Catalytic Autoantibodies in Multiple Sclerosis: Pathogenetic and Clinical Aspects

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The significance of catalytic autoantibodies abzymes in the pathogenesis of multiple sclerosis was evaluated in patients with different disease patterns and severity of disability.

Key Words: *multiple sclerosis; catalytic autoantibodies; abzymes; protoabzymes; DNA abzymes*

The problem of creating serological test systems for the diagnosis and prediction of the course and outcome of multiple sclerosis (MS) remains unsolved. A vast family of autoantibodies (autoAB) to myelin components plays an important role in the pathogenesis of MS [6,9,10,13]. Natural autoAB — abzymes (autoAB with catalytic activity) are detected at the early (preclinical) stages of autoimmune diseases and are actively involved in their pathogenesis [2,3,11].

We compared the content and levels of catalytic autoAB in the sera collected from patients with MS.

MATERIALS AND METHODS

Sera from 68 MS patients (evaluation of disability degree and diagnostic criteria are described previously [12]) were collected in Moscow Regional Research and Clinical Institute (Table 1).

Salts, agarose, acrylamide, enzymes, FITC, substrates, adsorbents, labeled AB, and other reagents (Pharmacia-LKB, Sigma, Bio-Rad, Merck, Life Technologies, *etc.*), nitrocellulose (NC) membranes (Amersham), and radioactive isotopes [32P]-γ-ATP (Izotop Firm) were used.

Polyclonal IgG were isolated from the sera by column chromatography [1,2]. Activity of AB preparations was evaluated by proteolysis of nonspecific BSA and specific myelin basic protein (human MBP) substrates.

Serum concentrations of anti-MBP autoAB, anti-MOG (myelin oligodendrocytic glycoprotein), and anti-MAG (myelin-associated glycoprotein) were evaluated by enzyme immunoassay.

MBP-binding autoAB were isolated from the sera by affinity chromatography on immobilized MBP [1,4].

Antibodies with high proteolytic activity were isolated from total AB preparations by fractionation on adsorbent with affinity ligand (aprotinin) [1,4].

Blood samples were screened for DNA abzymes by a previously developed method of affinity adsorption with evaluation of DNA-hydrolyzing activity in AB preparations [8].

RESULTS

Autoantibodies presented by a heterogeneous group of immunoglobulins were detected in the liquor and peripheral blood proteins of MS patients [7,9,13,14].

Preparations of anti-MBP autoAB from 42 of 68 sera (62%) possessed proteolytic activity towards BSA, which was seen from fluorescence shifts (surpassing 250-300% in some patients) in comparison with the control (Fig. 1).

Further studies were carried out on fractions containing anti-MBP autoAB with high proteolytic activity obtained by affinity adsorption on immobilized

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TABLE 1. Clinical Characteristics of MS Patients

Parameter/criterion of disease	Number of patients	Percentage
Number of patients	68	100
females	22	32.4
males	46	67.6
Disease duration, years		
<1	14	20.5
1-5	38	55.9
5-10	10	14.7
>10	6	8.9
Patient age, years		
<20	7	10.3
20-30	37	54.4
30-40	14	20.6
>40	10	14.7
EDSS index, score		
<3.0	44	64.8
3.5-5.0	12	17.6
>6.0	12	17.6
Disease pattern		
remittent	31	45.6
remittent progressive	14	20.6
primary progressive	12	17.6
secondary progressive	11	16.2
Disease stage		
exacerbation	28	41.2
remission	28	41.2
stabilization	12	17.6

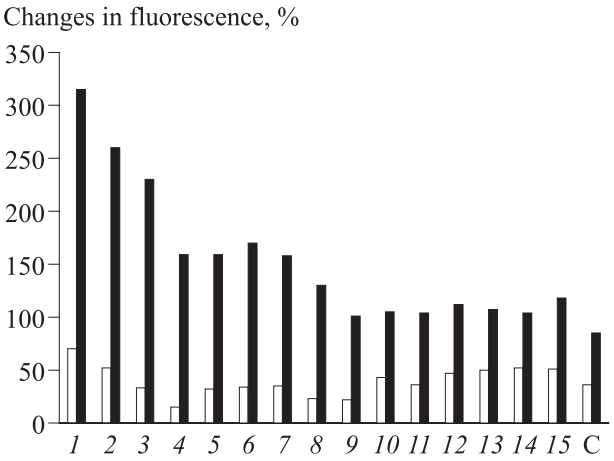

Fig. 1. Nonspecific proteolytic activity of anti-MBP autoantibodies from the sera of patients with multiple sclerosis (MS), recorded by the fluorescent method. C: control sample (BSA-FITC without antibodies) incubated under the same conditions; *1-15*: patients' numbers.

aprotinine. Preincubation of these AB with polyclonal rabbit anti-IgG led to complete disappearance of proteolytic activity, while AB specifically recognizing variable domain of human IgM had virtually no effect on this activity. The catalytic nature of proteolytic activity was confirmed in special studies with protease inhibitors [1,4].

In MS patients proteolytic activity of anti-MBP autoAB is similar to proteins hydrolysis with trypsin (Fig. 2, track *8*; Fig. 3). Activity towards MBP [7,9] was completely suppressed after preincubation with immobilized antispecies AB (data not presented), which confirms catalytic nature of detected activity and rules out the risk of enzyme contamination. IgG isotype AB from donor sera (Fig. 2, track *7*) exhibited no proteolytic activity towards MBP.

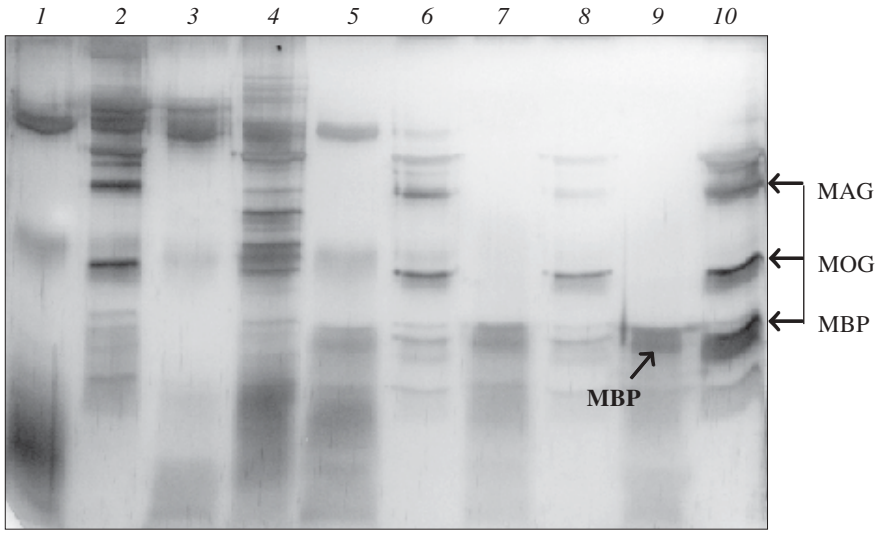

Fig. 2. Hydrolysis of myelin basic protein (MBP) with IgG autoantibodies from the sera of MS patients. Sera: tracks *1-6*) MS patients; *7*) donor; *8*) MBP hydrolysis by trypsin; *9-10*) antigen (MBP and MAG+MOG+MBP, respectively) incubated without antibodies.

TABLE 2. Clinical Immunological Parameters of MS Patients

Group	Sex		Pattern				Stage			EDSS, score
	male	female	R	RP	PP	SP	Rm	E	St	
With protoabzyme catalytic activity (*n*=50; 100%)	34 (68%)	16 (32%)	16 (32%)	10 (20%)	16 (32%)	8 (16%)	12 (24%)	26 (52%)	12 (24%)	4.5-5.0
Without protoabzymes (*n*=18; 100%)	12 (66.7%)	6 (33.3%)	14 (77.8%)	4 (22.2%)			16 (88.9%)		2 (11.1%)	1.5
With DNA abzyme catalytic activity (*n*=40; 100%)	26 (65%)	14 (35%)	10 (25%)	10 (25%)	12 (30%)	8 (20%)	10 (25%)	22 (55%)	8 (20%)	3.5-4.0
Without DNA abzymes (*n*=28; 100%)	22 (78.6%)	6 (21.4%)	20 (71.3%)	4 (14.3%)	2 (7.2%)	2 (7.2%)	18 (64.3%)	6 (21.4%)	4 (14.3%)	2.0
With proto- and DNA abzyme catalytic activities (*n*=34; 100%)	20 (58.8%)	14 (41.2%)	6 (17.6%)	8 (23.6%)	14 (41.2%)	6 (17.6%)	4 (11.7%)	22 (64.7%)	8 (23.6%)	(4.0-4.5)
Without proto- and DNA abzymes (*n*=12; 100%)	8 (66.7%)	4 (33.3%)	10 (83.3%)			12 (16.7%)	10 (83.3%)		2 (16.7%)	1.0

Note. R: remittent; RP: remittent progressive; PP: primary progressive; SP: secondary progressive ; Rm: remission; E: exacerbation; St: stabilization.

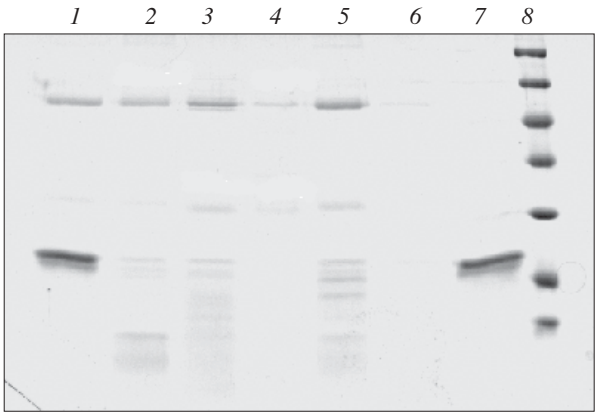

Fig. 3. Specific proteolysis of MBP by anti-MBP autoIgG from the sera of MS patients. Tracks *2*, *4*) antibodies from MS patients bound to MBP adsorbent; *1*, *3*) antibodies from MS patients not bound to MBP adsorbent; *5*, *6*) summary fractions of IgG antibodies; *7*) homogenous MBP preparation.

MBP-specific proteolytic activity associated with anti-MBP autoAB was detected in 73.5% patients with MS (in 50 of 68 patients).

Antimyelin autoAB (IgG isotype) were previously detected in the blood of MS patients [1]. The incidence of autoAB is the highest in patients with secondary progressive (88%) and remittent (62%) MS. Analysis revealed a correlation between serum concentration of antimyelin autoAB and levels of specific proteolytic activity in anti-MBP autoAB preparations. No correlation was detected between autoAB concentrations and levels of nonspecific proteolytic activity in AB preparations from MS patients.

Autoantibodies with low avidity, polyspecific by their nature, can provoke the development of syndromal forms of the disease in MS patients; hence, these AB can be regarded as regulators of physiological functions essential for the maintenance of equilibrium between health and autoimmune disease [1,4].

Screening of sera (immunoenzyme assay) from MS patients for anti-DNA autoAB confirmed their presence in 15 of 68 patients (22%), 14 of these 15 patients (93%) were triple seropositive (anti-DNA, anti-MBP, and anti-MOG autoAB).

DNA-hydrolysing activity associated with DNA-binding autoAB was detected in 58.8% of MS patients (40 of 68 patients).

Catalytic activity was detected in 50 (73.5%) patients with MS; 34 (68%) of these suffered from progressive disease, remittent variant was diagnosed in 16 (32%), and 12 (24%) patients were in remission (Table 2).

No catalytic activity of protoabzymes was observed in 18 (26.5%) of 68 patients with appreciably lower disability index (EDSS score). No disease exacerbations were observed in this group (Table 2).

DNA-hydrolyzing activity associated with DNA-binding autoAB was detected in 40 (58.8%) patients

with MS. The disease ran a progressive course in 30 (75%) of these (Table 2).

No catalytic activity of DNA abzymes was detected in 28 (41.2%) patients with appreciably lower disability indexes according to the EDSS score. Remittent variant with remission stage was observed in the majority of these cases (Table 2).

Catalytic activity was detected in 34 (50%) patients with MS. The disease was progressive in 28 (56%) and remittent in 6 (17%) of these patients (Table 2).

No catalytic activity of protoabzymes and DNA abzymes was detected in 12 (17.6%) patients. The disease was remittent at the stage of remission in the majority of cases (Table 2).

The presence of high-affinity anti-MBP auto-IgG in the serum was paralleled by their high proteolytic activity towards the specific substrate, while the level of this activity correlated with serum titers of these AB and with the variants of MS course and severity of patient's disability.

Serum and liquor DNA abzymes in MS patients are truly informative object for the evaluation of the formation of signs of secondary syndromal abnormalities [2].

Functional activity of abzymes and their association with MS patterns confirm practical significance of these molecular instruments for monitoring of therapeutic measures and predicting of the disease course.

REFERENCES

1. I. I. Vorob'ev, N. A. Ponomarenko, A. V. Reshetnyak, *et al.*, *Zh. Mol. Med.*, **3**. 48-55 (2004).
2. T. E. Naumova, O. M. Durova, A. G. Gabibov, *et al.*, *Zh. Nauch. Prakt. Revmatol.*, No. 1, 11-14 (2003).
3. N. A. Ponomarenko, A. M. Durova, I. I. Vorob'ev, *et al.*, *Dokl. Akad. Nauk*, **395**, 120-123 (2004).
4. S. V. Suchkov and A. G. Gabibov, *Zh. Allergol. Immunol.*, **2**, No. 1, 137-147 (2001).
5. A. G. Baranovskii, N. A. Ershov, V. N. Buneva, *et al.*, *Immunol. Lett.*, **76**, No. 3, 163-167 (2001).
6. M. Buntinx, P. Stinissen, P. Steels, *et al.*, *Crit. Rev. Immunol.*, **22**, Nos. 5-6, 391-424 (2002).
7. M. P. Burgoon, D. H. Gilden, and G. P. Owens, *Front. Biosci.*, **9**, 786-796 (2004).
8. A. G. Gabibov, G. V. Gololobov, O. I. Makarevich, *et al.*, *Appl. Biochem. Biotechnol.*, **47**, Nos. 2-3, 293-302 (1994).
9. D. A. Hafler, *J. Clin. Invest.*, **113**, No. 6, 788-794 (2004).
10. J. H. Noseworthy, C. Lucchinetti, M. Rodriguez, and B. G. Weinshenke, *N. Engl. J. Med.*, **343**, No. 13, 938-952 (2000).
11. N. A. Ponomarenko, O. M. Durova, I. I. Vorobiev, *et al.*, *J. Immunol. Methods*, **269**, Nos. 1-2, 197-211 (2002).
12. C. M. Poser, D. W. Paty, L. Scheinberg, *et al.*, *Ann. Neurol.*, **13**, 227-231 (1983).
13. L. Steinman and S. Zamvil, *Nat. Rev. Immunol.*, **3**, No. 6, 483-492 (2003).
14. F. Waldron-Lynch and M. J. Hennesy, *Ir. Med. J.*, **97**, No. 1, 5-6 (2004).