anti-idiotypic protease antibodies. *J Immunol Methods* 2002;269:5–12.
- Ponomarenko NA, Aleksandrova ES, Vorobiev II, Durova OM, Kozyr AV, Kolesnikov AV, et al. (2000) Natural antibody catalytic activities in mice with autoimmune disorders. *Doklady biochemistry : proceedings of the Academy of Sciences of the USSR* Vol.375 (November 2000) ISSN 224-7
- Ponomarenko, N. A., O. M. Durova, I. I. Vorobiev, A. A. Belogurov, Jr., I. N. Kurkova, A. G. Petrenko, G. B. Telegin, S. V. Suchkov, S. L. Kiselev, M. A. Lagarkova, et al. 2006. Autoantibodies to myelin basic protein catalyze site-specific degradation of their

- antigen. *Proceedings of the National Academy of Sciences of the United States of America*. Vol.103, No.2 (January 2006) ISSN 281-6
- Raso, V. & Stollar, B. D. (1975) *Biochemistry* 14, 591-599.
- Raz E, Ben-Bassat H, Davidi T, Shlomai Z, Eilat D.(1993) Cross-reactions of anti-DNA autoantibodies with cell surface proteins. *European journal of immunology*. Vol.23, No.2 (February 1993) ISSN 383-90.
- Reindl, M., Linington, C., Brehm, U., Egg, R., Dilitz, E., Deisenhammer, F., Poewe, W. & Berger, T. (1999) Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study. *Brain* Vol.122, No.11 (November 1999) ISSN 2047-56.
- Rekvig OP, Kalaaji M, Nossent H. (2004) Anti-DNA antibody subpopulations and lupus nephritis. *Autoimmunity reviews*. Vol.3, No.2 (February 2004) ISSN 1-6;
- Sashchenko L.P., Khaidukov S.V., Kozyr A.V., Luk'yanova T.I., Gabibov A.G., Suchkov S.V., Bobik T.V., Alekberova Z.S., Gnuchev N.V. Caspase-dependent cytotoxicity of anti-DNA autoantibodies. *Dokl. Biochem. Biophys.* (in russian) 2001, 380:313-319
- sclerosis. *Proceedings of the National Academy of Sciences of the United States of America* Vol.102, No.29 (July 2005) ISSN 10273-8.
- Steinman L, (1996) Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* Vol.85, No.3 (May 1996) ISSN 299-302
- Suchkov S.V., Gabibov A.G., Alekberova Z.S., Gnuchev N.V. DNA-abzymes and their clinical significance in systemic lupus erythematosus. *Ter. Arkh.* (in russian) 2001, 73:58-65
- Tellier C. Exploiting antibodies as catalysts: potential therapeutic applications. *Transfus. Clin. Biol.* 2002, 9(1):1-8
- Tomassini, V., L. De Giglio, M. Reindl, P. Russo, I. Pestalozza, P. Pantano, T. Berger, and C. Pozzilli. (2007). Anti-myelin antibodies predict the clinical outcome after a first episode suggestive of MS. *Multiple sclerosis : clinical and laboratory research* Vol.13, No.9 (November 2007) ISSN 1086-94
- Tramontano, A., Janda, K. D. & Lerner, R. A. (1986) *Science* 234, 1566-1570.
- Viau, M. & Zouali, M. (2005) *Clin. Immunol.* 114, 17-26.
- Wentworth P. Jr. Evidence for antibody-catalyzed ozone formation in bacterial killing and inflammation. *Science* 2002, 296(5576):2247-2249
- Wentworth, P., Jr., and K. D. Janda. (2001). Catalytic antibodies: structure and function. *Cell biochemistry and biophysics*. Vol.35, No.1 (2001) ISSN 63-87.
- Wentworth, P., Jr., L. H. Jones, A. D. Wentworth, X. Zhu, N. A. Larsen, I. A. Wilson, X. Xu, W. A. Goddard, III, K. D. Janda, A. Eschenmoser, and R. A. Lerner. (2001). Antibody catalysis of the oxidation of water. *Science* Vol.293, No.5536 (September 2001) ISSN 1806-11
- Wentworth, P., Jr., McDunn, J. E., Wentworth, A. D., Takeuchi, C., Nieva, J., Jones, T., Bautista, C., Ruedi, J. M., Gutierrez, A., Janda, K. D., et al. (2002) *Science* 298, 2195-2199.
- Wilkinson RW, Lee-MacAry AE, Davies D, Snary D, Ross EL. (2001) Antibody-dependent cell-mediated cytotoxicity: a flow cytometry-based assay using fluorophores. *Journal of immunological methods* Vol.258, No.1-2 (December 2001) ISSN 183-91
- Zhou Y.X., Karle S., Taguchi H., Planque S., Nishiyama Y., Paul S. Prospects for immunotherapeutic proteolytic antibodies. *J. Immunol. Methods* 2002, 269:257-268



## **Autoimmune Disorders - Current Concepts and Advances from Bedside to Mechanistic Insights**

Edited by Dr. Fang-Ping Huang

ISBN 978-953-307-653-9

Hard cover, 614 pages

**Publisher** InTech

**Published online** 14, November, 2011

**Published in print edition** November, 2011

Autoimmune disorders are caused due to break down of the immune system, which consequently fails in its ability to differentiate "self" from "non-self" in the context of immunology. The diseases are intriguing, both clinically and immunologically, for their diversified clinical phenotypes and complex underlying immunological mechanisms. This book offers cutting-edge information on some of the specific autoimmune disease phenotypes, respective diagnostic and prognostic measures, classical and new therapeutic options currently available, pathogenesis and underlying mechanisms potentially involved, and beyond. In the form of Open Access, such information is made freely available to clinicians, basic scientists and many others who will be interested regarding current advances in the areas. Its potential readers will find many of the chapters containing in-depth analysis, interesting discussions and various thought-provoking novel ideas.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Dmitry Kostyushev, Dmitry Gnatenko, Mikhail Paltsev, Aleksandr Gabibov and Sergey Suchkov (2011). Antibody-Proteases in the Pathogenesis of Autoimmune Demyelination and Monitoring Patients with Multiple Sclerosis, *Autoimmune Disorders - Current Concepts and Advances from Bedside to Mechanistic Insights*, Dr. Fang-Ping Huang (Ed.), ISBN: 978-953-307-653-9, InTech, Available from: <http://www.intechopen.com/books/autoimmune-disorders-current-concepts-and-advances-from-bedside-to-mechanistic-insights/antibody-proteases-in-the-pathogenesis-of-autoimmune-demyelination-and-monitoring-patients-with-mult>

**INTeCH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen