Research Article

Journal of Immunology (ISSN 2995-861X)

Autoantibodies-Abzymes with Amylase Activity in Experimental Autoimmune Encephalomyelitis Mice

Andrey E. Urusov, Kseniya S. Aulova and Georgy A. Nevinsky[1*]

[1]Institute of Chemical Biology and Fundamental Medicine, SB of the Russian Academy of Sciences, Russia, Novosibirsk.

Corresponding author

Prof. Georgy A. Nevinsky, Institute of Chemical Biology and Fundamental Medicine, SB of the Russian Academy of Sciences, Lavrentiev Ave., 8, Novosibirsk 630090, Russia.
Tel : +7-383-3635126,
Fax : +7-383-3635153,
Email : nevinsky@niboch.nsc.ru

Received Date : May 06, 2024
Accepted Date : May 07, 2024
Published Date : June 07, 2024

ABSTRACT

The exact mechanisms of multiple sclerosis evolution are still unknown. However, the progress in C57BL/6 mice of experimental autoimmune encephalomyelitis (EAE, similar to human multiple sclerosis) happens as a result of the contravention of bone marrow hematopoietic stem cells differentiation profiles integrated with the production of toxic for human's auto-antibodies-abzymes splitting myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), histones, DNA and RNA as well as IgGs with catalase and peroxidase activities. Here, we first analyzed the change in the relative amylase activity of IgGs from C57BL/6 mice blood over time at different stages of the EAE evolution: onset, acute, and remission phases. It was shown that the amylase activity of IgGs during spontaneous development of EAE first noticeably increases from 50 to 80, then sharply decreases to 100-130, and again sharply increases to 150 days of mice life. Immunization of 3-month-old mice (time zero) with the DNA-histone complex leads to a decrease, while their treatment with MOG results in a substantial increase in the amylase activity of IgGs. The relative activities of all IgG-abzymes indicated above corresponding to various stages of EAE development were compared. The data shows that spontaneous EAE and immunization of mice with DNA-histones complex and MOG leads to a different and very expanded formation of various B lymphocytes, producing abzymes with several diverse catalytic activities. In addition, the relative number of lymphocytes producing diverse antibodies with distinct enzymatic activities (including their relative activities) significantly depend both on the stages of spontaneous and antigen-induced accelerations of EAE development.

Keywords : C57BL/6 mice; EAE model; Catalytic antibodies; amylase activity

INTRODUCTION

Various abzymes (Abzs) against chemically stable analogs of transition states of many chemical reactions catalyzing more than 200 various reactions are obtained, and they are novel important enzymes (for review, see (1-7)). Natural catalytic auto-Abzs degrading polysaccharides, nucleic acids, different oligopeptides, and proteins were detected in the blood of patients with several autoimmune diseases (AIDs; for review, see (8-25)).

Multiple sclerosis (MS) is chronic demyelinating central nervous system (CNS) pathology. The MS etiology is still unclear, and the widely used theory of its pathogenesis presumes a leading role of inflammation associated with autoimmune reactions leading to myelin destruction (26-30).

Several experimental autoimmune encephalomyelitis (EAE) mice models exist that mimic specific features of human MS (for a review, see (31-40)), including C57BL/6. EAE evolution in C57BL/6 mice proceeds spontaneously and has a chronic-progressive course. Different Abs-abzymes were usually obtained with a dramatically higher incidence in autoimmune-prone mice strains than in conventionally used control mice strains (41,42). Some AIDs were supposed first may be a consequence of defects of hematopoietic stem cells (HSCs) (43). The spontaneous and accelerated by mice immunization with DNA of SLE-prone MRL-lpr/lpr mice progress of systemic lupus erythematosus (SLE) (23-25, 44) and experimental autoimmune encephalomyelitis in EAE- C57BL/6 mice (23-25,
45-48), has been shown later are conditioned by a specific reorganization of bone marrow HSCs. In AIDs, the immune system contravention-defects bound with distinct-specific changes in the bone marrow profile of HSCs differentiation, which is associated with the production of particular catalytic antibodies-abzymes hydrolyzing nucleic acids, histones, pep-

Journal of Immunology (ISSN 2995-861X)

tides, proteins, and polysaccharides, (17,23-25,44-48). With some exceptions, abzymes with DNase, RNase, and protease catalytic activities are absent in the blood of conditionally healthy volunteers, or they usually have shallow activities (17,23-25,44-48). The auto-Abs-abzymes with several enzymatic activities were revealed as the first statistically significant and the earliest markers of many AIDs beginning and development (17,23-25, 44-48).

Free histones in the blood of animals are cytotoxic and stimulate the development of autoimmune diseases (49). Complexes of DNA with histones are the most important auto-antigens in production Abs and abzymes against DNA and histones (26), Anti-DNA Abs have been recently found as a major component of intrathecal IgGs in the brain and CSF cells of multiple sclerosis patients (50).

DNase and histone-hydrolyzing Abzs of SLE and multiple sclerosis patients are detrimental and cytotoxic because they induce cell apoptosis, which accelerates the evolution of autoimmune diseases (51,52). Abzs against myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) possessing protease activity in MS and SLE patients may attack specific proteins of the axons myelin-proteolipid sheath. Consequently, these auto-abzymes could also play a negative detrimental role in MS pathoC57BL/6 mice; genesis (23-25, 44-48).

EAE C57BL/6 mice were used recently to study possible mechanisms of spontaneous, DNA-histones complexes and MOG-accelerated development of EAE (23-25, 44-48). It was demonstrated that immunization of C57BL/6 mice with DNA-histones complexes and MOG (23-25, 44-48) accelerates EAE development. The acceleration is associated with specific changes in HSCs differentiation profiles, an increase in lymphocyte proliferation, and apoptosis repression in different mice organs (23-25,44-48). These changes in parallel are associated with the production of auto-Abzs splitting DNAs, RNAs, nucleotides, polysaccharides, proteins, and peptides (23-25, 44-48). After immunization of mice with MOG or DNA-histones, changes in HSCs differentiation profiles occur several times, corresponding to the onset of the pathology by 7-8 days (the appearance of abzymes), a sharp exacerbation in the acute phase at 18-20 days (maximum activity of abzymes) followed by a slow transition to the remission stage and a decrease in the activity of abzymes in the hydrolysis of all mentioned above substrates (23-25, 44-48). It was shown that during the spontaneous development of EAE, the levels of DNA, MBP, MOG, histones, and microRNA hydrolysis by Abzs increase slowly-gradually (44-48). The catalase activity of abzymes also increases slowly (47). However, immunization of mice with MOG or DNA-histone complex resulted in a sharp increase in the activity of all abzymes at periods of the onset and acute phases.

Amylases are enzymes catalyzing the degradation of glycosides in glycogen, starch, and dextrin (22,23). Amylases are widespread and present in almost all living organisms (53,54). According to the various ways of starch hydrolysis, they are divided into three majority types: α-amylases, β-amylases, and γ-amylases (53-57). 1,4-α-D-glucan-glucanohydrolase or α-amylase is the most common extracellular enzyme hydrolyzing starch and glycogen (55). Amylase plays a vital role in carbohydrate metabolism in vivo, and alpha-amylase was found in different organisms: bacteria (26), fungi (57), plants (27), and animals (58).

As mentioned above, abzymes with some activities were not found in the blood of apparently healthy donors (23-25, 44-48). Abzymes with amylase activity are an exception. Abzymes from healthy donors have been shown to have very low but reliably testable activity (17). At the same time, their activity increases dozens of times with the development of a number of autoimmune diseases, including SLE and multiple sclerosis (61-63). Interestingly, the relative activity of abzymes with amylase activity in the multiple sclerosis cerebrospinal fluids of patients is ~30 times higher than in the blood of the same patients (62). To date, in experimental animals predisposed to AIDs, antibodies with amylase activity have been studied only in SLE-prone MRL-lpr/lpr mice (23-25). It was shown that the amylase activity of IgGs slowly increases during spontaneous SLE development. It was interesting to analyze at what stages of AIDs development the production of Abzs with amylase activity can occur. Therefore, we used EAE-prone C57BL/6 mice, in which case it was possible to analyze changes in the amylase activity of IgGs during the onset, acute phase, and remission of this disease. Here, we investigated the amylase activity of IgGs from the blood of C57BL/6 mice corresponding to spontaneous, MOG- and DNA-histones complex accelerating the development of EAE.

MATERIALS AND METHODS

Reagents

All chemicals and an equimolar mixture of five histones (H9250) were from Sigma (St. Louis, MO, USA). Superdex 200 HR 10/30 (17-5175-01) and Protein G-Sepharose columns (17061801) were from GE Healthcare Life Sciences (New York, NY, USA). MOG35–55 (2568/1) was from EZBiolab (Munich, Germany). Maltoheptaose was from Boehringer Mannheim (0 139 041; Mannheim, Germany). These preparations were free from possible contaminants.

Experimental animals

C57BL/6 mice from 50 to 90 days of life (3-month-old) were grown in mice special vivarium using special free of any pathogens conditions of the ICG (Institute of Cytology and Genetics) (17-21). All analyses have been implemented following the ICG Bioethical Committee's protocols conforming

Journal of Immunology (ISSN 2995-861X)

to known specifications of working with animals of Directive 86/609/CEE of the European Communities Council. The Bioethical committee of the ICG approved our study. To analyze the changes in the amylase activity of antibodies during the spontaneous evolution of EAE, C57BL/6 mice from 50 to 150 days after birth were used. Immunization experiments were conducted using three-month-old mice (90 days of life; zero time).

Immunization of mice

In this article, we analyzed IgGs, which were used earlier by us for the investigation of different parameters characterizing the evolution of EAE in C57BL/6 mice before and after their treatment with MOG and DNA complex with five histones (H1-H4) (44-48). On the zero time (3-month-age mice), the mice were immunized with MOG or DNA-histones complex according to (44-48). For isolation of individual IgGs, 0.8-1 mL of the blood was collected at different times before and after mice treatment by decapitation using standard approaches (44-48). Methods of mice immunization were published earlier (44-48) and are described in more detail in Combined Supplementary data; Part 1, Immunization of mice).

IgG purification

Electrophoretically and immunologically homogeneous IgGs were obtained as in (17-21) by chromatography of animal's blood plasma proteins on Protein G-Sepharose; additional cleaning of IgGs was performed using FPLC gel filtration in harsh conditions (pH 2.6). For the protection of antibodies from possible contaminations, all samples were filtered using special 0.1 μm filters. Aliquots of solutions obtained were kept at -70o C before being used in different experiments. SDS-PAGE of IgGs for assay of homogeneity was performed using 5-15% gradient gels as in (44-48). All possible proteins after electrophoresis were visualized by gels silver staining (a more detailed method of IgGs purification is given in Part 2—IgG purification in Combined Supplementary data.

IgG preparations were separated by SDS-PAGE as in (44-48) to exclude possible artifacts due to hypothetically possible contaminating amylases. After SDS-PAGE was used to restore the amylase activity, SDS was removed by incubation of the gel for 3 h at 23°C with Tris-HCl buffer (pH 7.5) and washed 15 times with this buffer. The longitudinal strips of gel were cut into small pieces (2.0-3.0 mm), carefully pounded, and placed in centrifuge tubes containing 50 μL, Tris-HCl (pH 7.5). During 10 days all tubes kept at +4° C with periodic shaking. Then, gel particles were removed using centrifugation, and the supernatants were used to determine amylase activity. The parallel gel strips were used to detect the IgG's position by silver staining. This amylase activity was detected only in the band corresponding to intact IgGs, and there were no other protein bands and peaks of amylase activity.

Assay of amylase activity

The relative amylase activity of IgGs was determined using optimal conditions as in (44). The reaction solution (20 μl) containing 50 mM Tris-HCl, pH 7.5, 1 mM NaN3, 1.7 mM maltoheptaose, and 0.001 - 0.2 mg/mL of IgGs was incubated for 1-6 h at 37oC. Products of maltoheptaose hydrolysis were identified by thin layer chromatography on TLC Silica gel 60 W (HX68188587; Merck, Darmstadt, Germany) using 1-butanol-acetic acid-H2O (12:4:4). After TLC plates were air dried, then sprayed with a 5% H2SO4 solution in 1-propanol, and incubated at 110°C for 8 min. The activities of IgGs preparations were estimated from the scanning data as a d forms. All measurements were performed within the linear regions of the time courses and IgGs concentration curves. If the Abs activity was low (< 5-10 % of hydrolysis), the incubation time was increased to 5-20 h. If oligosaccharide degradation exceeded 40%, the concentration of IgGs preparations was decreased 2-10-fold depending on the sample analyzed. Finally, the relative catalytic activities were normalized to one mg/mL IgGs and 1 h of incubation.

Statistical analysis

All data are the mean and standard deviation of at least 2-3 independent experiments for each IgG sample, averaged over seven various mice of each group. A significance of differences (p) between groups was calculated using the Mann-Whitney test.

RESULT

Experimental groups of mice

The development of EAE results in the immune system of C57BL/6 mice specific reorganization associated with significant changes in the differentiation profile of mice HSCs and the increase in proliferation of lymphocytes in different organs (23-25,44-48). All these defects-changes in mice's immune systems lead to increased proteinuria and the production of catalytically active Abs hydrolyzing DNA, MBP, and MOG (23-25,44-48). It was interesting to see how these defects-changes in the immune system can affect possible alterations in IgG relative activities in the hydrolysis of oligosaccharides. Besides, it seemed essential to compare the overtime patterns of changes in the relative activity of Abs with amylase activity in comparison with abzymes hydrolyzing MBP, histones, MOG, RNA, and DNA during mice EAE development analyzed earlier (23-25,44-48).

In the study of amylase activity, we used homogeneous IgGs preparations containing no any canonical enzymes before and after immunization of C57BL/6 mice with MOG (45) and DNA-histones (46); these preparations were obtained and described in (45-48).

To demonstrate the processes of violation of the immune

Journal of Immunology (ISSN 2995-861X)

status in C57BL/6 mice, Supplementary integral data indicate overtime changes in a number of mice bone marrow BFU-E, CFU-E, CFU-GM, and CFU-GEMM colony forming units (Supplementary Figure S1), the relative amount of lymphocytes in bone marrow, spleen, thymus, and lymph nodes (Supplementary Figure S2) for untreated mice, as well as after their treatment with DNA-histones complex and MOG described in (45-48).

Amylase activity

The IgGs used in this work were obtained from the blood of C57BL/6 mice previously and described in (45-48). These Abs were electrophoretically homogeneous and were active in the hydrolysis of DNA, micro-RNA, MBP, and histones (45-48), as well as in the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 3,3'-diaminobenzidine (DAB), and some other substrates (63) and possess catalase activity (47). It was shown that all catalytic activities of these polyclonal antibodies are their own properties, and they do not contain admixtures of any canonical enzymes (45-48,60-63). It was interesting to see whether these antibodies exhibit amylase activity.

The amylase activity of IgGs was estimated from the efficiency of IgGs to hydrolyze maltoheptaose (MHO), according to (44). Figure 1 shows an analysis of MHO hydrolysis with 12 different IgG preparations using thin-layer chromatography.

Figure 1

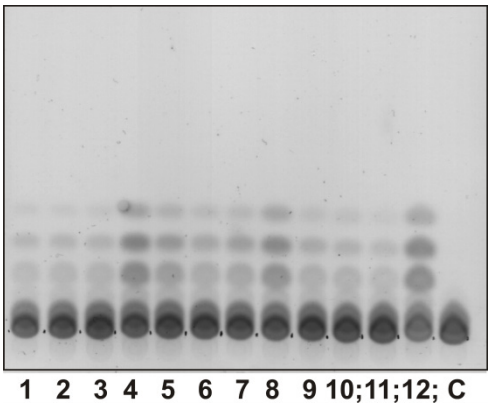

FIGURE 1. Typical examples of maltoheptaose hydrolysis by 12 different IgG preparations (0.1 mg/mL) using thin-layer chromatography. Line C corresponds to control – incubation of MHO without antibodies. The relative activity of antibodies was calculated from the percentage ratio of the hydrolysis products and the initial non-hydrolyzed MHO in every line.

Figure 2 demonstrates nine typical kinetic curves of MHO hydrolysis in the presence of nine different individual IgG preparations.

Figure 2

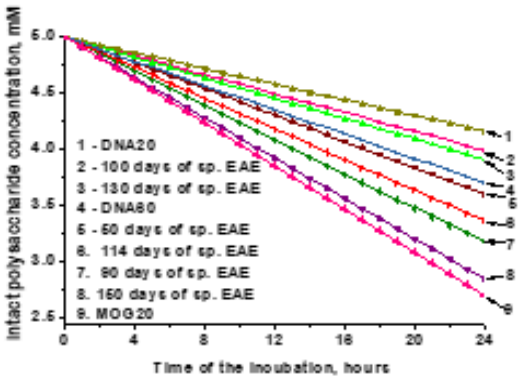

FIGURE 2. Typical examples of the time-dependences of MHO hydrolysis in the presence of 9 individual IgG preparations (0.1 mg/mL) corresponding to nine different groups of mice. All designations are given on the Panel. IgGs marked as 50-150 days sp. EAE corresponds to antibodies of spontaneous EAE development from 50 to 150 days. Individual IgGs labeled as DNA20 and DNA60 correspond to 20 and 60 days after mice treatment with DNA-histones complex, while MOG20 – 20 days after mice immunization with MOG.

Such kinetic curves were obtained for all individual IgG preparations corresponding to different groups of mice. All IgGs hydrolyze MHO but at different rates. To prove that the amylase activity belongs directly to IgGs and they do not contain any admixtures of canonical amylases, we used an equimolar mixture of 35 IgG preparations (IgGmix). After SDS-PAGE and SDS removal, the gel was cut into small fragments (2-3 mm), and all possible components of these fragments were eluted from the gel. The eluates obtained were used to analyze potential proteins capable of hydrolyzing MHO (Figure 3).

Figure 3

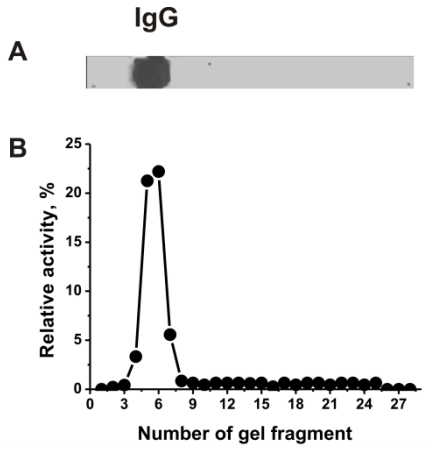

FIGURE 3. SDS-PAGE analysis of the electrophoretic homogeneity IgGmix corresponding to a mixture of 35 individual IgG preparations (19 μg) in 4–18% gradient gels followed by silver staining (A). After SDS-PAGE, the gels were incubated using the special solution to

Journal of Immunology (ISSN 2995-861X)

remove SDS and IgGs renaturation. The relative amylase activity was estimated using 15 µl extracts of 2.0-3.0-mm fragments of one longitudinal gel slice (B). The error from 2 independent experiments in the initial rate determination did not exceed 8–10%.

The amylase activity was detected only in the gel fragments corresponding to 150 kDa IgGmix. Since SDS dissociates all protein complexes, detecting the amylase activity only in the gels peaches of IgGmix intact and the absence of any other proteins and amylase activity peaks corresponding to other gels fragments gives the direct evidence that IgGmix preparation possesses intrinsic amylase activity (Figure 3). In time changes in the amylase activity during the development of EAE Previously, it was shown that significant changes in the profile of differentiation bone marrow stem cells begin in C57BL/6 mice at about three months of their life (zero time). In addition, only in 3-month-old mice were reliably detected abzymes of hydrolyzing DNA, MBP, MOG, and histones were revealed. Taking this into account, the spontaneous and MOG- or DNA-histones accelerated development of EAE in these mice was carried out using 3-month-old mice (90 days, zero time) (44-48). Later, in the study of micro-RNA hydrolyzing and catalase activity, mice were used from 50 to 150 days after birth (47,48). In this work, mice 50–150 days after delivery were also used to analyze amylase activity. The changes in relative amylase activity of individual IgGs during EAE spontaneous development were first analyzed. For further analysis, the averaged data corresponding to 7 mice in each group corresponding to 50-150 days of spontaneous EAE were used (Figure 4).

Figure 4

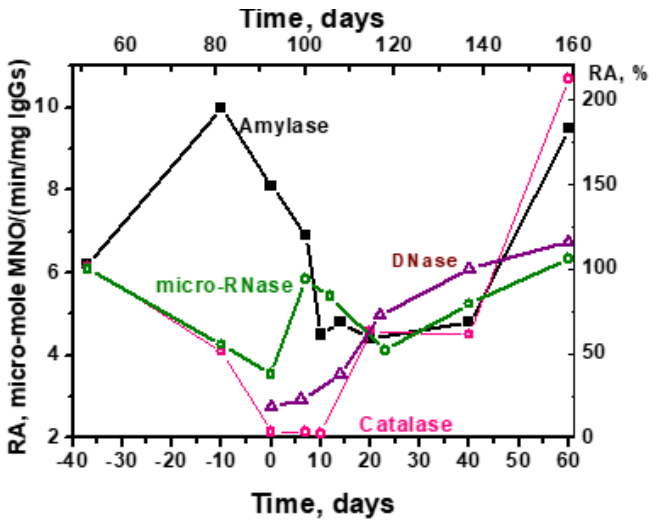

FIGURE 4. In time changes of an average amylase activity of IgG (7 mice in each group) during 50-150 days of spontaneous development of EAE (□; left scale). The upper scale shows the total number of experiment days, while the lower scale indicates the beginning of the experiments (zero time) with immunization of mice with MOG and the DNA-histones complex. For comparison, the Figure shows previously obtained data on in-time changes in catalase (o) (20), micro-RNase (o) (21), and DNase (Δ) (17) relative activities (RA) over time (all activities right scale). Because of the big difference between the enzymatic activities of antibodies in the catalysis of three various reactions, they are shown on the right scale in relative percent.

At 50 days of mice age, the blood of C57BL/6 mice contains IgG-abzymes with amylase activity (Figure 4). The spontaneous development of EAE during 60 days from 50 to 100 days after mice birth (upper scale) first leads to a 1.6-fold increase in amylase activity to 80 days ($p < 0.05$). Then, the activity remarkably decreased to 100 days and remained almost unchanged until 130 days of the mice's life. However, it rises sharply by 150 days of their life.

In this regard, it seemed interesting to compare the time course of changes in the amylase activity of antibodies with those found earlier for DNA-, MBP-, MOG-, micro-RNA-hydrolyzing, and catalase activities. To this aim on Figure 4 are summarized the data for these activities that were published earlier (44-48). Since the level of all activities is different, to compare the dynamics, all activities are given using relative percent. Interestingly, while amylase activity increases from 50 to about 80-90 days (time zero), catalase and miRNA hydrolyzing activities are significantly reduced. DNase activity, starting from 90 days (zero time), gradually increases up to 150 days (Figure 4, (45,46)). Similar gradual increases in activity during 90-150 spontaneous development of EAE were observed for MBP-, MOG, and histones-hydrolyzing and other activities (see, Supplementary Figures S3 and S4) (45-48). Wherein for amylase, catalase, and micro-RNA-hydrolyzing activities after zero time, complex dependences of their changes are observed. However, in general, starting from 90-100 days, they also increase to 150 days (Figure 4).

Three-month-old mice were immunized with MOG and DNA-histones complex (45-48). According to (23-25,31-33), 7-8 days after C57BL/6 mice treatment with MOG correspond to the onset, while 19-20 days to the acute phase of EAE evolution, when the stage of a remission begins later after about 25-30 days. By 18-20 days, DNase and protease activities are maximal (Supplementary Figure S3) (45,46), and they sharply decrease during the stage of remission (>25-30 days).

Figure 5 shows data on the relative amylase activity of IgG preparations before and after mice treatment with MOG and DNA-histones complex. Whereas spontaneous development by day 20 after zero time first results in a ~1.8-fold decrease in amylase activity ($p < 0.05$), by day 60 it increases 1.2 times. Interestingly, at 20 days after immunization of mice with a DNA-histones complex, the amylase activity of antibodies is 2.3 times, while at 60 days 1.4-fold lower than at time zero. Treatment of mice with a DNA-histone complex leads to a different change in the differentiation profile of bone

Journal of Immunology (ISSN 2995-861X)

marrow stem cells than their immunization with MOG. 20 days after treatment of mice with MOG, the amylase activity of antibodies increases by 2.0 times ($p < 0.05$) and in 4.7 times ($p < 0.05$) in comparison with zero time and 110 days after spontaneous development of EAE, respectively (Figure 5).

Figure 5

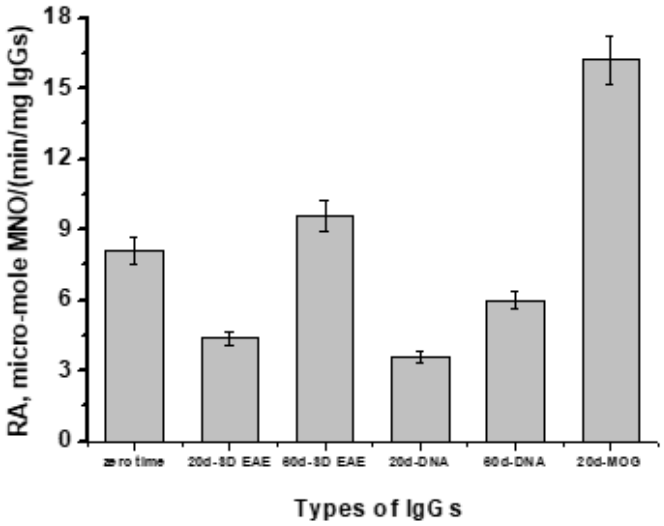

FIGURE 5. Relative average amylase activity IgGs of different mice groups: zero time (3-month-old mice), 20 (20d-SD EAE) and 60 (60d-SD EAE) days of spontaneous EAE development, 20 (20d-DNA) and 60 (60d-DNA) days after immunization of mice with DNA-histones complex and 20 days (20d-MOG) after mice treatment with MOG).

DISCUSSION

Different canonical enzymes, including amylases, are substantially present in various cells (53-59). Their activities in the blood are mostly low because they quickly lose their catalytic activities in the blood (64,65). Different IgGs are stable molecules in the blood; they are usually intact for several months (66). It cannot be ruled out that abzymes with amylase activity may play a specific role in the pathogenesis of autoimmune diseases. This hypothesis is supported by data on the appearance of antibodies with amylase activity in MRL-lpr/lpr mice during their spontaneous and DNA-accelerated development of SLE (44). In addition, the cerebrospinal fluid of MS patients contains antibodies, amylase activity of which is 30 times higher than in the blood of the same patients (62). Thus, a change in the differentiation profile of stem cells leads to the appearance in MS patients of bone marrow lymphocytes, which can efficiently hydrolyze oligosaccharides. In this study, it was first shown that the blood of EAE mice contains amylase antibodies, the relative activity of which changes greatly during the spontaneous development of EAE from 50 to 150 days of mice life (Figure 4). A comparison of amylase activity with catalase and micro-RNA-hydrolyzing activities from 50 to 90 days of mice life is particularly interesting. The patterns of changes in amylase, catalase, and micro-RNA-hydrolyzing activities before the immunization of mice are different (Figure 4). Interestingly, the spontaneous development of EAE leads to the testable appearance of DNase, MOG-, MBP, and histones-hydrolyzing activities at 3 months of mice life (90 days), which then gradually (almost linearly) increase up to 150 days as shown in Figure 4 in the example DNase activity. From 90 to 150 days of spontaneous development of EAE, there is an increase in amylase, catalase, and micro-RNA-hydrolyzing activities, but these dependencies are not gradual and demonstrate significant differences (Figure 4). One gets the impression that at about 3 months of mice's life, significant changes in the differentiation profile of bone marrow stem cells begin, which lead to the following development of EAE. However, these changes in the differentiation profile lead to the appearance of lymphocytes producing antibodies with DNase, amylolytic, catalase, MBP-, MOG-, histones-, and micro-RNA-hydrolyzing activities to some extent at different times. The activity of IgGs with catalase activity begins to increase significantly only at 100, while amylase activity after 130 days of mice life (Figure 4).

Interestingly, immunization of mice with DNA-histones complex and MOG has different effects of changing the amylase activity of antibodies. There is a significant decrease in the activity after immunization of mice with DNA-histones complex, while treatment of mice with MOG leads to an increase of amylase activity compared to that at zero time (Figure 5). Interestingly, the maximum level of increase in various abzyme activities after immunization of mice with MOG in comparison with that at zero time differed significantly (-fold): DNase (24.0), MBP- (4.4), MOG-hydrolyzing (5.3) (45), catalase (57.3) (47), hydrolysis of different micro-RNAs (1.8-3.1) (48), peroxidase 5.3 (63), and amylase (2.0, Figure 5). The question arises of how one can explain the appearance of abzymes with different activities during the spontaneous and MOG-induced development of EAE.

At first glance, immunization of mice with MOG should lead to the production of abzymes that hydrolyze MOG. At the same time, the immunization of mice with MOG and DNA-histones complex leads to an expanded formation of many different lymphocytes producing abzymes with at least several of very different catalytic activities. This may mean that each of these antigens can change the differentiation profile of bone marrow stem cells not unambiguously but in several somehow associated ways. At the same time, the relative number of lymphocytes producing antibodies with different enzymatic activities, including their relative activity, may depend both on the stage of spontaneous and on the used antigens for acceleration of EAE development. In this regard, it is useful to note previously obtained data. In the example of mice not prone to the development of AIDs (CBA and BALB/c), it was

shown that their immunization with DNA-histones complex does not change the profile of stem cell differentiation (44). However, the blood of such mice contains IgGs with significant DNase activity. Interestingly, their appearance is not associated with a violation of the differentiation profile of stem cells and is a consequence of the specific additional differentiation of bone marrow lymphocytes in different organs of mice (44). In addition, immunization of these mice resulted in the appearance of DNase abzymes but no other potential proteins, nucleotides, and polysaccharide substrates. Thus, the expansion of substrates for hydrolysis with antibodies is an exceptional feature of mice predisposed to the spontaneous development of AIDs. It should be assumed that the appearance of abzymes with amylase activity in C57BL/6 mice is also associated with an extended effect of MOG and DNA-histones complexes on differentiating bone marrow stem cells.

In principle, Abzs can be formed to different molecules mimic the transition state of different chemical reactions (1-8). In addition, in AIDs, anti-idiotypic abzymes can be induced by enzyme catalytic centers and may also show some of the characteristics of catalytic activities of parent enzymes (66-69). Thus, abzymes with catalase activity can be antibodies against the active centers of catalases of mammalian blood and cells. MS is at least a two-phase AID (74). The cascade of several reactions at the first inflammatory stage is very complex, including different enzymes, chemokines, cytokines, and many other compounds. The coordinated action of B and T cells, mediators of inflammation, complement system, and auto-antibodies leads to the formation of demyelination nidi and infringement of conductivity. The neurodegenerative stage of MS appearing later and it is directly associated with the patient's neural tissue destruction (74). At different stages of this pathology development, different types of changes in the differentiation profile of stem cells and catalases in complexes with different cells and blood molecules can induce the formation of antibodies with many different catalase activities.

CONCLUSIONS

C57BL/6 autoimmune-prone mice are characterized by the spontaneous and accelerated development of EAE after their Immunization with MOG and DNA-histones complex associated with changes in the differentiation profile of bone marrow stem cells, and production of abzymes with catalase, peroxidase activity, and hydrolyzing DNA, RNA, MBP, MOG, and histones. Specific defects-changes in the immune status of C57BL/6 mice during spontaneous development of EAE also lead to an increase in the amylase activity of autoantibodies. Immunization of mice with DNA-histones complex leads to a decrease while with MOG to increase in amylase activity of IgGs in comparison with zero time (3-month-old mice).

Supplementary Materials

The following are available online; Combined supplementary data including Supplementary Figures S1-S4 and Supplementary methods (Part 1, Immunization of mice and Part 2, IgG purification).

Acknowledgments

This study was mostly funded by the Russian Science Foundation (Grant number 22-15-00103); antibodies from the blood of healthy mice were isolated with the support from Russian State-funded budget project of ICBFM SB RAS 0245-2021-0009 (121031300041-4.

Disclosures

We declare that we have no conflicts of interest.

Abbreviations used : Abs: antibodies; auto-Abs, autoantibodies; BFU-E, erythroid burst-forming unit (early erythroid colonies); CFU-GM, granulocyte-macrophage colony-forming unit, CFU-E, erythroid burst-forming unit (late erythroid colonies) CFU-GEMM, granulocyte-erythroid-megacaryocyte-macrophage colony-forming unit; CNS, central nervous system; HSCs, hematopoietic stem cells; EAE, experimental autoimmune encephalomyelitis; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus.

REFERENCES

1. Lerner, R.A.; Tramontano, A. Antibodies as enzymes. Trends Bioch. Sci. 1987, 12, 427–438.

2. Stewart, J.D.; Benkovic, S.J. Recent developments in catalytic antibodies. Int. Rev. Immunol. 10, 229-240.

3. Martin, A.B.; Schultz, P.G. Opportunities at the interface of chemistry and biology. Trends Cell Biol. 1999, 9, 24-28.

4. Zhao, D.; Chen, J.; Hu, X.; Zhang, S. Catalytic Antibodies: Design, Expression, and Their Applications in Medicine. Appl. Biochem. Biotechnol. 2023, 195, 1514-1540.

5. Ostler, E.L.; Resmini, M.; Brocklehurst, K.; Gallacher, G.J. Polyclonal catalytic antibodies. Immunol. Methods. 2002, 269,111-124.

6. Padiolleau-Lefèvre, S.; Ben Naya, R.; Shahsavarian, M.A.; Friboulet, A.; Avalle, B. Catalytic antibodies and their applications in biotechnology: state of the art. Biotechnol. Lett. 2014, 36, 1369-1379.

7. Hanson, C.V.; Nishiyama, Y.; Paul, S. Catalytic antibodies and their applications. Curr. Opin. Biotechnol. 2005, 16, 631-636.

8. Paul, S. Natural catalytic antibodies Mol. Biotechnol. 1996, 5, 197-207.

9. Dimitrov, J.D.; Lacroix-Desmazes, S. Noncanonical functions of antibodies. Trends Immunol. 2020, 41, 379-393.

10. Wootla, B.; Lacroix-Desmazes, S.; Warrington, A.E.; Bieber, A.J.; Kaveri, S.V.; Rodriguez, M. Autoantibodies with enzymatic properties in human autoimmune diseases. J Autoimmun, 2011, 37, 144-50.

11. Kamalanathan, A.S.; Goulvestre, C.; Weill. B.; Vijayalakshmi, M.A. Proteolysis activity of IgM antibodies from Rheumatoid arthritis patients' sera: evidence of atypical catalytic site. Journal of Molecular Recogn., 2010, 23, 577-582.

12. Gabibov, A.G.; Ponomarenko, N.A.; Tretyak, E.B.; Paltsev, M.A.; Suchkov, S.V. Catalytic autoantibodies in clinical autoimmunity and modern medicine. Autoimmun. Rev. 2006, 5, 324-330.

13. Kozyr, A.V.; Gabibov, A.G. DNA-hydrolyzing Ab: is catalytic activity a clue for physiological significance? Autoimmunity. 2009, 42, 359-361.

14. Ponomarenko, N.A.; Vorobiev, I.I.; Alexandrova, E.S.; Reshetnyak, A.V.; Telegin, G.B.; Khaidukov, S.V.; Avalle, B.; Karavanov, A.; Morse, H.C 3rd, et al. Induction of a protein-targeted catalytic response in autoimmune prone mice: antibody-mediated cleavage of HIV-1 glycoprotein GP120. Biochemistry. 2006, 45, 324-330.

15. Belogurov, A. Jr.; Kozyr, A.; Ponomarenko, N.; Gabibov, A. Catalytic antibodies: balancing between Dr. Jekyll and Mr. Hyde. Bioessays. 2009, 31,1161-1171.

16. Keinan, E. (Ed) Catalytic antibodies, Wiley-VCH Verlag GmbH and Co. KgaA: Weinheim, Germany, 2005; pp. 1–586.

17. Savel'ev, A.N.; Eneyskaya, E.V.; Shabalin, K.A.; Filatov, M.V.; Neustroev, K.N. Antibodies with amylolytic activity. Protein Peptide Lett. 1999, 6, 179–181.

18. Paul, S.; Volle, D.J.; Beach, C.M.; Johnson, D.R.; Powell, M.J.; Massey, R.J. Catalytic hydrolysis of vasoactive intestinal peptide by human autoantibody. Science 1989, 244, 1158–1162.

19. Li, L; Paul, S.; Tyutyulkova, S.; Kazatchkine, M.D.; Kaveri, S.J. Catalytic activity of anti-thyroglobulin antibodies. J. Immunol. 1995, 154, 3328-332.

20. Kalaga, R.; Li, L.; O'Dell, J.R.; Paul, S. Unexpected presence of polyreactive catalytic antibodies in IgG from unimmunized donors and decreased levels in rheumatoid arthritis. J. Immunol. 1995, 155, 2695-2702.

21. Ponomarenko, N.A.; Durova, O.M.; Vorobiev, II.; Belogurov, A.A. Jr.; Kurkova, I.N.; Petrenko, A.G.; et al. Autoantibodies to myelin basic protein catalyze site-specific degradation of their antigen. Proc. Natl. Acad. Sci. U S A. 2006, 103,:281-286.

22. Shuster, A.M.; Gololobov, G.V.; Kvashuk, O.A.; Bogomolova. A.E.; Smirnov, I.V.; Gabibov, A.G. DNA hydrolyzing autoantibodies. Science 1992, 256, 665-667.

23. Nevinsky, G.A. Autoimmune processes in multiple sclerosis: Production of harmful catalytic antibodies associated with significant changes in the hematopoietic stem cell differentiation and proliferation. In Multiple sclerosis, Conzalez-Quevedo, A. Ed.; InTech: Rijeka, Croatia, 2016; pp. 100–147.

24. Nevinsky, G.A. Natural catalytic antibodies in norm and in autoimmune diseases, In Autoimmune Diseases: Symptoms, Diagnosis and Treatment, Brenner, K.J. Ed.; Nova Science Publishers Inc.: New York, NY, USA, 2010; pp. 1–107.

25. Nevinsky, G.A. The extreme diversity of autoantibodies and abzymes against different antigens in patients with various autoimmune diseases. Chapter in the book "Advances in Medicine and Biology" Nova Science Publishers, Inc.; New York, USA, 2021, 184, 1-130.

26. O'Connor, K.C.; Bar-Or, A.; Hafler, D.A. The neuroimmunology of multiple sclerosis: Possible roles of T and B lymphocytes in immunopathogenesis. J. Clin. Immunol. 2001, 21, 81–92.

27. Archelos, J.J.; Storch, M.K.; Hartung, H.P. The role of B cells and autoantibodies in multiple sclerosis. Ann. Neurol. 2000, 47, 694-706.

28. Hemmer, B.; Archelos, J.J.; Hartung, H.P. New concepts in the immunopathogenesis of multiple sclerosis. Nat.

Rev. Neurosci. 2002, 3, 291-301.

29. Niehaus, A.; Shi, J.; Grzenkowski, M.; et al. Patients with active relapsing-remitting multiple sclerosis synthesize antibodies recognizing oligodendrocyte progenitor cell surface protein: implications for remyelination. Ann. Neurol. 2000, 48, 362-371

30. Cross, A.H.; Trotter, J.L.; Lyons, J. B cells and antibodies in CNS demyelinating disease. J. Neuroimmunol. 2001, 112, 1-14.

31. Croxford, A.L.; Kurschus, F.C.; Waisman, A. Mouse models for multiple sclerosis: Historical facts and future implications. Bochim. Biophys. Acta 2011, 1812, 177-183.

32. Miller, S.D.; Karpus, W.J.; Davidson, T.S. Experimental A. Autoimmune Encephalomyelitis in the Mouse Curr Protoc Immunol. Chapter: Unit–15.1, 2007.

33. Mouse E.A.E. models. Overview and Model Selection. Hooke Laboratories, Inc.: Lawrence, MA, USA, 2011-2013.https://blog.crownbio.com/models-multiple-sclerosis (Published on June 15, 2018).

34. Klotz, L.; Kuzmanov, I.; Hucke, S.; Gross, C.C.; Posevitz, V.; Dreykluft, A.; Schulte-Mecklenbeck, A.; Janoschka, C.; et al. B7-H1 shapes T-cell-mediated brain endothelial cell dysfunction and regional encephalitogenicity in spontaneous CNS autoimmunity. Proc. Natl. Acad. Sci. USA 2016, 113, E6182-E6191.

35. Bettelli, E. Building different mouse models for human MS. Ann. NY Acad. Sci. 2007, 1103,11-18.

36. Bettelli; E.; Pagany, M.; Weiner, H.L.; Linington, C, Sobel, R.A.; Kuchroo. V.K. Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. J. Exp. Med. 2003, 197, 1073-1081.

37. Bettelli, E.; Baeten, D.; Jäger, A.; Sobel, R.A.; Kuchro, V.K. Myelin oligodendrocyte glycoprotein-specific T and B cells cooperate to induce a Devic-like disease in mice. J. Clin. Invest. 2006, 116, 2393–2402.

38. Krishnamoorthy, G.; Lassmann, H.; Wekerle, H.; Holz, A. Spontaneous opticospinal encephalomyelitis in a double-transgenic ouse model of autoimmune T cell/B cell cooperation. J. Clin. Invest. 2006, 116, 2385–2392.

39. Zhan, J.; Kipp, M.; Han, W.; Kaddatz, H. Ectopic lymphoid follicles in progressive multiple sclerosis: From patients to animal models. Immunology 2021, 164, 450–466.

40. Ransohoff, R.M. A mighty mouse: Building a better model of multiple sclerosis. J. Clin. Invest. 2006, 116, 2313–2316.

41. Nishi,Y. Evolution of catalytic antibody repertoire in autoimmune mice. J. Immunol. Methods. 2002, 269, 213-233.

42. Tawfik, D.S.; Chap, R.; Green, B.S.; Sela, M.; Eshhar, Z. Unexpectedly high occurrence of catalytic antibodies in MRL/lpr and SJL mice immunized with a transition-state analog: is there a linkage to autoimmunity? Proc Natl Acad Sci USA. 2002, 92, 2145-2149.

43. Ikehara, S.; Kawamura, M.; Takao, F. Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells. Proc.Natl. Acad. Sci. USA 1990, 87, 8341-8344.

44. Andryushkova, A.A.; Kuznetsova, I.A.; Orlovskaya, I.A.; et al. Antibodies with amylase activity from the sera of autoimmune-prone MRL/MpJ-lpr mice. FEBS Lett. 2006, 580, 5089-95.

45. Doronin, V.B.; Parkhomenko, T.A.; Korablev, A.; Toporkova, L.B.; Lopatnikova, J.A.; Alshevskaja, A.A.; Sennikov, S.V.; Buneva, V.N.; Budde, T.; Meuth, S.G.; et al. Changes in different parameters, lymphocyte proliferation and hematopoietic progenitor colony formation in EAE mice treated with myelin oligodendrocyte glycoprotein. J. Cell Mol. Med. 2016, 20, 81–94.

46. Aulova, K.S.; Toporkova, L.B.; Lopatnikova, J.A.; Alshevskaya, A.A.; Sedykh, S.E.; et al. Changes in cell differentiation and proliferation lead to production of abzymes in EAE mice treated with DNA-Histone complexes. J. Cell Mol. Med. 2018, 22, 5816-5832.

47. Urusov, A.E.; Tolmacheva, A.S.; Aulova, K.S.; Nevinsky, G.A. Autoantibody-Abzymes with Catalase Activity in Experimental Autoimmune Encephalomyelitis Mice. Molecules 2023, 28, 1330.

48. Nevinsky, G.A.; Urusov, A.E.; Aulova, K.S.; Ermakov, E.A. Experimental Autoimmune Encephalomyelitis of Mice: IgGs from the Sera of Mice Hydrolyze miRNAs. Int. J. Mol Sci. 2023, 24, 4433.

49. Chen, R.; Kang, R.; Fan, X.-G.; Tang, D. Release and

activity of histone in diseases. Cell Death. Dis. 2014, 5, e1370

50. Williamson, R.A.; Burgoon, M.P.; Owens, G.P.; Ghausi, O.; Leclerc, E.; Firme, L.; et al. Anti-DNA antibodies are a major component of the intrathecal B cell response in multiple sclerosis. Proc. Natl. Acad. Sci. USA 2001, 98, 1793-1798.

51. Sinohara, H.; Matsuura, K. Does catalytic activity of Bence-Jones proteins contribute to the pathogenesis of multiple myeloma? Appl. Biochem. Biotechnol. 2000, 83, 85-92.

52. Kozyr, A.V.; Kolesnikov, A.V.; Aleksandrova, E.S. et al. Autoantibodies to nuclear antigens, correlation between cytotoxicity and DNA-hydrolyzing activity. Appl. Biochem. Biotechnol. 1998, 75, 45-61.

53. Janeček, Š.; Svensson, B.; MacGregor, E.A. α-Amylase: an enzyme specificity found in various families of glycoside hydrolases. Cell Mol. Life Sci. 2014, 71, 1149-1170.

54. Whitcomb, D.C.; Lowe, M.E. Human pancreatic digestive enzymes. Dig. Dis. Sci. 2007, 52, 1-17.

55. Gupta, R.; Gigras, P., Mohapatra, H.; Goswami, V.K.; Chauhan, B. Microbial alpha-amylases: a biotechnological perspective. Process Biochemistry. 2003, 38, 1599–1616.

56. Palva, I. Molecular cloning of a-amylase gene from Bacillus amyloliquefaciens and its expression in B. subtilis. Gene. 1982, 19, 81–87.

57. Tsukagoshi, N.; Furukawa, M.; Nagaba, H.; Kirita, N.; Tsuboi, A.; Udaka, S. Isolation of a cdna-encoding aspergillus-oryzae taka-amylase-a—evidence for multiple related genes. Gene. 1989, 84, 319–327.

58. Rogers, J.C.; Milliman, C. Isolation and sequence analysis of a barley alpha-amylase cDNA clone. J. Biol. Chem. 1983, 258, 8169–8174.

59. Schibler, U.; Pittet, A.; Young, R.A.; Hagenbuechle, O.; Tosi, M.; Gellman, S.; et al. The mouse a-amylasemulti-gene family: sequence organization of members expressed in the pancreas, salivary gland and liver. Mol. Biol. 1982, 155, 247–266.

60. Neustroev, K.N.; Ivanen, D.R.; Kulminskaya, A.A.; Brumer, I.H.; Saveliev, A,N.; et al. Amylolytic activity and catalytic properties of IgM and IgG antibodies from patients with systemic lupus erythematosus. Human antibodies 2003, 12, 31-34.

61. Saveliev, A.N.; Ivanen, D.R.; Kulminskaya, A.A.; Ershova, N.A.; Kanyshkova, T.G.; et al. Amylolytic activity of IgM and IgG antibodies from patients with multiple sclerosis. Immunol. Lett. 2003, 86, 291-297.

62. Doronin, V.B.; Parkhomenko, T.A.; Castellazzi, M.; Cesnik, E.; Buneva, V.N.; Granieri, E.; Nevinsky, G.A. Comparison of antibodies with amylase activity from cerebrospinal fluid and serum of patients with multiple sclerosis. PLoS ONE 2016, 11, e0154688.

63. Tolmacheva, A.S.; Nevinsky, G.A. Essential Protective Role of Catalytically Active Antibodies (Abzymes) with Redox Antioxidant Functions in Animals and Humans. Int. J. Mol. Sci. 2022, 23, 3898.

64. Zenkov, N.K.; Lankin, V.Z.; Men'shikova, E.B. Oxidative stress. Biochemical and pathophysiological aspects. MAIK, Nauka/Interperiodica, Moscow, 2001; pp. 3-343.

65. Frei, B.; Stoker, R.; Ames, B.N. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc. Natl. Acad. Sci. USA 1988, 85, 9748.

66. Correia, I.R. Stability of IgG isotypes in serum. MAbs 2010, 2, 221-232.

67. Friboulet, A.; Izadyar, L.; Avalle, B.; Roseto, A.; Thomas, D.; Abzyme generation using an anti-idiotypic antibody as the "internal image" of an enzyme active site. Appl. Biochem. Biotechnol. 1994, 47, 229-237; discussion 237-239.

68. Kolesnikov, A.V.; Kozyr, A.V.; Alexandrova, E.S.; Koralewski, F.; Demin, A.V.; Titov, M.I.; Avalle, B.; Tramontane, A.; Paul, S.; Thomas, D.; Gabibov, A.G.; Friboulet, A. Enzyme mimicry by the antiidiotypic antibody approach Proceedings of the National Academy of Sciences 13526-13531.

69. Ponomarenko, N.A.; Pillet, D.; Paon, M.; Vorobiev, I.I.; Smirnov, I.V.; Adenier, H.; Avalle, B.; Kolesnikov, A.V.; Kozyr. A.V.; Thomas. D.; Gabibov. A.G.; Friboule, A. Anti-idiotypic antibody mimics proteolytic function of parent antigen. Biochemistry. 2007, 46, 14598-1609.

70. Steinman, L. Multiple sclerosis: a two-stage disease. Nat. Immunol. 2001, 2, 2762-2764.