Chapter 4

Autoimmune Processes in Multiple Sclerosis: Production of Harmful Catalytic Antibodies Associated with Significant Changes in the Hematopoietic Stem Cell Differentiation and Proliferation

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Additional information is available at the end of the chapter

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Abstract

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system. MS pathogenesis is not clear. Destruction of myelin by inflammation caused by autoimmune reactions has been proposed. Interestingly, healthy humans usually do not develop abzymes (Abzs). It was shown that DNase and MBP-hydrolyzing Abzs are easily detectable at the beginning of autoimmune diseases (ADs) including MS, when concentrations of antibodies to autoantigens are not yet significantly increased and correspond to levels in healthy donors. In addition, the relative enzymatic activity of antibodies from cerebrospinal fluid (CSF) is ~50-fold higher than that from the sera of the same MS patients. Experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice, a model mimicking relevant aspects of human MS was used. During development of spontaneous and MOG35-55-induced EAE in C57BL/6 mice, a specific reorganization of the immune system of mice was observed. It leads to a condition which was associated with the generation of catalytically active IgGs-hydrolyzing DNA, myelin basic protein (MBP), and MOG. Production of Abzs was associated with increased proteinuria, leading changes in differentiation of mice bone marrow hematopoietic stem cells (HSCs) and an increase in proliferation of lymphocytes in bone marrow, spleen, and thymus as well as a significant suppression of cell apoptosis in these organs. Treatment of control non-autoimmune CBA mice with MOG led to the different differentiation and proliferation of HSCs comparing with EAE C57BL/6 mice. The treatment of EAE mice with cuprizone inducing demyelination lead to a significant decrease in the size of the brain corpus callosum, but do not significantly change the differentiation profile of HSCs differentiation when compared with untreated mice. It indicates that cuprizone treatment is associated with demyelination, but not autoimmune reactivity. The possible differences in immune system reorganizations

during preclinical phases of the disease, acute and late EAE, leading to production of different autoantibodies and Abzs as well other changes are discussed.

Keywords: catalytic antibodies, autoimmunity, hematopoietic stem cells, multiple sclerosis, cell differentiation, cell proliferation

1. Introduction

Classically, antibodies (Abs) have been characterized as proteins produced by the immune system, which have the sole function of binding other molecules, called antigens, and eliciting an immune response. In this classical conception, Abs act similarly to enzymes in specific binding to other molecules. However, in contrast to enzymes, they do not have the ability to catalyze chemical conversions of their bound partners. For the vast majority of Abs, this observation is correct. However, it was shown that antibodies against chemically stable analogues of the transition states of chemical reaction can possess different enzymatic activities [1–8]. These artificial catalytic Abs were termed "abzymes" (derived from antibody enzymes). Abzymes (Abzs) catalyzing more than 100 distinct chemical reactions are novel biological catalysts that attracted interest in recent years reviewed repeatedly [1–8].

The first example of natural Abzs was an IgG found in bronchial asthma patients, which hydrolyzed intestinal vasoactive peptide [9], the second Abz was an IgG with DNase activity in a systemic lupus erythematosus (SLE) patient [10], and the third was an IgG with RNase activity in an SLE patient [11]. Later, catalytic IgGs and/or IgAs, IgMs hydrolyzing different oligopeptides, proteins, DNA, RNA, nucleotides, and polysaccharides were detected in the sera of patients with several autoimmune diseases (ADs) and some viral pathologies (for review see [8, 12–21] and refs therein).

Some Abs and auto-Abs with different catalytic activities may be induced spontaneously by primary antigens and can have characteristics of the primary antigen, including the catalytic activity of idiotypic and/or anti-idiotypic Abs [8, 12–21]. Healthy humans usually do not develop catalytic Abs or their activities are very low. Detection of Abzs was shown to be the earliest indicator of development of different ADs [8, 12–21]. At the early stages of ADs, the repertoire of Abzs is usually relatively narrow, but it greatly expands with the progress of the disease, leading to the generation of catalytically diverse Abs with various activities and functions [8, 12–21]. Some Abzs are cytotoxic and can play an important negative role in the pathogenesis of different ADs, while positive roles have been also proposed for other Abzs [8, 12–21]. Abzs activities increase in association with a specific reorganization of the immune system, such as differentiation and proliferation of bone marrow hematopoietic stem cells and lymphocyte proliferation in various organs of SLE mice [22–24]. Different mechanisms of Abzs production exist in healthy externally immunized animals and of autoimmune mammals during the development of pathological reactions were revealed ([8, 12–24], see below).

Catalysis by auto-Abzs is potentially applicable in many different fields, including efficient catalysts, the generation of new drugs, and evaluation of the functional roles of Abzs in innate

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and adaptive immunity, the understanding of self-tolerance and of destructive responses in ADs [25–27]. Abzs can be employed for the development of new of drugs, some of which may be useful for therapy.

In this review, Abzs with different catalytic activities in multiple sclerosis (MS) are compared with other Abzs in ADs. In addition, a role of defects of immune systems leading to changes in differentiation of mice bone marrow hematopoietic stem cells (HSCs) and an increase in proliferation of lymphocytes in bone marrow, spleen, and thymus as well as a significant suppression of cell apoptosis in these organs associated with the production of Abzs is discussed.

2. Features of the immune status of patients with multiple sclerosis

The development of ADs is characterized by spontaneous generation of primary Abs to proteins, nucleic acids and their complexes, polysaccharides, nucleotides, etc. [8, 16–21, 28–32]. The origin of natural Abzs is complex. On the one hand, they are similar to artificial Abzs may be directed against analogues of transitional states of catalytic reagents or even against substrates of enzymes acting as haptens. Some antigens may change conformation when they associate with other proteins, and their structure in such complexes could mimic that of a transitional state of the antigenic reaction. On the other hand, later in ADs anti-idiotypic Abs can be induced by a primary antigen and may show some of the characteristics of catalytic activity [8, 33, 34].

MS is known as a chronic demyelinating disease of the central nervous system. Its etiology to date is unclear, and the most widely accepted theory of MS pathogenesis assigns the main role in the destruction of myelin to the inflammation related to autoimmune (AI) reactions [32]. Activated CD4+ myelin-reactive T cells are generally considered as major mediators of MS. Several recent findings imply an important role of B cells and auto-Abs against myelin autoantigens in the pathogenesis of MS [32, 33, 35, 36]. Different studies suggest that a crucial role in MS immunopathogenesis can belong to auto-Abs against myelin autoantigens exercising Ab-mediated demyelination [36]. Auto-antibodies against oligodendrocyte progenitor cell surface protein could block remyelination by eliminating or incapacitating these cells [37]. An important dual role of auto-Abs is suggested: They may be harmful in lesion formation but also potentially beneficial in the repair [35]. It is appropriate to mention here that the main targets of both above-mentioned auto-Abs are glycoproteins: myelin oligodendrocyte glycoprotein that is expressed preferentially on the surface of the myelin sheath [38] and progenitor cell-specific surface glycoprotein AN2 [37], respectively. Elevated level of oligoclonal IgGs in the cerebrospinal fluid (CSF) and B cell accumulation in the CSF and in lesions of MS patients provide evidence for antibody involvement in demyelination [39].

After cloning, the IgG repertoire directly from active plaques and periplaque regions in MS brain and from B cells recovered from the cerebrospinal fluid of a patient with MS with subacute disease for understanding MS pathogenesis new keys have been proposed [40]. It was shown that in the MS patients' high-affinity anti-DNA Abs are the major components of

intrathecal IgG response. Furthermore, DNA-specific monoclonal Abs obtained from patients with MS and DNA-specific Ab derived from a SLE patient bound efficiently to the oligodendrocytes and surface of neuronal cells. Cell-surface recognition of these Abs was DNA-dependent. The results obtained indicate that anti-DNA Abs may promote important neuropathological mechanisms in chronic inflammatory disorders, such as MS and SLE [40]. It should be mentioned that SLE and MS demonstrated some similarities in the development of the same medical, biochemical, and immunological indexes including anti-DNA antibodies. Relative levels of auto-Abs to native (nat) and denatured (den) DNA in the blood of 49 MS patients and healthy donors were compared [41, 42]. The levels of anti-nat-DNA and anti-den-DNA Abs in 18% and 53% of the patients, respectively, were significantly higher than in controls. The titters of Abs to den-DNA in MS patients are usually higher than to nat-DNA. The correlation coefficients between titters of Abs to nat-DNA and den-DNA for a complete group (0.88) and its subgroups with remission (0.81), primary progressing (0.88), and secondary chronic-progressive (0.89) state of the disease were estimated [41, 42]

Interestingly, the titters of Abs against human myelin basic protein (MBP) in SLE patients 2.2-fold higher than in healthy individuals, but 2.1-fold lower than in patients with MS [43, 44]. In the case of 49 patients with MS, a possible correlation between titters of Abs to DNA [41, 42] and to MBP [44] and 13 different standard clinical parameters including Poser criteria (indexes for evaluation of damage to functional systems: pyramidal functions; cerebellar functions; functions of brain stem; sensitive functions; functions of intestines and urinary bladder; visual functions; cerebral (psychical) functions and sum of these characteristics) [45] was carried out [21, 44]. High percent of MS patients showed significantly higher anti-MBP and anti-DNA Abs levels as compared to healthy subjects. For the whole group of MS patients, the absolute values of positive CCs between titters of anti-DNA or anti-MBP Abs and clinical Poser indexes were very low (between 0.01 and 0.19), absent (~0), or were negative (−0.02 to −0.07) and statistically insignificant. Several CCs become higher increasing values up to 0.1–0.55 and −0.04 to −0.47 after the cohort was divided into subgroups of patients with primary progressing, secondary progressing, and remitting course of the disease [44].

The groups of secondary progressing, primary progressing, and remitting course of MS patients were not "homogenous" in relation to the characteristics of the patients, and their further subdivision using cluster and factorial analysis showed high statistically significant correlation coefficients [44]. For example, a direct dependence between titters of anti-MBP and symptoms of damage of the pyramidal tract for one sub-subgroup of the remitting course subgroup was observed (CC = 0.92). In some cases, correlations of the opposite sign were found for the same pairs of analyzed parameters for the three subgroups with different MS courses and their sub-subgroups obtained by cluster analysis.

Interestingly, in the case of different MS subgroups, the level of anti-DNA Abs correlates with various clinical parameters, more often with the disturbance in the function of cerebrum, bladder, and intestines, and, to a less extent, cerebellum [41, 42]. In contrast to anti-DNA Abs, a positive correlation of anti-MBP titters with disturbed brain stem function was revealed in a relatively large fraction of patients (~37%), while a negative correlation was shown with pyramid function and with the rate of disease progression [42, 44]. The correlation between

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Abs to DNA and to MBP in MS patients was also very weak. On overall, the correlation coefficients between the same two analyzed parameters can be either positive or negative in the case of whole group, different subgroups, and their sub-subgroups [41, 42, 44]. The absence of a definite dependence between titters of anti-DNA and anti-MBP Abs and these parameters with the standard clinical indices may be due to the several reasons. Taken together, during analysis of biochemical, immunological and clinical indices must taken into account the current phase of the disease. It should be mentioned; quite different characteristics of pathologic processes can be obtained in individual patients, as the disease progresses against the background of the continually changing immunoregulation including exhaustion of different compensatory and adaptive mechanisms and systemic metabolic changes. This causes the clinical course in individual MS patients hardly predictable [46, 47]. Since each patient can be characterized by an individual combination of genetic, environmental, chronic, inflammatory, autoimmune, demyelinating, neurodegenerative, and other factors [41, 42, 44], it is not surprising that we could not find statistically significant correlation of titters of antibodies to DNA and MBP with the standard clinical parameters in the whole group of 49 MS patients.

3. Catalytic antibodies of MS patients

Natural Abzs from the sera of patients with various diseases are usually polyclonal in origin and may be products of different immuno-competent cells ([13–24] and references cited here). Natural abzyme purification is one of the most complicated aspects of their study; it was discussed in detail in reviews [13, 19]. In study of Abzs with different activities from the sera and CSF of MS patients and healthy donors, electrophoretically and immunologically homogeneous Ab fraction (IgG+IgM + IgA) was first purified by affinity chromatography of the serum or CSF proteins on Protein A-Sepharose under conditions that remove nonspecifically bound proteins. Then IgMs were separated from IgAs and IgGs by FPLC gel filtration [48–56]. 150 kDa IgG, 170 kDa IgA, and ~900 kDa IgM did not contain any contaminating proteins detectable by silver staining under non-reducing and reducing conditions (e.g., **Figure 1**) [48–56].

The application of rigid criteria allowed the authors of the first article concerning natural Abzs [9] to conclude that vasoactive intestinal peptide-hydrolyzing activity is an intrinsic property of Abs from the sera of patients with asthma. Later several additional rigid criteria were proposed (for review see [13, 19]). We applied a set of strict criteria worked out previously [9, 13–21] for the analysis of DNase [52, 54–56], MBP-hydrolyzing [48–51, 53] and amylase [57, 58] activities as intrinsic properties of IgGs, IgAs, and IgMs from the sera and IgGs from the CSF of MS patients [52, 53]. They may be summarized as follows: (a) the IgGs, IgAs, and IgMs were electrophoretically homogeneous (e.g., **Figure 1**); (b) FPLC gel filtration of these Abs using conditions dissociating strong noncovalent complexes in an acidic buffer did not eliminate analyzed activities, and the peaks of the activities and intact Abs exactly coincided (**Figure 2A**); (c) immobilized polyclonal mouse IgGs against the light chains of human antibodies completely absorbed the activities; peaks of these activities coincided with the peak of IgGs (IgA or IgM) eluted with an acidic buffer (e.g., **Figure 2B**). (d) F(ab) and F(ab)2

fragments obtained using digestion by proteases of corresponding catalytic IgGs showed comparable levels of the activities comparing with intact Abs. The fulfillment of these criteria was observed for MS Abzs with all activities mentioned above.

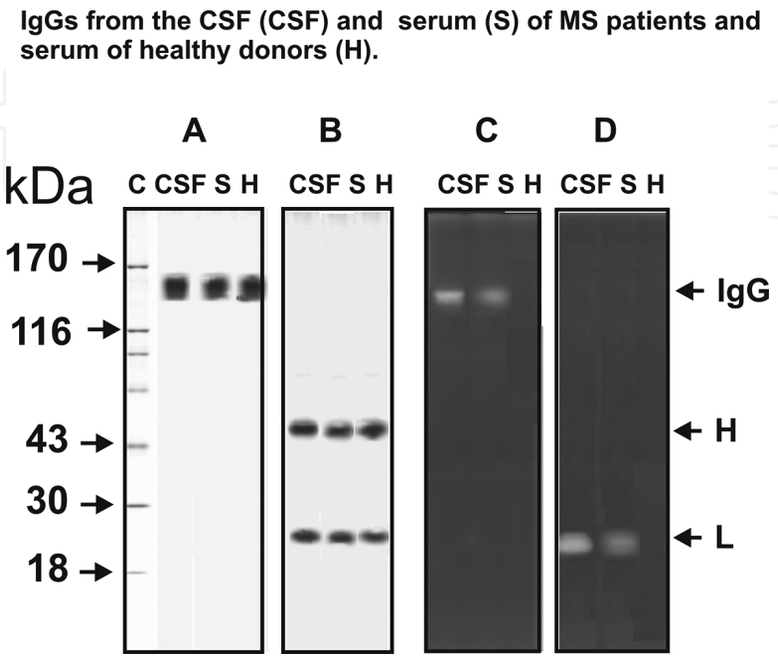

Figure 1. SDS-PAGE analysis of IgGmix (7 μg) corresponding to equimolar mixtures of 13 IgG preparations of CSFs and 13 IgGs from sera of MS patients, and 10 healthy donors in 3–16% gradient gel before (A) and after treatment with DTT (B) followed by silver staining [52, 54–56]. The arrows (lane C) indicate the positions of molecular mass markers. In situ gel assay of DNase activity of IgGmix (15 μg) corresponding to CSFs and to sera of MS patients, and healthy donors in a gel containing DNA before (C) and after treatment with DTT (D). DNase activity was revealed by ethidium bromide staining as a dark band on the fluorescent background. A part of the gel corresponding to Panels C and D was stained with Coumassie R250 to show the position of intact IgGs and separated light and heavy chains (the arrows of Panels C and D).

To exclude possible artifacts due to the hypothetical traces of contaminating enzymes, IgGs from sera and CSF were subjected to SDS-PAGE in a gel co-polymerized with calf thymus DNA, and their DNase activity was detected by incubating the gel in the standard reaction buffer (**Figure 1C** and **D**). Ethidium bromide staining of the gels after the electrophoresis and refolding of IgGs revealed sharp dark bands against a fluorescent background of DNA. In addition, after incubation of IgGs with DTT only light chains of MS csf-IgGmix and serum-IgGmix demonstrated DNase activity (**Figure 1D**). Since SDS dissociates all protein complexes, the revealing of the DNase activity in the gel zones of only to intact IgGs (Panel C) and separated light chains (Panel D) together with the absence of any other activity or protein bands (**Figure 1**), guaranty direct evidence that IgGs from sera and CSFs of MS patients cleavage DNA and they do not contain canonical DNases [52, 54–56].

MS IgGs and IgAs were separated by SDS-PAGE, respectively, under non-reducing and reducing conditions, and their MBP-hydrolyzing and amylase activities were detected after the extraction of Abs from excised gel slices (for example, **Figure 2C**) [44, 48–51, 53, 57, 58].

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The detection of MBP-hydrolyzing and amylase activities in the gel region corresponding only to intact IgGs, together with the absence of any other bands of the activity or protein, provided direct evidence that IgG possesses MBP-hydrolyzing and amylase activities [44, 48–51, 57, 58]. Similar results were obtained for MS IgAs- and IgMs-hydrolyzing MBP [49]. In addition, it was shown that the fractions of IgGs (IgAs or IgMs) having affinity to MBP-Sepharose hydrolyze effectively only human and MBPs, but not other control proteins [48–50].

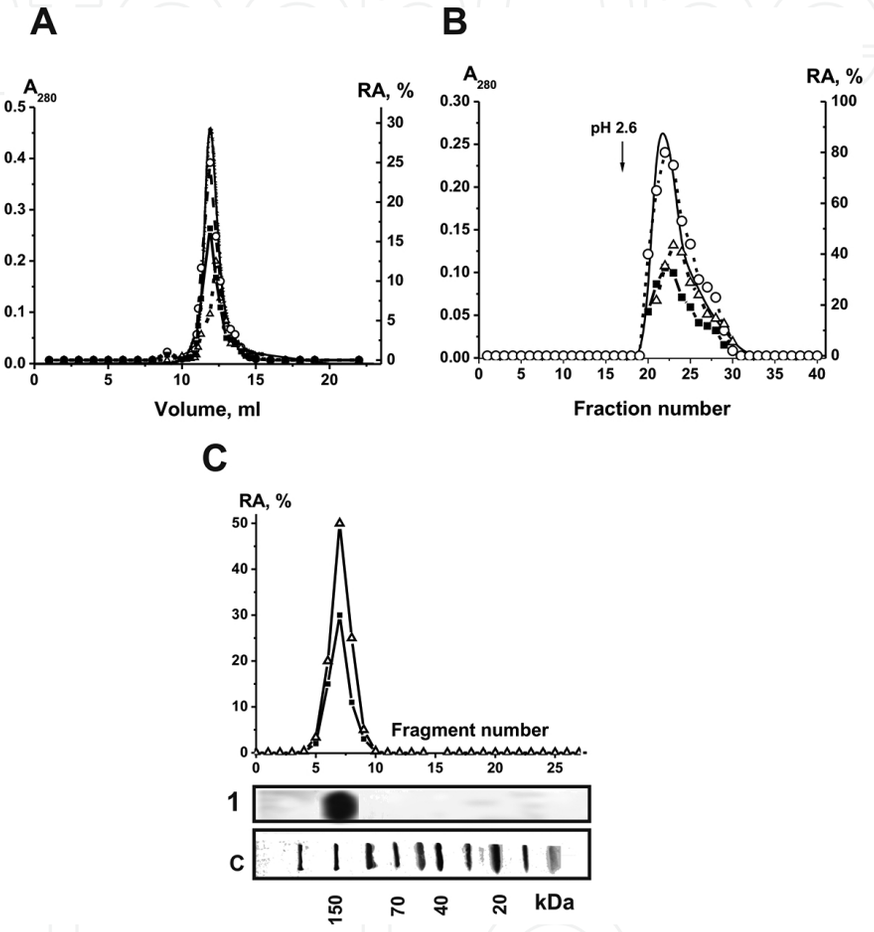

Figure 2. Analysis of strict criteria fulfilment to show that the enzymatic activities are intrinsic properties of IgGs from cerebrospinal fluid of MS patients. FPLC gel filtration of mixture of equal amounts of electrophoretically homogeneous IgGs from 15 preparations of CSF (csf-IgGmix) on a Superdex 200 column in an acidic buffer (pH 2.6) destroying immunocomplexes after Abs incubation in the same buffer (A) and csf-IgGmix affinity chromatography on Sepharose bearing mouse IgGs against human IgGs (B): (—), absorbance at 280 nm (A280); relative activity (RA) of IgGs in the hydrolysis of DNA (○), BMP (■), and maltoheptaose (Δ). In-gel assay of MBP-hydrolyzing (■) and amylase (Δ) activities of csf-IgGmix (15 μg) (C). The relative MBP-hydrolyzing and amylase activities (RA, %) were revealed using the extracts of 2–3-mm fragments of one longitudinal slice of the gel. The RA of IgGs corresponding to complete hydrolysis of the substrates was taken for 100%. The second control longitudinal slice of the same gel was stained with Coomassie Blue (Panel C, lane 1); lane C shows positions of protein markers. The average error in the initial rate determination from three experiments did not exceed 7–10%.

It is known that catalytic centers of nuclease and proteolytic Abzs are usually located on the light chain, while the heavy chain is more often responsible for specific antigen recognition and increased antigen affinity for Abs [13–21]. It was shown that catalytic centers of DNase,

protease, and amylase Abzs from MS patients are located on the light chains of these Abs [48–53, 54, 56–58]. Intact proteins usually interact with both light and heavy chains of Abzs, thus ensuring the specificity of the target protein recognition and its cleavage. Overall, it is obvious that nuclease, amylase, and MBP-hydrolyzing activities are intrinsic properties of Abzs from sera and CSF of MS patients and do not due to the admixture of any possible canonical enzymes.

4. Comparison of the relative catalytic activity of Abs from different MS patients

4.1. Abzymes with DNase and RNase activities

Anti-DNA Abs are detectable even in the sera of healthy mammals, but their titters vary significantly [59]. In comparison with healthy donors, concentrations of anti-DNA antibodies are higher in patients with rheumatoid arthritis (7% of patients) [59], myasthenia gravis (6%), Sjogren's syndrome (18%), multiple sclerosis (17–18%), primary Hashimoto's thyroiditis (23%), and systemic lupus erythematosus (SLE, 36%). Anti-DNA Abs are directed against histone-DNA nucleosomal complexes appearing in the circulation from internucleosomal cleavage during apoptosis in many SLE patients [60].

Healthy humans do not develop Abzs with detectable DNase activity, their levels being usually on the borderline of sensitivity of the detection methods [13–21]. The RAs of Abs from the sera of MS patients vary markedly from patient to patient [54]. **Figure 3A** illustrates cleavage of plasmid DNA by Abs from sera of ten MS patients after 2 h of incubation. During this time, some Abs cause only single breaks in one strand of plasmid supercoiled DNA converting it to the relaxed form (lanes 1–3), whereas others cause multiple breaks causing DNA linearization (lanes 4–6). The most active Abs hydrolyze DNA into short- and medium-length oligonucleotides (lanes 7–10). Relative activity (RA) of different MS IgG preparations was first estimated using arbitrary units (a/u) from 0 to 10 a/u (**Figure 3A**).

IgGs from 53 of 55 MS patients (~96%) demonstrated detectable RAs from 1 to 10 a/u; the average values of IgG DNase activity were 5 ± 4 a/u. MS Abzs hydrolyze single- and double-stranded DNAs of different sequences and length [52, 54–56]. The affinity of Abzs with DNase activity for DNA is usually high (1–10 nM) and corresponds to the typical affinity of Abs for nucleic acids and ~104- and 105-fold higher than that for canonical DNases [54–56].

Recently, we have shown, for the first time, that average concentration of total proteins (132-fold), total IgGs (194-fold), and anti-DNA antibodies (1986-fold; **Figure 3B**) in the sera (average value 437 ± 311 A450 units) is significantly higher than that in the CSF (average value 0.22 ± 0.008 A450 units, **Figure 3B**) of fifteen MS patients [52]. In the sera and CSFs, the relative activities of total protein varied remarkably from patient to patient. Specific DNase activity of the total protein of CSF reparations were surprising approximately 198-fold higher than the serum ones. We present first evidence showing that IgGs from CSF not only bind but efficiently hydrolyze DNA and that average specific DNase activity of homogeneous

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antibodies from CSF (543.3 ± 239.7 pmole DNA/1 mg of Ab/1 h) is unpredictably ~49-fold higher than that from the sera (average value 11.2±4.3 pmole DNA/1 mg of Ab/1 h) of the same MS patients (**Figure 3C**) [52].

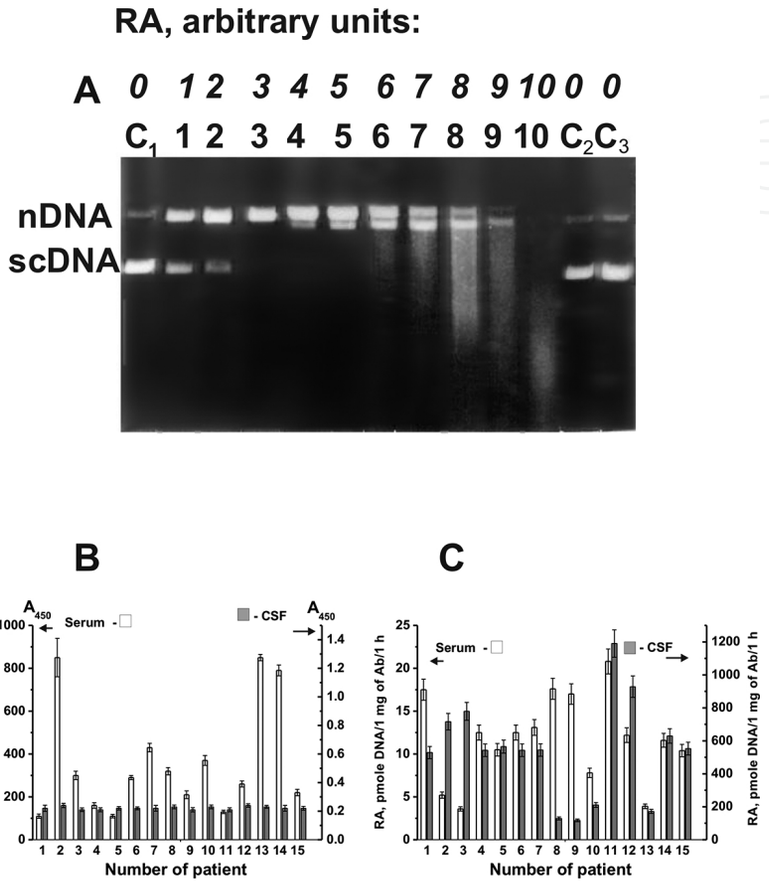

Figure 3. DNase activities of catalytic IgGs from sera of ten different patients with MS in the cleavage of supercoiled (sc) and nicked (n) plasmid DNA (A). Lanes 1–10, IgGs from the sera of 10 different patients; C1, scDNA incubated alone; C2 and C3, scDNA incubated with Ab from the sera of two healthy donors [54, 55]. Relative content (A450 units) of anti-DNA IgGs determined by ELISA in serum (the right hand axis) and in CSF (the left hand axis) preparations of fifteen MS patients (B) and RAs of polyclonal IgGs purified from these fifteen serum and CSF preparations in the hydrolysis of supercoiled DNA (C) [52].

4.2. Abzymes with protease activity

It was shown that IgGs from sera of healthy donors do not hydrolyze MBP [13–21]. We have compared the RAs of IgGs, IgAs, and IgMs from sera of 35 MS patients in the hydrolysis of MBP (**Figures 4A** and **B**) [48, 49]. Specific activities of IgGs from sera of any single patient were usually significantly lower than those of IgMs and sIgAs. Specific inhibitors of thiol and acidic proteases had a weak effect on protease activity of IgGs and IgMs. But, specific inhibitors of serine proteases (PMSF and AEBSF) significantly suppressed proteolytic activity of the Abzs. IgGs, IgMs, and IgAs hydrolyze specifically human MBP, but not many other tested proteins [48, 49].

The sera of healthy donors demonstrated ~four- and fivefold lower concentration of anti-MBP concentration (average value 0.08 ± 0.05 A450 units) than that for MS patients (average value 0.32 ± 0.08 A450 units) [43, 44]. We used ELISA to compare the relative levels of Abs against MBP in the sera and CSFs of 15 MS patients (**Figure 4C**) [53]. The average relative content of anti-MBP Abs in the sera of MS patients is approximately 230-fold higher than in the corresponding CSFs (**Figure 4C**). We present first evidence showing that IgGs from CSF efficiently hydrolyze MBP and that their average specific catalytic activity is unpredictably ~54-fold higher than that of Abs from sera of the same MS patients (**Figure 4D**) [53].

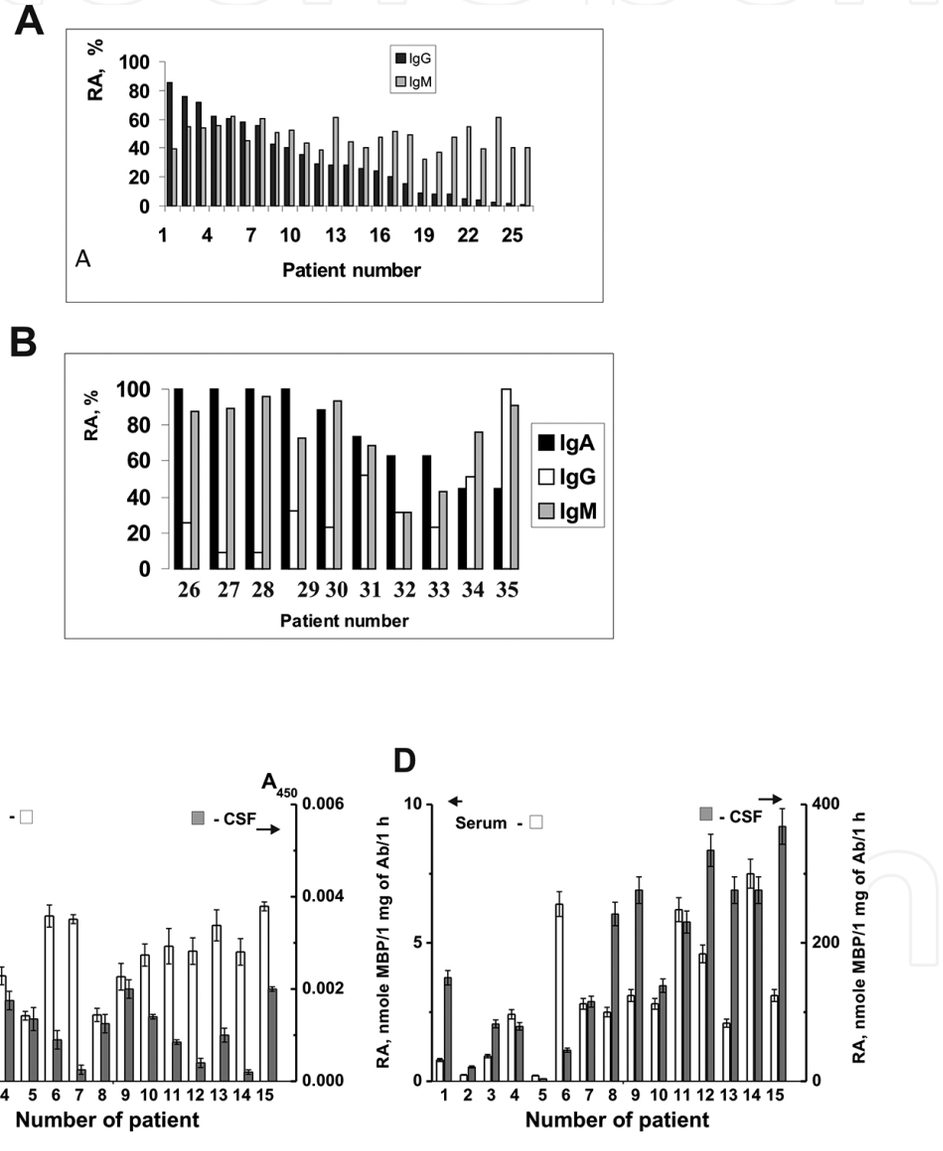

Figure 4. The relative activities (RA) of polyclonal IgGs and IgMs from the sera of 25 different MS patients (A) and of IgGs, IgMs, and IgAs from the sera of other 10 MS patients (B) in the hydrolysis of MBP. The RAs were determined from decrease of initial MBP; the relative intensity of the main bands of MBP incubated alone for 3 h was taken as 100% [49]. Relative content (A450 units) of anti-MBP IgGs determined by ELISA in serum (the right hand axis) and in CSF (the left hand axis) preparations of fifteen MS patients (C) and RAs of polyclonal IgGs purified from these fifteen preparations of biological fluids in the hydrolysis of supercoiled MBP (D) [53].

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IgGs and sIgAs from breast milk demonstrated high ATPase activity [61]. It has recently been shown that during spontaneous development of a profound SLE-like pathology in MRL-lpr/lpr mice leads to a production of DNase, protease, ATPase, and amylase Abzs [22–24]. Interestingly, individual IgGs from sera and CSF of MS patients did not possess detectable ATPase activity [62].

IgGs and IgMs from sera of patients with several autoimmune diseases [57, 58, 63–66] and sIgAs from human breast milk [67] possess amylase activity; however, the maximal activity was observed for Abs from sera of patients with MS [57, 58, 63] and SLE [63, 65, 66]. Individual IgGs and IgMs isolated from patients with MS and SLE had approximately three orders of magnitude higher specific amylolytic activity than that for healthy donors [57, 58, 63–66].

The ability of the presence of MBP- and DNA-hydrolyzing antibodies in the cerebrospinal fluid of MS patients may be related to the fact that anti-MBP and anti-DNA Abs may play an important role in the pathogenesis of this disease. Anti-MBP Abzs can attack MBP of the myelin-proteolipid shell of axons [48–51]. In addition, DNase Abzs of MS patients [16, 21] similarly to SLE patients [68] are cytotoxic and induce cell apoptosis, which can play an important role in SLE and MS pathogenesis. At the same time, the involvement of antibodies with amylase activity in the pathogenesis of any autoimmune diseases has not yet been identified. We have recently shown the first unpredictable evidence showing that IgGs from CSF (average value 9.0 ± 4.9 μM/1 h/mg) possess amylase activity and efficiently hydrolyze maltoheptaose; their average specific Ab activity is ~30-fold higher than that of antibodies from sera (average value 0.30 ± 0.14 μM/1 h/mg) of the same MS patients [62]. Specific average RA for IgGs from healthy volunteers was approximately ~1000 lower than that for MS patients [57, 58]. It was shown that a relative RA of total proteins of CSF (including Abs) is ~15-fold lower than that for purified IgGs, while the RAs of the total sera protein is higher than that of sera IgGs by a factor of 1033. This result speaks in favor of the fact that amylolytic activity of CSF proteins is mainly caused by the activity of amylase Abzs. One cannot exclude that amylase Abzs of CSF can also play a, as yet unknown, role in the pathogenesis of MS.

As it was mentioned above, the correlation coefficients between titters of Abs to DNA and to MBP and 13 different standard clinical parameters, including Poser criteria in the case of 49 patients with MS, were very low [21, 44]. We have not revealed high correlation coefficients between different RAs characterizing IgGs of CSF and serum as well as RAs of these IgGs and total proteins corresponding CSF and serum. All CCs varied from −0.03 to +0.18 except unpredicted correlation of RAs for IgGs from serum and total protein corresponding to CSF (CC = 0.7). Similar situation was observed earlier for CCs between RAs of these IgGs and total proteins corresponding CSF and serum in the hydrolysis of DNA (CCs = −0.05 − +0.03) [52]. In addition, we calculated a possible correlation between RAs characterizing 15 IgGs in the hydrolysis of DNA [52], MBP [53], and oligosaccharide [62] (**Table 1**). All CCs were relatively low (−0.009 − +0.2) except some of them: IgG amylase activity of CSF correlates with MBP-hydrolyzing activity of serum (CC = +0.41) and CSF (CC = +0.45); DNase activity of serum IgGs correlates with MBP-hydrolyzing activity of serum (CC = +0.44) and CSF (CC = +0.61), as well as MBP-hydrolyzing activity of serum and CSF (CC = +0.59). However, all these correlation coefficients were very low or relatively low. Thus, an additional question is why there is no

good correlation between various indexes, characterizing different MS patients. As mentioned above, each patient can be characterized by an individual combination of genetic, environmental, chronic, inflammatory, autoimmune, demyelinating, neurodegenerative, and other factors [46, 47]. This can be the main reason for the lack of a good correlation between the different indicators of MS development by various patients. However, taking all data together, it is reasonable to propose that MBP-, DNA-, and polysaccharide-hydrolyzing Abzs can probably cooperatively promote important neuropathological mechanisms in MS.

Number of patient	**Amylase activity of sera IgGs**	**Amylase activity of CSF IgGs**	**DNase activity of sera IgGs**	**DNase activity of CSF IgGs**	**MBP-hydrolyzing activity of plasma IgGs**	**MBP-hydrolyzing activity of CSF IgGs**
Parameter number	**1**	**2**	**3***	**4***	**5***	**6***
Correlation coefficient	1/2: 0.22	1/3: 0.03	1/4: - 0.009	1/5: - 0.13	1/6: 0.1	2/3: 0.22
	2/4: 0.03	2/5: 0.41	2/6: 0.45	3/4: 0.11	3/5: 0.44	3/6: 0.61
	4/5: 0.34	4/6: 0.11	5/6: 0.59	–	–	–

* Parameters 1 and 2 [62], 3 and 4 [52] and 5 and 6 [53]. Here the same 15 preparations of IgGs from the same MS patients were compared.

Table 1. Correlation coefficients between relative activities of 15 IgGs from sera and CSFs of MS patients in the hydrolysis of oligosaccharide, DNA, and MBP.

4.3. Extreme diversity of MS Abzs

The extreme diversity of RNase and DNase activity of IgG and/or IgM Abzs from the sera of autoimmune patients and also of MRL-lpr/lpr mice was observed [13–21, 69–76]. It was demonstrated that different patients (and animals) may have an extremely large a relatively small content of polyclonal nuclease Abzs containing different relative amounts of kappa and lambda light chains and demonstrating at various optimal pHs maximal activity, possessing a different net charge, dependent or not on different metal ions, and characterized by different substrate specificities.

Different auto-Abs, including Abs to DNA, and different DNA-dependent enzymes can interact with DNA [13–21, 77, 78]. DNase, RNase, and MBP-hydrolyzing IgGs from the sera of autoimmune patients are usually very heterogeneous in their affinity for these substrates and can be separated into many fractions by chromatography on DNA-cellulose and MBP-Sepharose ([13–21] and refs therein). MS Abs with DNase [54–56] and MBP-hydrolyzing [43] activities were distributed all over the profiles of chromatographies (e.g., **Figure 5A**). The affinity of abzyme fractions for these substrates (in terms of Km values) was increased gradually with the increase in eluting salts concentrations. In contrast to MS IgGs, Abzs from SLE patients are more sensitive to EDTA (**Figure 5B** and **C**) and less sensitive to specific inhibitors of serine-like proteases. Similar data of extreme diversity of DNase activity of IgGs were observed in the case of other autoimmune diseases and autoimmune mice [13–21, 75]. All these data are indicative of the extreme diversity of Abzs with different activities in their affinity for specific substrates and in their relative specific activities.

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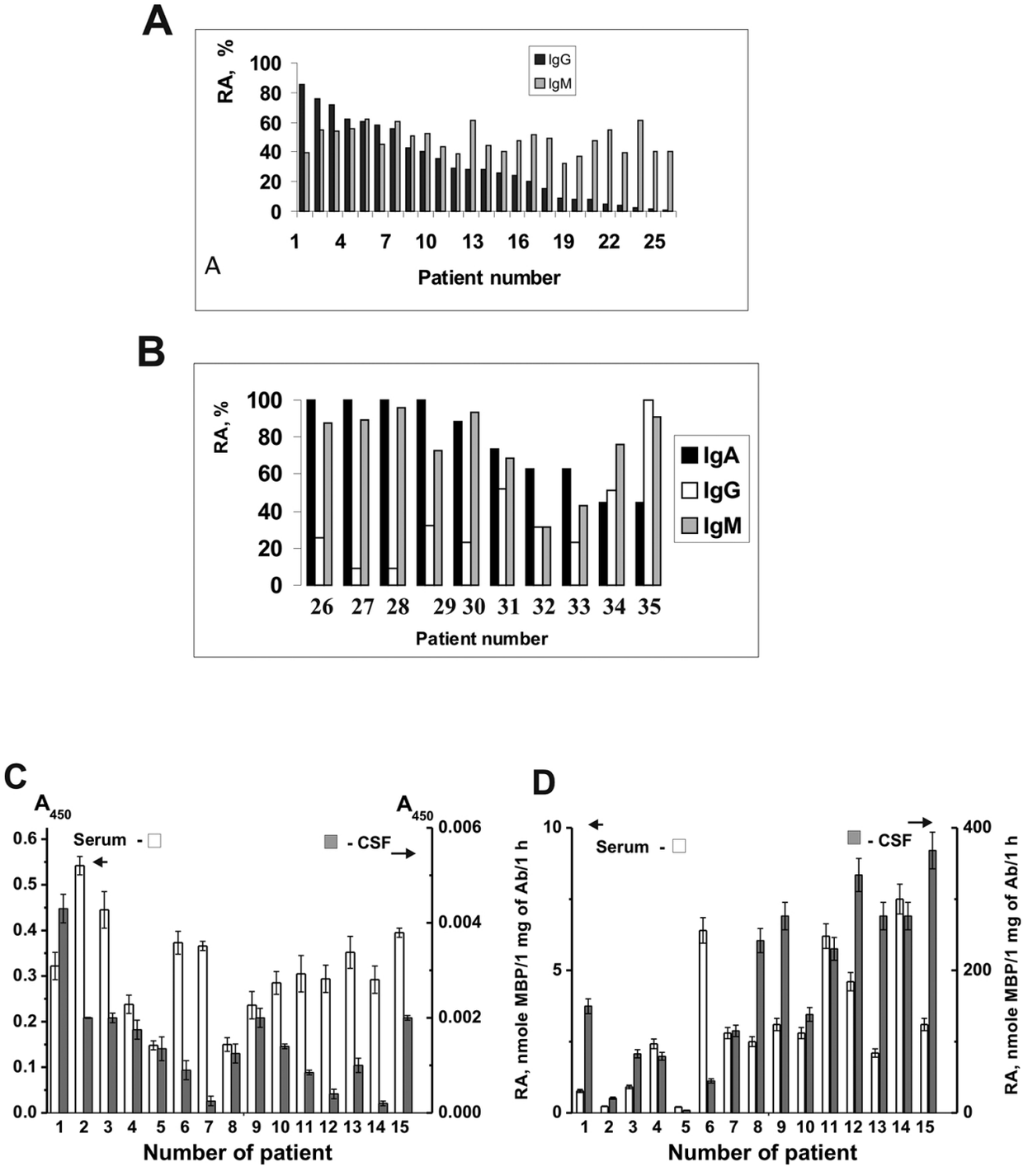

Figure 5. Affinity chromatography of MS IgGs on DNA cellulose: (–), absorbance at 280 nm; columns, relative DNase activity (A) [54, 56]. Affinity chromatography of mixtures of IgGs from sera of 15 SLE (B) and 15 MS (C) patients on MBP-Sepharose [43]: (—), absorbance at 280 nm; the relative catalytic activities (RA) of IgGs of different fractions in the absence (□) and in the presence of EDTA (■) was measured. Depending on the RA, the reaction mixtures were incubated for 0.3–16 h in the presence of 0.01–0.1 mg/ml IgGs and then the RAs were normalized to the standard conditions: the complete transition oligopeptide corresponding to MBP and DNA to their products was taken for 100%.

Theoretically, the human immune system can produce up to 106 specificities of Abs against one antigen. Affinity chromatography data (**Figure 5**) do not give possibility to estimate a

possible number of monoclonal DNase antibodies that correspond to the polyclonal IgGs, eluting at various Ab peaks. For this evaluation, we have used the analysis of possible number of monoclonal Abs with DNase activity [79–81]. An immunoglobulin kappa light chain library from blood of SLE patients was cloned into a phagemid vector. Phage particles displaying recombinant monoclonal light chains (MLChs) capable of binding DNA were isolated by affinity chromatography on DNA cellulose. The phage particles containing MLChs bound with DNA-cellulose were distributed between eleven peaks (11 different fractions) eluted during chromatography, and all fractions obtained were active in the hydrolysis of DNA. The fraction eluted by 0.5 M NaCl (peak 7) and an acidic buffer (pH 2.6; peak 11) were used for preparation of individual monoclonal light chains (MLChs, ~28 kDa). Then, 45 of 451 and 33 of 687 individual colonies corresponding respectively to peaks 7 and 11 were randomly chosen for study of MLCh DNase activity [79–81]. Fourteen of 45 and nineteen of 33 clones, respectively, contained MLChs with DNase activity. Totally, all 33 of 78 purified MLChs (42%) containing no canonical DNases demonstrated various enzymatic properties including different k_{cat} values, one or two pH optima, were inactive after dialysis against EDTA, but could be activated by several externally added metal ions; the ratio of relative activity in the presence of Mn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+} was individual for each MLCh, with Mn^{2+}, Mg^{2+}, and Co^{2+} being among the best activators of their DNase activity [79–81]. In the presence of Mn^{2+} or Mg^{2+}, the DNase activity of some MLChs did not require K_+ or Na^+, while the others could be activated by KCl or NaCl at optimal concentrations from 1 to 100 mM. It should be mentioned that not only Abs from sera of patients with different ADs containing kappa, but also lambda light chains possess DNase activity [13–21]. In addition, we have analyzed MLChs corresponding only to two of eleven peaks obtained [79–81], while Abs of all 11 peaks were catalytically active. It means that the sera of SLE (and MS) patients in principle can contain some hundreds of catalytic DNase lambda- and kappa-IgGs with different catalytic properties.

In order to compare the substrate specificity of Abzs, the hydrolysis of various oligonucleotides by MS IgGs was analyzed [55]. The oligonucleotides (ODNs) cleavage patterns varied from patient to patient. Some Abs demonstrated sequence-independent hydrolysis of ODNs, while other produced in parallel both 5′-phosphate terminated products (similar to those of DNase I) or 3′-phosphate terminated ODNs, which are typical for DNase II. Several IgGs demonstrate sequence-dependent manner, while for other the products correspond to both 3′- and 5′-exonuclease activities. The hydrolysis of ODNs by different IgGs was also strongly dependent on the reaction conditions used including addition of MgCl2, EDTA, increase in NaCl or potassium phosphate concentration [55]. All the data obtained show that MS IgGs can demonstrate different combinations of endo- and exonuclease activities and properties of the MS DNase IgGs distinguished them from all known canonical DNases [55]. The extreme diversity of RNase and DNase IgG and/or IgM Abzs in their affinity to DNA (and in k_{cat} values) was also revealed for several other autoimmune diseases [13–21, 55, 68, 69, 76].

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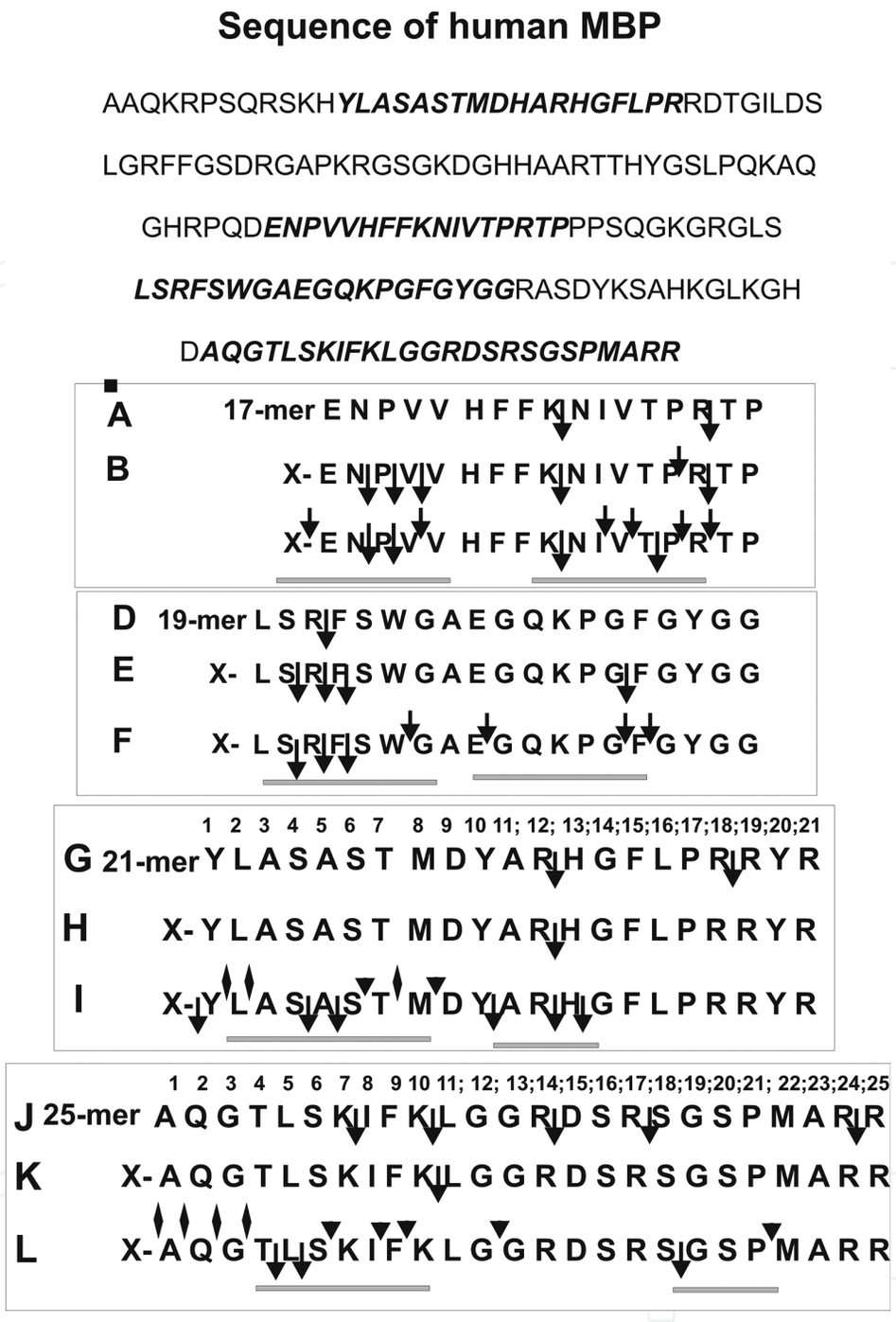

Figure 6. Complete sequence of human MBP (on the top); the positions of OP17, OP19, OP21, and OP25 sequences in the human MBP sequence are shown in bold. All sites of cleavage of X-OP17 (B–C) and X-OP19 (E–F) (X is fluorescent label of OPs), corresponding to detectable major and minor products of these OPs digestion after their mild (B and E) and deep (C and F) hydrolysis by equimolar mixture of 15 SLE IgGs are shown [80]. Panels A and D show the major cleavage sites, which were found previously in the case of hydrolysis of globular intact MBP by MS and SLE IgGs [43, 78]; trypsin hydrolyzes these OPs at the same sites. All sites of the OP21 (I) and OP25 (L) corresponding to major and moderate products of the cleavage are shown by long and short arrows respectively, while corresponding to minor ones by diamonds [81]. Panels G and J show all possible sites of these OPs cleavage by trypsin, while panels H and K demonstrate the major cleavage sites of MBP, which were found previously in the case of hydrolysis of globular intact MBP by MS and SLE IgGs [43, 78]. Clustered major and minor sites of cleavage are underlined.

Anti-MBP Abzs of MS patients' cleavage MBP at several sites corresponding to four known immunodominant regions of human MBP [82]. It was demonstrated that anti-MBP Abzs of SLE patients hydrolyze MBP at the same four immunodominant regions of MBP [43]. Four different encephalytogenic peptides corresponding to known antigenic determinants (AGDs) of MBP can play a negative role in the MS and SLE pathogenesis [82]. Therefore, it was interesting to study the specific sequences of MBP in more detail. Interestingly, MS and SLE anti-MBP IgGs hydrolyze nonspecific tri- and tetrapeptides with an extremely low efficiency and they cannot efficiently cleavage longer oligopeptides corresponding AGDs of HIV-1 integrase [83]. To identify all MBP cleavage sites corresponding to anti-MBP IgGs in the case of four AGDs of MBP, we have used a combination of MALDI mass spectrometry, reverse-phase chromatography, and thin layer chromatography for revealing of the cleavage products of four (17-, 19-, 21, and 25-mer) encephalytogenic oligopeptides corresponding to these AGDs [84, 85]. Several clustered major and minor sites of cleavage were revealed in the case of all oligopeptides (**Figure 6**). It was shown that the number of oligopeptide cleavage sites is greater than that of intact MBP. The active sites of anti-MBP Abzs are located on their light chains, while the heavy chains are responsible for Abs increased affinity for protein substrates. Interactions of both light and heavy chains of Abzs with intact globular proteins provide high affinity and specificity of intact MBP hydrolysis. The affinity of anti-MBP Abzs for oligopeptides was ~103-fold lower than that for the intact MBP. These data indicate that a relatively short oligopeptides interact mainly with the light chain of different monoclonal Abzs of total pool of IgGs, which possesses lower affinity for substrates, and therefore, depending on the oligopeptide sequences, their hydrolysis may be less specific [84, 85].

IgGs and IgMs from the sera of patients with MS and SLE were found to possess amylolytic activity hydrolyzing α—(1-4)-glucosyl linkages of maltooligosaccharides, glycogen, and several synthetic substrates [57, 58, 63–67]. The specific amylolytic activity of individual IgGs and IgMs from MS patients was about three orders of magnitude higher than that of healthy donors [57, 58]. Fractions of auto-Abs from human milk [67] and from sera of patients with different autoimmune pathologies [57, 58 63–65] revealed different modes of action in the hydrolysis of maltooligosaccharides, p-nitrophenyl maltooligosaccharides, p-nitrophenyl, and α-D-glucopyranoside; several samples of MS Abzs demonstrated β-xylosidase activity, which is not observed in known mammalian polysaccharide-hydrolyzing enzymes.

Proteolytic Abs from the sera of patients with various autoimmune diseases [9, 86] casein-hydrolyzing Abzs from human milk [87], casein-, human serum albumin-, and HIV reverse transcriptase-hydrolyzing Abs from AIDS patients [88] are serine-like proteases, and their activity is most strongly reduced by specific serine protease inhibitors PMSF or AEBSF. Specific inhibitors of acidic and thiol proteases demonstrated a weak effect on proteolytic activity of MS polyclonal IgGs and IgMs [48–51]. However, specific inhibitors of serine proteases significantly inhibited proteolytic activity. In addition, MS polyclonal IgGs contained several chelated metals and the relative amount of which decreases in the order: $Fe^{2+} > Ca^{2+} > Cu^{2+} > Zn^{2+} > Mg^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+}$ [50]. After removing of Mg^{2+} ions by dialysis against EDTA, MS IgGs have not completely lost proteolytic activity, which was increased after addition of different external Me^{2+} ions. After chromatography of MS IgGs on Chelex-100, a minor metal-

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dependent fraction did not cleavage MBP in the absence Mg^{2+} ions, but it was activated after addition of metal ions: $Ca^{2+} < Cu^{2+} < Mn^{2+} < Mg^{2+}$ [50]. Protease activities of several MS IgGs were also activated by other metal ions (Ni^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}, and Co^{2+}) and especially Ni^{2+}. Observed properties of MS Abzs distinguish them from other known mammalian metalloproteases and demonstrate their pronounced catalytic diversity. Metal-dependent IgG from MS patients was the first example of Abzs with metal-dependent proteolytic activity [50]. Later, we have revealed an important metal-dependent casein-hydrolyzing sIgA from human milk [89] and MBP-hydrolyzing IgGs from SLE patients [43]. We have recently shown that anti-integrase IgGs and IgMs from AIDS patients can contain Abzs cleaving HIV integrase by small subfractions resembling thiol, serine, acidic, and metal-dependent proteases, the ratio of which may be individual for every HIV-infected patient [90, 91]. Since anti-MBP from sera of MS and SLE patients are very similar, we have analyzed in more details monoclonal Abzs from SLE patients [92, 93].

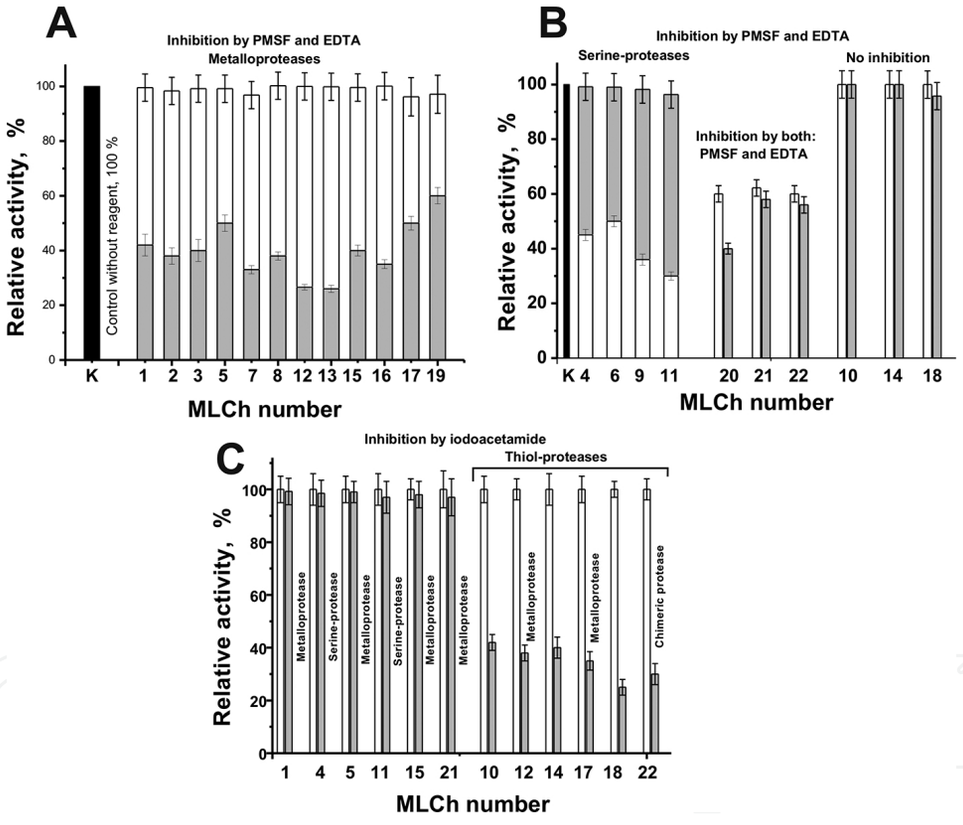

Figure 7. The relative MBP-hydrolyzing activity of 22 MLChs after their pre-incubation with specific inhibitors of different type proteases [92]. Various MLChs (0.1 mg/ml) were pre-incubated in the absence of other components (black bars, control—C), in the presence of 50 mM EDTA (gray bars) or 1 mM PMSF (white bars) and then added to standard reaction mixture containing MBP substrate (A and B). Panel C shows several examples of the relative activity of MLChs with metalloprotease activity (numbers: 1, 5, 12, 15, and 21) and serine-like activity (numbers: 4 and 11), which do not change their activity after pre-incubation with iodoacetamide; three MLChs (numbers: 10, 14, and 18) demonstrating negative response to PMSF and EDTA as well as chimeric MLCh-22 after their pre-incubation with iodoacetamide leading to a significant decrease in the activity. White and grey bars correspond respectively to the activity before (control) and after these MLChs pre-incubation with iodoacetamide (Panel C). The relative activity of all MLChs before their pre-incubation with different inhibitors was taken for 100% [92].

An immunoglobulin light chain phagemid library corresponding to peripheral blood of SLE patients lymphocytes was used [92, 93]. Small pools of phage particles displaying light chains with different affinity for MBP were obtained using affinity chromatography on MBP-Sepharose. For preparation of the individual monoclonal light chains (MLChs, 27–28 kDa), the fraction eluted with 0.5 M NaCl was used. Seventy two of 440 individual colonies were randomly chosen, expressed in E. coli in a soluble form, and individual MLChs were purified by metal chelating chromatography. 22 of 72 MLChs efficiently cleaved MBP showing various pH optima in a 5.7–9.0 range and different substrate specificity in the hydrolysis of four various MBP oligopeptides. Four light chains demonstrated serine-like, three MLChs—thiol protease-like activities, while eleven MLChs were metal-dependent (**Figure 7**). PMSF and EDTA inhibited by the activity of three chimeric MLChs, two other chains were supressed by EDTA and iodoacetamide, and one by PMSF, EDTA, and iodoacetamide. In the presence of Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+}, the ratio of relative activity was individual for each of 22 MLCh preparations [92, 93]. These observations suggest an extreme diversity of anti-MBP Abzs in SLE (and most probably MS) patients.

As it was mentioned above, iodoacetamide weakly (5–15%) suppress MBP-hydrolyzing activity of polyclonal Abs from sera of patients with MS and SLE [43, 48–51]. It was shown that the relative number of iodoacetamide-dependent MLChs is only approximately 27% of all MLChs analyzed, and several of them possess at the same time activity of serine- and metalloproteases (**Figure 7**) [92, 93]. Thus, a relative contribution of thiol-like proteolytic activity to a total MBP-hydrolyzing activity of polyclonal Abzs pools in SLE and MS patients can be remarkably lower than that of antibodies with serine-like and metalloprotease activities and may be estimated for total IgGs depending on patients comparable to 5–15%, as found previously [43, 48–51]. Using different methods, it has been proven that recombinant monoclonal kappa light chain NGTA2-Me-pro-ChTr possess two different activities—trypsin-like and metalloprotease activities [93], while other with trypsin-like, metalloprotease and DNase activities (Timofeeva AM, personal communication). Thus, it reasonable to believe that the immune system of SLE and MS patients can produce anti-MBP Abzs not only with one type but also with a combined structure of the active center, carrying amino acid residues typical for different proteases with metal-dependent, serine-, thiol-, and acidic-like activities.

Mammalian blood DNase II and DNase I demonstrate only one pronounced pH optimum in hydrolysis of scDNA (pH 5.2 and 7.0, respectively) [94–96]. In contrast to all human DNases, all polyclonal MS IgGs demonstrated high or detectable activity at a wide range of pH values between 5.0 and 9.5 [56]. The heights of the peaks corresponding to various optima were different, and the ratios of RAs at these pH values were individual for each polyclonal IgG preparation. **Figure 8A** demonstrates typical data for two individual MS patients. Similar results were obtained for DNase IgGs and/or IgMs from patients with other autoimmune diseases [13–21].

It is well known that mammalian, bacterial, and plant canonical proteases, depending on their biological function, can have optimal pHs ranging from acidic (2.0) to neutral and alkaline (8–10) [97, 98]. We have measured the relative activity of polyclonal IgGs at pH from 2.6 to 10.5. In contrast to canonical proteases, the pH profile of each preparation of polyclonal IgGs from

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MS [51] and SLE [43] patients was unique (**Figures 8C** and **8D**). In contrast to all human proteases having one pronounced pH optimum, catalytic MS and SLE polyclonal IgGs demonstrated high specific MBP-hydrolyzing activity within a wide range of pH values (2.6–10). Taking this into account, one cannot exclude that human immune system could, in principle, produce Abzs with very different proteolytic activities including similar to that of stomach acidic proteases. The above results clearly demonstrate that polyclonal IgGs from individual MS and SLE patients are very heterogeneous and can consist of different sets of catalytic IgG subfractions demonstrating quite distinct pH dependencies.

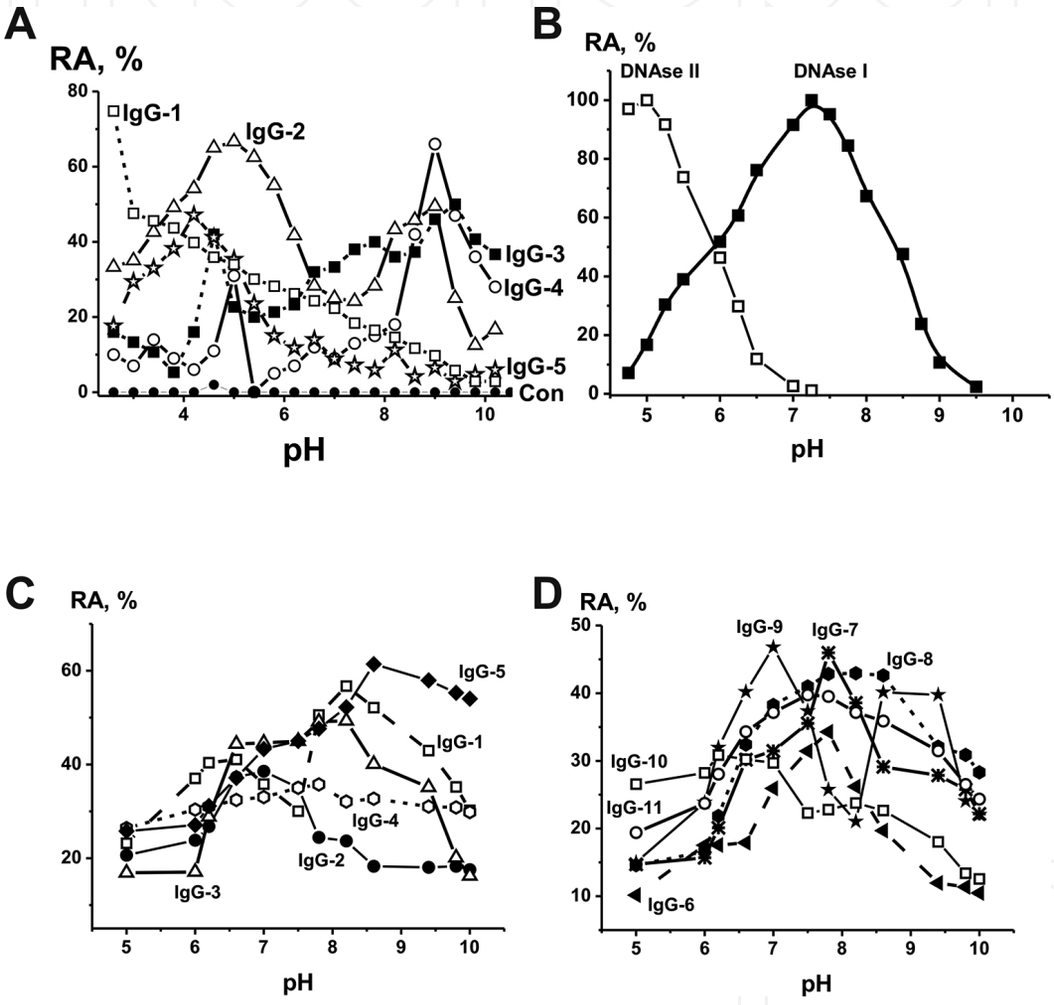

Figure 8. pH dependences of the RAs in the hydrolysis of plasmid DNA by five individual MS polyclonal IgGs (IgG-1–IgG-5) (A) and by canonical DNase I and DNase II (B) [56] as well as pH dependences of the relative MBP-hydrolyzing activity of five individual IgGs from the sera of five different MS (C) and six various SLE (D) patients [43, 51]. Hydrolysis of DNA incubated alone was used as control (A, "Con.") The relative DNase and protease activities corresponding to a complete transition of substrates to their shorter products as well as maximal activities of DNase I and DNase II were taken for 100%.

The next question concerning structural diversity of MS abzymes is related to a possibility of existence of catalytic Abs of different subclasses. It was interesting whether the cerebrospinal fluid similarly to serum of MS patients can contain IgG antibodies of all four subclasses. We

have recently analyzed CSF and sera of MS patients, the average content of lambda- and kappa-IgGs as well as IgGs of four different subclasses (IgG1-IgG4) [53]. The average relative content of lambda-IgGs and kappa-IgGs in the case of CSFs (8.0 and 92.0%) and sera (12.3 and 87.7%) are comparable, while IgG1, IgG2, IgG3, and IgG4: CSF—40.4, 49.0, 8.2, and 2.5% of total IgGs, respectively, and the sera—53.6, 36.0, 5.6, and 4.8%, decreased in different order [53].

To analyze an "average" situation concerning a possible catalytic heterogeneity of MBP- and DNA-hydrolyzing IgGs from sera, we have prepared a mixture of equal amounts of IgGs from the sera of ten MS patients [51, 99]. The purity of IgGs of all types was confirmed by ELISA. It was shown that small fractions of IgGs of all four subclasses (IgG1–IgG4) are catalytically active in the DNA cleavage and their relative activity (nM supercoiled DNA/1 mg IgG/1 h) on average decreases in the order: IgG4 (4.1) < IgG3 (1.4) < IgG2 (0.94) < IgG1 (0.58), while their approximate relative contribution to the total activity of Abzs (taking into account their relative content in the sera) decreases in the order: IgG4 (65.6%) > IgG2 (18.2%) > IgG3 (9.3%) > IgG1 (6.9% [99]. In the hydrolysis of DNA on average, k-IgGs are several folds more active than λ-IgGs. By different physico-chemical methods of Abs analysis, it was shown that the immune systems of MS patients can generate a variety of different type of anti-DNA Abzs and with various catalytic properties, which can play an important role in MS pathogenesis.

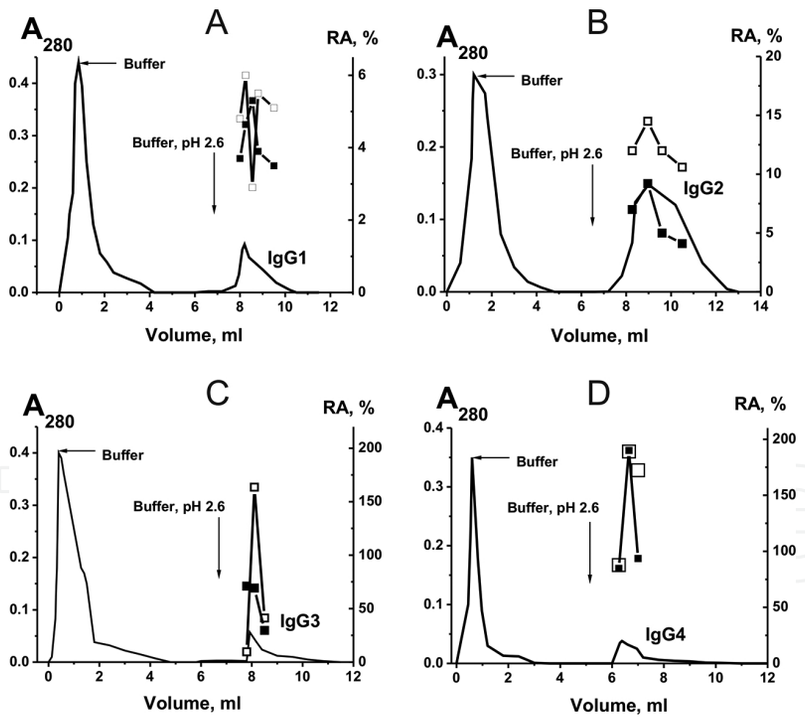

Figure 9. Affinity chromatography of polyclonal IgGs (mixture of 10 preparations from sera of MS patients) on anti-IgG1 (A), anti-IgG2 (B), anti-IgG3 (C), and anti-IgG4 (D) Sepharoses [84]: (—), absorbance at 280 nm, (□) and (■), relative catalytic activities (RA) in the hydrolysis of MBP and OP-19 corresponding to one of immunodominant region of MBP, respectively. Depending on the RA, the reaction mixtures were incubated for 0.3–16 h in the presence of 10–100 μg/ml IgGs and then the RAs were normalized to the standard conditions: the complete transition of 0.19 mg/ml MBP and 0.33 mM OP-19 to their hydrolyzed forms in the presence of 0.1 mg/ml IgGs after 1 h of incubation was taken for 100%. The average error in the initial rate determination from two experiments in each case did not exceed 7–10%.

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It was shown that IgGs from sera of MS patients containing k- and λ-types of light chains demonstrated comparable relative activities in the hydrolysis of MBP [51]. IgGs of all four subclasses (IgG1–IgG4) demonstrate catalytic activity (**Figure 9**), their contribution to the total activity of Abzs in the hydrolysis of MBP and its 19-mer oligopeptide increasing in the order: IgG1 (1.5–2.1%) < IgG2 (4.9–12.8%) < IgG3 (14.7–25.0%) < IgG4 (71–78%). Interestingly, the RAs of DNase polyclonal IgGs from MS patients increased in the same order (IgG1 < IgG2 < IgG3 < IgG4 [99]) as the RAs of MBP-hydrolyzing IgGs from SLE patients [43]. These data provide the evidence that all types of human MS and SLE IgGs, IgG1–IgG4 can possess various catalytic activities, but differ in their RAs and contribution into the total activity of MBP- and DNA-hydrolyzing polyclonal Abzs.

4.4. Relative catalytic activity of MS abzymes

The catalysis mediated by artificial Abzs is usually characterized by relatively low k_{cat} values, which are 102- to 106-fold lower than those for canonical enzymes [1–8]. The known k_{cat} values for natural Abzs from AI patients vary in the range of 0.0001–40 min^{-1} [8–21]. The k_{cat} of polyclonal IgGs with various activities from sera of MS patients is in average varied in different ranges. Overall, the k_{cat} values characterizing hydrolysis of DNA, RNA, MBP, oligosaccharides in the reactions catalyzed by non-separated polyclonal MS IgGs and their subfractions separated by affinity chromatographies are comparable with or even higher than those for other known Abzs [8–21]. In addition, the RAs of DNase non-separated polyclonal Abs from patients with different diseases increase approximately in the order: diabetes [100] ≤ viral hepatitis [101] ≈ thick bone encephalitis [102] < polyarthritis ≤ Hashimoto's thyroiditis [71, 76, 103] ≤ schizophrenia [104] < AIDS [105] ≤ MS [54–56, 99] < SLE [10, 68–70]. At first glance, the k_{cat} values characterizing some polyclonal Abs may seem relatively low compared with those for canonical enzymes with the same enzymatic functions. At the same time, some DNA-dependent enzymes with very important functions, for example, repair enzymes and EcoRI endonuclease demonstrate the k_{cat} values comparable or even lower [106] than those for Abzs analyzed. In addition, the RAs of RNase IgGs and IgMs from the sera of patients with different autoimmune diseases including MS may be 10- to 1000-fold higher than those of DNase Abzs from the same patients. RNase activities of autoimmune Abzs were more often 0.1–20% of that of RNase A and of six known human sera RNases, while poly(A) was hydrolyzed by MS and other autoimmune Abs 2–10 times faster than by RNase A, one of the most active RNases known [11, 13–21, 54, 101]. The specific activity of IgGs of several MS patients was 40–400% of that of RNase A [101].

Notably, currently there are no methods that could efficiently separate Abzs from catalytically inactive antibodies against the same substrate (antigen). The specific activities were calculated using the total concentrations of polyclonal Abs, and affinity chromatography on DNA-cellulose or MBP-Sepharose leads only to partial enrichment of individual fractions with proteolytic and DNase activities. Thus, the specific substrate-hydrolyzing activities of the individual monoclonal Ab subfractions in pool of polyclonal Abs may be significantly higher than those of the non-fractionated or partially fractionated polyclonal IgGs, IgAs, and IgMs. In addition, it is impossible not to take into account catalytic activities of Abs from patients

with MS and other autoimmune diseases in the analysis of their possible role in the pathogenesis of these pathologies, since some of them may be very harmful for people health.

5. Specific reorganization of immune system of SLE prone MRL-lpr/lpr mice

According to our data, the catalytic activity of nuclease and protease Abzs in sera of autoimmune patients is usually very easily detectable at the beginning of autoimmune diseases, when concentrations of Abs to DNA or other autoantigens are not yet significantly increased, and correspond to levels in healthy donors [13–21]. It was shown that detection of Abzs with different activities in human serum may be considered a good indicator of the onset or a significant progression of autoimmune reactions associated with several pathologies [13–21]. In this regard, is of great interest to understand what mechanisms underlie the development of autoimmune processes and how they associated with the production of catalytic antibodies. Some observations suggest that autoimmune diseases can be originated from defects in the hematopoietic stem cells (HSCs) [107]. Therefore, one can assume that in the development of different Ads, there may be some common objective laws. Taking this into account, it seems reasonable first to consider what regularities were found by us in the development of SLE in MRL-lpr/lpr mice. First, it was shown that IgGs from the sera of 2-month-old to 7-month-old control non-autoimmune BALB/c and (CBAxC57BL)F1 mice and conditionally healthy 2-month-old to3-month-old autoimmune prone MRL-lpr/lpr mice are catalytically inactive [22–24].

It is known that MRL-lpr/lpr mice spontaneously developing a lupus-like AI disorder are characterized by visual symptoms of the pathology (pink spots, baldness of head, and parts of the back, general health deterioration, etc.). The sera of these mice contain Abzs with DNase, amylase and ATPase activities [22–24, 108]. Appearance of proteinuria (≥3-mg/ml concentration of protein in urine) correlated well with pronounced visual symptoms [22–24]. The highest levels of anti-DNA Abs, catalytic activities of Abzs, visible markers of SLE and proteinuria were observed usually at 5–12 months of age, which agrees with previously reported data for typical period of signs of pathology appearance in MRL-lpr/lpr mice [109]. We have analyzed spontaneously diseased mice with all visible symptoms no older than 7 months. Although the state of "health" in the case of AI-prone mice should be considered very provisional, the mouse SLE pathology is nevertheless spontaneous and autoimmune reactions, leading to deep pathology develop gradually. To distinguish various levels of the pathology development, MRL-lpr/lpr mice demonstrating no typical SLE indices and Abzs activities (similar to healthy control non-autoimmune mice) were conditionally designated (independently of age) as healthy MRL-lpr/lpr mice, whereas the animals demonstrating no visual or biochemical SLE indices but having detectable abzyme activities were conditionally designated as pre-diseased mice.

A specific reorganization of immune system of these mice after spontaneous development of deep SLE-like pathology results in conditions associated with a production of Abs hydrolyzing

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DNA, ATP, and polysaccharides with low catalytic activities (conditionally pre-diseased mice). A significant increase in amylase and especially DNase, ATPase relative activities was associated with a transition from pre-diseased to deep diseased mice correlating with additional changes in differentiation and proliferation of mouse bone marrow hematopoietic stem cells and lymphocyte proliferation in different organs. The highest increase in activities of all Abzs was revealed in 3-month healthy mice immunized with DNA, which are characterized by a different profile of HSC differentiation and by a significant suppression of cell apoptosis in comparison with pre-diseased and diseased mice. Overall, all mouse groups investigated were characterized by a specific relationship between abzyme activities, HSC differentiation profiles, levels of lymphocyte proliferation, and cell apoptosis in different organs [22–24]. We came to the conclusion that the appearance of ATPase and DNase activities in diseased mice [22–24] similarly to SLE and MS patients [13–21] may be considered as the earliest statistically significant marker of spontaneous SLE and a further significant increase in catalytic activities correlates with the appearance of SLE visible markers and with an increase in proteinuria and concentrations of anti-DNA Abs. Some differences in immune system reorganizations at pre-disease and deep disease leading to the production of different autoantibodies and Abzs comparing with healthy mice were revealed [22–24].

6. Change of some indexes characterizing development of EAE in C57BL/6 mice

We tried to compare several different indexes associated with development of ADs in autoimmune prone mice MRL-lpr/lpr and experimental autoimmune encephalomyelitis (EAE; C57BL/6) mice, the models of human SLE and MS, respectively. When for acceleration of SLE development in MRL-lpr/lpr DNA is usually used [22–24], the stimulation of EAE in C57BL/6mice is usually carried out using mouse myelin oligodendrocyte glycoprotein (MOG35-55), which is an antigen of the myelin sheath [112, 113].

In our study, we have used four experimental groups of C57BL/6 and (CBA × C57BL) F1 or CBA mice:

- untreated control EAE C57BL/6 mice [110],
- MOG-immunized C57BL/6 mice [110],
- control untreated non-autoimmune CBA mice [111],
- control MOG-immunized non-autoimmune CBA mice [111].

In MOG-induced EAE mice, first clinical symptoms appear at 5–7 days after immunization, while the maximum stage of the disease is usually manifested at 14–16 days after immunization [112, 113]. A possible change in the relative weight, different immunological and biochemical parameters at 3 months of age (zero time; control) as well as MOG-treated C57BL/6 and CBA mice during consecutive 40 days was analyzed. After 6 days but not at later stages an average decrease in weight of EAE mice was observed in MOG treated compared

to untreated control mice (**Figure 10A**), but the weight of CBA mice increase slower, than untreated mice during 40 days (**Figure 10B**). In other models of autoimmunity including MRL-lpr/lpr, the appearance of pronounced visual symptoms usually correlates well with proteinuria (≥3 mg/ml concentration of protein in urine) [22–24]. Control non-autoimmune CBA mice at 7 months demonstrated no proteinuria (<2 mg/ml) [22–24]. At the same time, EAE C57BL/6 mice at three months of age are usually characterized by a significantly higher level of proteinuria (up to 10–12 mg/ml) [114]. We have analyzed the time-dependent change in proteinuria for immunized and untreated C57BL/6 mice (**Figure 10C**) and control CBA mice (**Figure 10D**). In seven mice of the EAE control group, the average proteinuria was 7.2 ± 0.8 mg/ml at time zero (3 months of age) and was nearly the same until day 20, but increased to 12 ± 0.8 mg/ml at 40 days (**Figure 10C**). For control CBA untreated mice, there was no remarkable change in concentration of urine proteins during 40 days (**Figure 10D**). In EAE mice treated with MOG, a steady increase in proteinuria was observed: day 0: 7.2 ± 0.8 mg/ml; day 30: 13.6 ± 2.5 mg/ml; day 40: 21.8 ± 3.8 mg/ml. The increase after 40 days became significantly different from control animals (**Figure 10C**). CBA mice from 0 to 40 days demonstrated increase in urine protein concentration from 1.6 ± 0.08 to 2.5 ± 0.23 mg/ml (**Figure 10D**). High level of EAE mice proteinuria at three months may be due to the fact that these mice demonstrate spontaneous development of autoimmune processes, which is stimulated by their immunization by MOG. Immunization of non-autoimmune CBA mice also leads to remarkable increase in proteinuria, but after 40 days it is lower 3 mg/ml (**Figure 10D**).

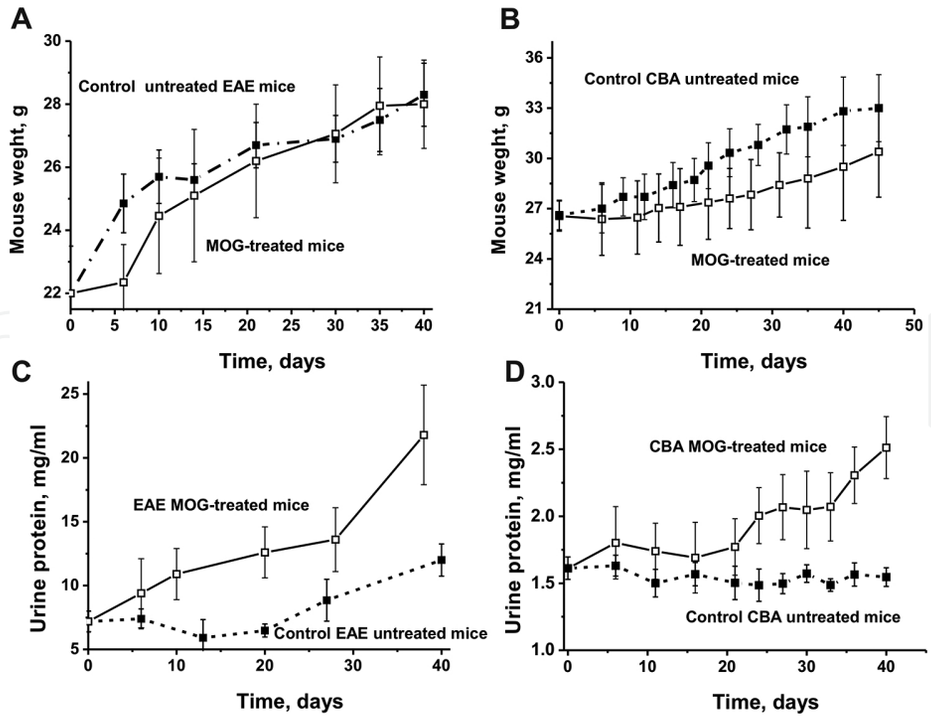

Figure 10. In time changes in weight of C57B2/6 (A) and CBA (B) mice, as well as relative concentration of urine proteins of C57B2/6 (C) and CBA (D) mice in the case of MOG-treated and untreated animals (see Panels) [110, 111].

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The sera of healthy humans and mammals usually contain auto-Abs to many various antigens, including DNA [13–21, 59]. For example, the average concentration of anti-DNA Abs for BALB and CBA non-autoimmune mice is approximately 0.03–0.04 A450 units, which is comparable with that for healthy MRL-lpr/lpr mice (0.032 A450 units); then after the spontaneous development of SLE it increases to 0.2 A450 units [24]. Untreated C57BL/6 mice demonstrated an approximately 3.5-fold higher concentration of anti-DNA Abs (0.12 ± 0.04 A450 units) at 3 months of age (day 0) comparing with MRL-lpr/lpr mice. For untreated C57BL/6 mice, the change in anti-DNA Abs during 0–40 days was statistically insignificant (**Figure 11A**). Comparing to healthy control individuals and other neurological diseases of non-AI origin, the sera of MS patients contain anti-DNA Abs at a higher concentration (0.22 A450 units) [19, 21]. Immunization of C57BL/6 mice with MOG during 30 days (0.24 A450 units) led to a near-linear statistically significant twofold increase in anti-DNA Ab concentration, and then to a further additional increase to 0.42 A450 units, which is higher than that for spontaneous disease (0.12 A450 units) (**Figure 11A**), but lower than that for MRL-lpr/lpr mice immunized with DNA (0.6 A450 units) [24].

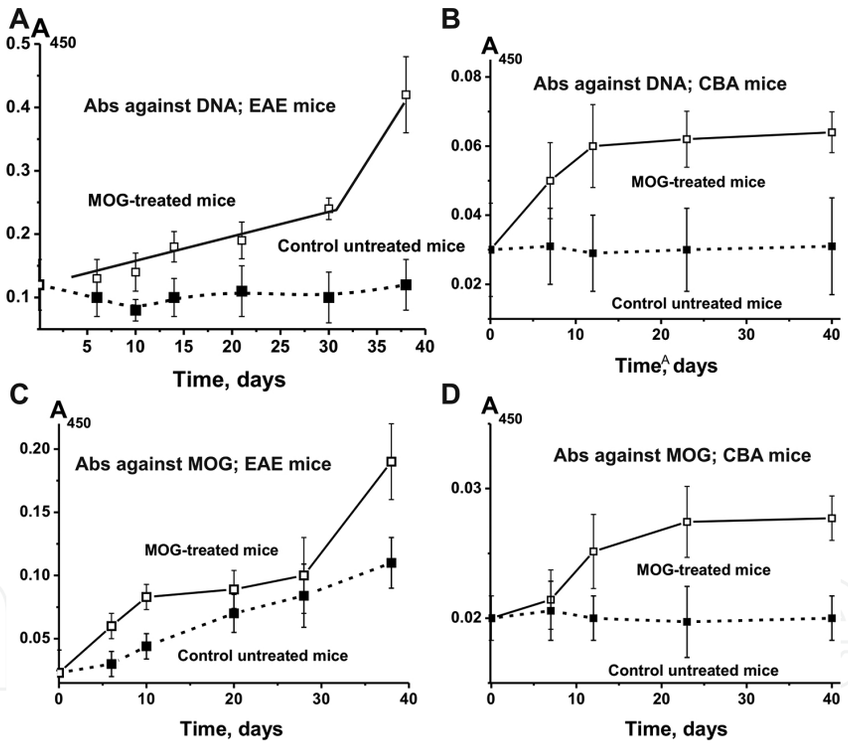

Figure 11. Relative concentrations of Abs to DNA in the case of C57B2/6 (A) and CBA (B) mice as well as to MOG for C57B2/6 (C) and CBA (D) untreated and treated with MOG (see Panels) [110, 111]. Anti-MOG and anti-MBP antibody concentrations in the sera of C57B2/6 and CBA mice were measured by ELISA (sera was diluted 50-fold). The concentration of plasma anti-DNA Abs was determined using standard ELISA plates with immobilized double-stranded DNA (sera was diluted 100-fold).

Untreated CBA mice demonstrated at zero time fourfold lower average concentration of anti-DNA Abs (0.03 A450 units) comparing with EAE mice (0.12 A450 units), and their concentration was not increase remarkably during 40 days (**Figure 11B**). After CBA mice treatment with

MOG, the average concentration of anti-DNA Abs increased ~twofold at 12 days (0.06 A450 units) and then was the same up to 40 days (**Figure 11B**). Finally at 40 days, anti-DNA concentration in treated CBA mice was ~sevenfold lower than for EAE mice immunized with MOG (**Figure 11**). Thus, the immunization of CBA mice with MOG can somehow stimulate their immune response to DNA in these healthy mice, but it is significantly lower than that in the case of autoimmune EAE C57BL/6 mice.

Human healthy donors show a relative average index of anti-MBP Abs – 0.08 ± 0.04 A450, which is ~four- and fivefold lower than that for MS patients (0.32 ± 0.08 A450 units) [44]. The concentration of anti-MOG Abs in the sera of C57BL/6 control (untreated) mice demonstrated a near-linear statistically significant approximately 4.8-fold increase during 40 days (from 0.023 to 0.11 A450 units; **Figure 11C**). After treatment of mice, a significant rise in the titter at 10 days (from 0.023 to 0.083 A450 units) was revealed, followed by a temporary plateau and then an additional increase to 0.18 A450 units. The concentrations of anti-MOG Abs at zero time for C57BL/6 and CBA mice were to some extent comparable (~0.02 A450 units). However, in contrast to EAE mice, CBA mice did not demonstrate remarkable change in anti-MOG Abs over time (**Figure 11D**). The treatment of CBA mice with MOG led to an increase in the average concentration of anti-MOG Abs ~1.4-fold, but this value (0.028 A450 units) is ~6.5- and ~fourfold, respectively lower than those for treated (0.18 A450 units) and untreated (0.11 A450 units) EAE mice (**Figure 11**). Thus, the immunization of CBA mice with MOG stimulates formation of Abs against this antigen, but these healthy mice in contrast to EAE mice do not demonstrate spontaneous development of autoimmune reactions leading to the formation of anti-MOG antibodies. These data indicate that the mice treatment with MOG leads to the formation of Abs against MOG and to DNA. At the same time, the relative concentrations of Abs to different antigens are not always good indicators of the real development of autoimmune diseases, because the relative titters to autoantigens in healthy humans and mammals can be very different [13–21, 59]. The titters of antibodies against autoantigens in sick patients and animals are usually higher than those in healthy individuals only in very late stages of ADs. As we have shown previously, the detection of DNase Abs in human sera may be considered to be a good indicator for the beginning of, or a significant development in, AI reactions associated with several ADs [13–21]. Moreover, like in AI patients, only DNase and ATPase activities of IgGs can be considered as statistically significant indicators of pre-disease conditions of spontaneous SLE in AI prone MRL-lpr/lpr mice [22–24]. Notably, well detectable DNase and ATPase Abs in MRL-lpr/lpr mice can sometimes be revealed 1–2 months earlier than a statistically reliable increase in Abs against DNA, as well as detection of visual and biochemical indexes of mouse SLE [22–24]. We have estimated the relative DNase and proteolytic activities of IgGs from untreated EAE and CBA and MOG-treated mice (**Figure 12**). Surprisingly, detectable levels of IgGs with all activities (in contrast to MRL-lpr/lpr) were revealed the sera of untreated C57BL/6 mice [22–24] even at the beginning of the experiment (at 3 months of age) and then it increased near-linearly. After 40 days, all activities were statistically significantly higher than at day 0: DNase (6.1-fold), MOG-hydrolyzing (1.8-fold), MBP-hydrolyzing (2.8-fold) (**Figure 12**), while there was no statistically significant increase in the concentrations of anti-DNA Abs (**Figure 11**). Furthermore, the detectable increase in abzymes from immunized EAE mice hydrolyzing DNA, MOG, and MBP was observed at a

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time corresponding to the outset and acute phase of EAE (6–8 days), followed by a statistically significant increase in their activities at 14 days, and maximal increases at 22 days after treatment with MOG (**Figure 12**). Interestingly, a significant decrease in the activities was revealed at the transition from the acute (18–22 days) to the severe chronic phase of EAE (40 days) induced by MOG.

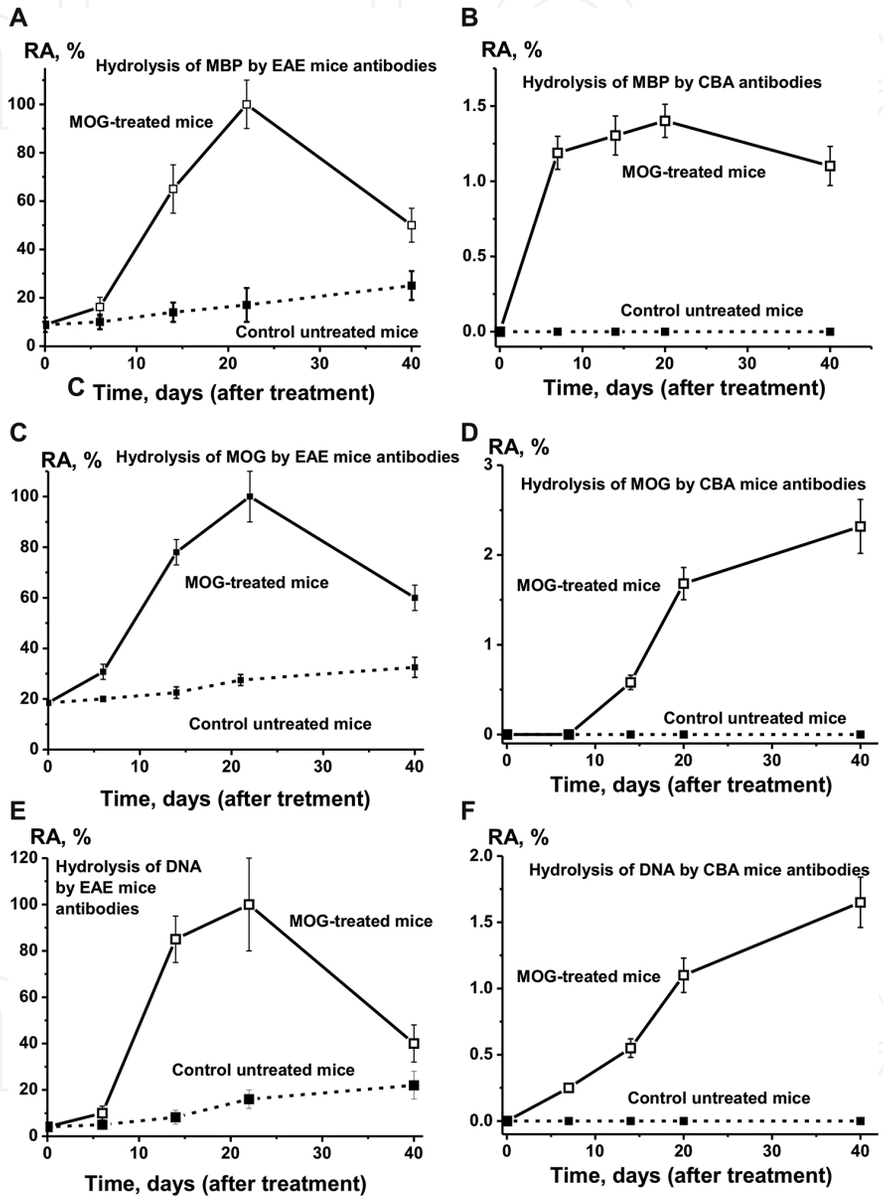

Figure 12. In time changes in the relative average activity of IgGs from sera of untreated and MOG-treated EAE C57B2/6 and CBA mice in the hydrolysis of MBP (A, B), MOG (C, D) and DNA (E, F) [110, 111]. To quantify the MOG-, MBP-, and DNA-hydrolyzing activities of IgGs of different groups, we determined a concentration for each individual IgG preparation (0.03–0.2 mg/ml) and time of incubation (1–24 h) providing hydrolysis (10–40%) of these substrates to their products. Since all measurements (initial rates) were taken within the linear regions of the time courses and Abs concentration curves, the measured RAs for IgGs were normalized to standard conditions and maximal enzymatic activities were taken as 100% of activity analyzed. The average relative activities (RAs) of IgGs corresponding to 7 individual mice for each group are given.

It was shown earlier that Abs from sera of healthy mice and rabbit are completely inactive in the hydrolysis of different substrates including DNA and proteins [13–21]. Immunization of healthy animals with complex DNA and methylated BSA (m-BSA) led to the formation of Abs-hydrolyzing DNA and RNA [116, 117]. The averages relative activities of Abs from sera of immunized animals are usually 100- to 1000-fold lower those from patients with autoimmune diseases [13–21, 115, 116].

IgGs from sera of CBA untreated mice were completely inactive in the hydrolysis of MBP, MOG, and DNA (**Figure 12**). After treatment of CBA mice, IgGs demonstrated detectable activity in the hydrolysis of these substrates, but their maximal relative activities in the hydrolysis of MBP, MOG, and DNA were approximately 40- to 70-fold lower than those for Abs of immunized EAE mice (**Figure 12**). In addition, IgGs from sera of EAE mice at three months of age were significantly more active than maximal activity of Abs of immunized CBA mice in the hydrolysis of all substrates: MBP (6.4-fold), MOG (6.5-fold), and DNA (2.5-fold) (**Figure 12**). It means that spontaneous autoimmune processes and the appearance of the cells producing different Abzs begin to develop in EAE mice before 3 months of their age.

Interestingly, during 40 days, the average relative DNA-hydrolyzing activity increased in EAE mice by a factor of 6.1 (**Figure 12E**), while statistically significant increase in the titters of anti-DNA Abs, there was not observed (**Figure 11A**). Since it was previously shown that the relative content of Abzs to different antigens in the total pools of Abs is usually ≤ 0.01—3%, this phenomenon is not surprising, [13–21]. Therefore, the production of a small fraction of specific DNase and protease Abzs may have no detectable effect on the total concentration anti-DNA and anti-MOG Abs.

6.1. Proliferation and apoptosis of lymphocytes in different organs of EAE prone mice

Immunization of healthy and AI-prone mammals usually leads to in an increase in the level of lymphocytes differentiation and proliferation in different organs [19–21]. Different lymphocytes producing Abzs were obtained with a dramatically higher incidence in autoimmune mouse strains than in conventionally used control mouse strains [117, 118]. At the same time, immunization of animals with some antigens usually leads to an increase in apoptosis of some lymphocytes. Apoptotic cells are considered as the primary source of antigens and immunogens in SLE triggering the recognition, perception, processing, and/or presentation of apoptotic autoantigens by antigen-presenting cells and can cause AI processes [60]. DNA-hydrolyzing DNase Abzs from patients with SLE [68] and MS [16], as well as DNase Bence-Jones proteins from multiple myeloma patients [119] are cytotoxic, cause nuclear DNA fragmentation, and induce cell apoptosis. A significant decrease in apoptosis can be an important factor providing the enhancement in the level of specific lymphocytes producing auto-Abs and Abzs, which are normally eliminated in different mammalian organs. Thus, the total number of lymphocytes of various types in different organs after immunization of animals depends on the ratio of possible levels of cell proliferation and apoptosis. The overall level of cytotoxic (harmful) or beneficial to organisms antibodies may depend on this ratio.

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We have compared possible lymphocyte proliferation (**Figure 13**) and apoptosis (**Figure 14**) in different organs of EAE and CBA mice. Lymphocyte proliferation in different organs at 3 months of age (zero time) for EAE and CBA mice is to some extent comparable (**Figure 13**).

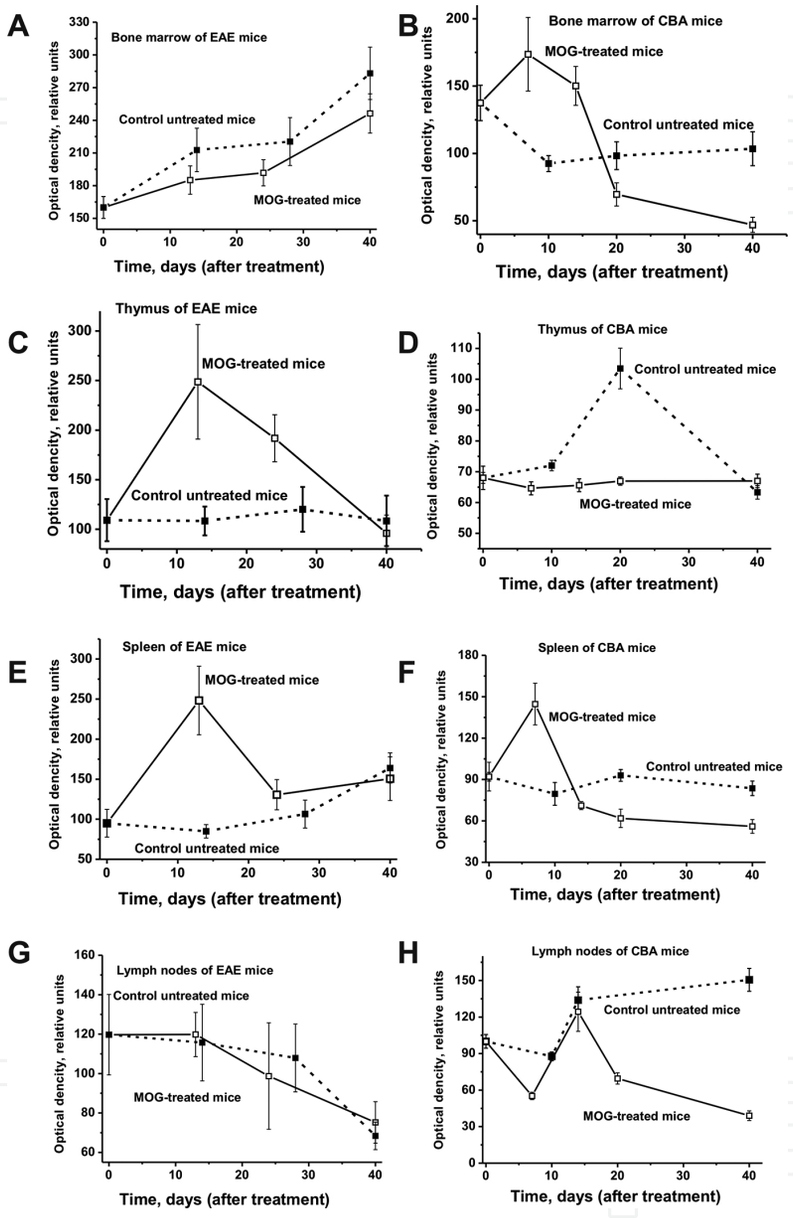

Figure 13. In time changes in lymphocyte proliferation (optical density) in bone marrow, thymus, spleen, and lymph nodes in the case of untreated and MOG-treated EAE and CBA mice; average values corresponding to 7 mice of each group are given [110, 111]. The error in the lymphocyte determination from three independent experiments in the case of every mouse of each group did not exceed 7–10%. Types of mice and their organs are given on panels A–H.

Proliferation of lymphocytes in bone marrow of untreated EAE mice increases constantly over time, and their treatment with MOG slightly decreases the proliferation level (**Figure 13A**). At the same time, untreated CBA mice demonstrate a remarkable decrease in the cell proliferation level over time. However, immunization of these mice with MOG increases their proliferation

in the period from 10 to 15 days, but then it is significantly decreased (**Figure 13B**). Maximal observed level of lymphocytes proliferation in bone marrow CBA mice (~26%) is ~threefold lower than that for EAE mice (~77%). The total number of lymphocytes resulting in the brains of EAE mice can be significantly higher than the observed one, since the average level of cell apoptosis in the brains of these mice is about 14.2% and increased up to 22.4% after their treatment with MOG (**Figure 14A**). Interestingly, at three month of age, CBA mice (4.2%) demonstrate ~3.4-fold lower the average level of cell apoptosis comparing with EAE mice, while their immunization with MOG increase it only to 4.6% (**Figure 14B**), 4.9-fold lower than that for EAE mice (**Figure 14A**).

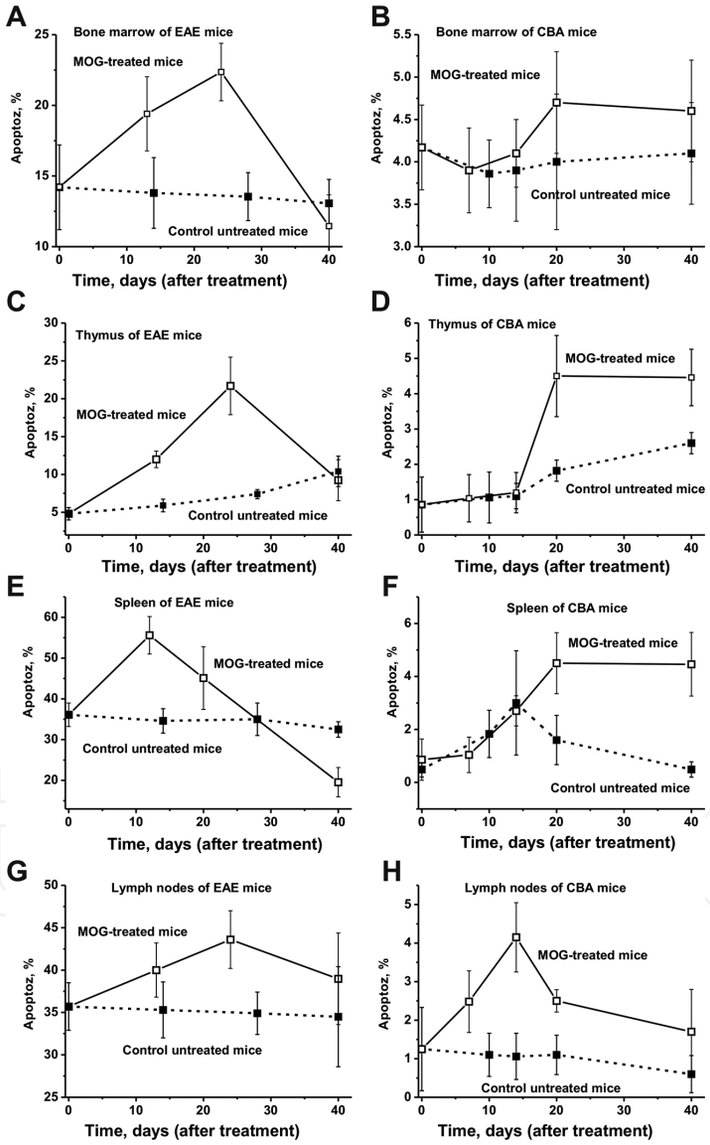

Figure 14. In time changes in cell apoptosis (%) in bone marrow, thymus, spleen, and lymph nodes in the case of untreated and MOG-treated EAE and CBA mice; average values corresponding to 7 mice of each group are given [110, 111]. The error in the cell apoptosis determination from three independent experiments in the case of every mouse of each group did not exceed 7–10%. Types of mice and their organs are given on panels A–H.

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The level of lymphocyte proliferation in thymus of untreated EAE mice almost does not change in time (**Figures 13C**), while it increases significantly at 20 days and then return to the level observed at zero time at 40 days (**Figure 13D**). The converse situation is observed for levels of the lymphocytes proliferation of EAE and CBA mice after their immunization with MOG (**Figures 13C** and **D**). Interestingly, the level of the thymus cell apoptosis in healthy CBA (~0.9%) at three month of age is about fivefold lower than that for EAE mice (~4.8%); after mice treatment with MOG, it increases in EAE mice up to 22%, but remains relatively low (~4.8%) in CBA animals. Thus, in contrast to EAE animals, the apoptosis does not affect significantly on the level of lymphocytes proliferation in thymus of CBA mice.

While the level of lymphocytes proliferation in the spleen of untreated EAE mice remarkably increases over time, in CBA mice, it has tendency of only slight decrease (**Figures 13 E** and **F**). At the beginning of autoimmune processes in MOG-immunized EAE mice (7–15 days), there is a strong increase in spleen lymphocytes proliferation for EAE and CBA mice, but the maximum value for autoimmune mice is significantly higher (**Figures 13E** and **F**). Interestingly, the relative level of spleen cell apoptosis in non-immunized EAE mice at three months is very high (~36%) compared to the bone marrow (14.2%) and especially to the thymus (4.8%), but it remains practically unchanged during the spontaneous development of the EAE pathology (**Figure 14E**). At three months of age, CBA mice are characterized by a very low level of spleen cell apoptosis, 0.5%, and its increases at 14 days to 3%, but it returns to 0.5% at 40 days (**Figure 14F**). The treatment of EAE mice leads first (12 days) to an increase in the proliferation from 36% to 55.6%, and then to significant decrease to 19.5% at 40 days. Approximately after 20 days of CBA mice treatment with MOG the cell proliferation increase to ~5%, this is ~11-fold lower than that for EAE mice (**Figure 14F**). Thus, immune processes in the spleen of control healthy CBA mice before and after their treatment with MOG vary relatively weak over time. However, a slow increase in the number of lymphocytes in the spleen of EAE mice during spontaneous develop of the disease and its acceleration after treatment with MOG are associated with strong parallel opposite processes in the lymphocytes proliferation and apoptosis (**Figures 13** and **14E** and **F**). These data are consistent with the fact that immunization of MRL-lpr/lpr mice at three months of age with DNA also leads to a very strong activation of the proliferation and apoptosis in their spleen [22–24].

Lymph nodes of EAE and CBA mice demonstrate different profiles of the cell proliferation over time (**Figures 13 G** and **H**). The level of cell proliferation in lymph nodes of EAE mice before and after animals' treatment with MOG is nearly the same; the cell proliferation is constantly decreased from 3 to 40 days. The level of lymphocytes proliferation in the lymph nodes of CBA mice at 6–10 days before and after their treatment is remarkably decreased (**Figure 13H**). Then, untreated and treated CBA mice show remarkable increase in the proliferation level, but finally control mice at 40 days demonstrate additional increase, while immunized mice—significant decrease in cell proliferation. It should be mentioned that lymph nodes (~36%) similarly to spleen (~36%) of EAE mice are characterized by very high level of cell apoptosis at three months of age, while this level for CBA mice is very low (~1.3%) (**Figure 14H**). Parallel high-level differently directed processes of the cell proliferation and apoptosis in lymph nodes of EAE mice can probably to some extent counterbalance each other. High

level of the apoptosis in the lymph nodes of non-treated, but more powerful apoptosis in treated EAE mice can be a reason of a slow decrease in lymphocytes of this organ of autoimmune mice leading to a comparable profiles of the changes over time (**Figures 13** and **14**). The relative changes in the levels of proliferation and apoptosis in lymph nodes of CBA comparing with EAE mice are relatively low (**Figures 13** and **14**).

Overall, the autoimmune EAE and non-autoimmune CBA mice demonstrate very different profiles of differentiation and proliferation of bone marrow stem cells, as well as levels of proliferation and apoptosis of lymphocytes in various organs.

Primary differentiation of lymphocytes begins in the bone morrow of people and animals, and additional differentiation occurs in their various organs. Therefore, possible relative levels of cell differentiation, proliferation, and apoptosis in various organs of mice depends on what type of hematopoietic stem cells was "arrived" to these organs from their bone marrow. As it was mentioned above, autoimmune diseases can originate from defects in hematopoietic stem cells [107]. Therefore, it was very important to understand a possible difference and similarity in differentiation and proliferation of stem cells in the bone marrow of mice, which are prone and unwilling to autoimmune diseases.

6.2. Differentiation of stem cells in the bone marrow of mice

The specific reorganization of the immune system in a profound SLE-like pathology of MRL-lpr/lpr mice during its spontaneous development is associated with changes in the differentiation profile of bone marrow HSCs, leading to the rise of the level of lymphocyte proliferation in combination with the production of different Abzs [22–24]. Since similar to MRL-lpr/lpr, C57BL/6 mice also reveal features of autoimmune-prone mice, it was reasonable to expect similar changes in the HSCs: erythroid burst-forming unit (early erythroid colonies; BFU-E), erythroid burst-forming unit (late erythroid colonies; CFU-E), granulocytic-macrophagic colony-forming unit (CFU-GM), and granulocytic-erythroid-megacaryocytic-macrophagic colony-forming unit (CFU-GEMM) in these mice before and after their immunization with MOG. But it was also very interesting to compare changes in the HSCs for autoimmune C57BL/6 and non-autoimmune CBA mice.

The relative amount of different colonies (BFU-E + CFU-E + CFU-GM + CFU-GEMM) of three monthly autoimmune EAE and normal healthy CBA mice was different. In order to calculate the relative amount of four different types of colonies, we have estimated a relative percent of every type of the colonies with respect to the total number of colonies taken as 100% (**Table 2**). It was shown that the average relative number of BFU-E (57.3 ± 20.1 and 54.5 ± 7.0%), CFU-GM (34.0 ± 3.9 and 28.2 ± 2.0%) colonies, respectively, for C57BL/6 and CBA mice at three months of age was to some extent comparable (**Table 2**). At the same time, the relative average number of CFU-E (3.2 ± 1.7%) for EAE mice was 4.6 lower than that for CBA mice (14.7 ± 10.5%), while in the case of CFU-GEMM, the reverse situation was observed; this average value for EAE mice (5.6 ± 1.7%) was ~2.2-fold higher than that for CBA mice (2.6 ± 0.78%) (**Table 2**). In time changes in the proliferation profiles of the HSCs, during 40 days, it was observed for untreated and MOG-treated autoimmune C57BL/6 and non-autoimmune CBA mice.

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Mice	The average relative content, %			
	BFU-E	CFU-E	CFU-GM	CFU-GEMM
EAE, C57BL/6 (1)**	57.3±20.1	3.2±1.7	34.0±3.9	5.6±1.7
Healthy, CBA (2)	54.5±7.0	14.7±10.5	28.2±2.0	2.6±0.78
Ratio of values 1 and 2 or (2 and 1)	1.1 (0.95)	0.22 (4.6)	1.2 (0.83)	2.2 (0.46)

*
Total amount of four types of colonies was taken for 100 %; the average meaning± average deviation are given.
**
Parameters 1 and 2 from [110] and [111], respectively.

Table 2. The average relative content of different colonies of EAE and CBA mice at three month of age (zero time).

It was revealed that there was a constant increase in the relative amount of BFU-E in untreated EAE mice to 28–30 days and then was observe a small decrease (**Figure 15A**). After the treatment of EAE mice with MOG, there was a significant decrease in BFU-E colonies up to 18–20 days and then there was a significant increase (**Figure 15A**). In the case of healthy CBA mice, we revealed quite opposite situation; the relative number of BFU-E in time for untreated mice was decreased, while after treatment with MOG it was significantly increased (**Figure 15B**).

In time changes of CFU-E, before and after treatment of EAE mice was comparable; first there was significant increase from 0 to 10 days and then from 10 to 40 days the number of these colonies slowly, but strongly decreased (**Figure 15C**). CFU-E colonies demonstrated different changes over time in the case of CBA mice. Before and after CBA mice treatment, the relative number of CFU-E cells was remarkably decreased from 0 to approximately 10–12 days (**Figure 15D**). At the same time, later we observed significant increase in CFU-E for MOG-treated CBA mice, but brightly expressed decrease of the cells in the case of the untreated mice (**Figure 15D**).

Quite opposite types in the differentiation were also observed for CFU-GM forming units in the case of the EAE and CBA mice (**Figures 15 E** and **F**). While the relative number of CFU-GM cells in EAE mice over time was dramatically decreased (0–30 days), in the case of CBA mice, it was remarkably increased (0–40 days). The MOG-treatment of EAE mice led to a slight increase in the number of the CFU-GM colonies, while for CBA mice there was observed a constant decrease the number of these colonies (**Figure 15E** and **F**).

Interestingly, a significant decrease in the CFU-GEMM units in treated EAE mice was observed at 10–40 days, while the level of their decrease for untreated mice was observed only after 20 days (**Figure 15G**). In the case of untreated CBA mice, the relative number of CFU-GEMM units was significantly increased from 0 to 25 days, but then it began to decrease, while treatment of CBA mice with MOG stimulated a constant decrease in the number of these colonies (**Figure 15H**). Thus, the autoimmune C57BL/6 and non-autoimmune CBA mice of three-month age demonstrate comparable percent of BFU-E and CFU-GM colonies, but significant difference in the relative number of CFU-E and CFU-GEMM units (**Figure 15**). In addition, these mice show a completely opposite profile of stem cells differentiation before

and after their treatment with MOG (**Figure 15**). However, changes in the differentiation profile in the case of untreated CBA mice are not associated with the appearance of cells producing Abzs; Abs from the sera of these mice are completely catalytically inactive. Immunization of CBA mice with MOG stimulates production of Abs-hydrolyzing MBP, MOG, and DNA, but the activities of these Abzs are very low comparing with those for EAE mice. Specific differentiation of bone marrow stem cells of EAE mice even before three months of their age leads to the appearance of cells producing abzyme with relatively high activity. During spontaneous development of EAE in C57BL/6 mice, there is a significant increase in such cells and Abzs, while their immunization stimulates a very strong increase in the Abzs activities.

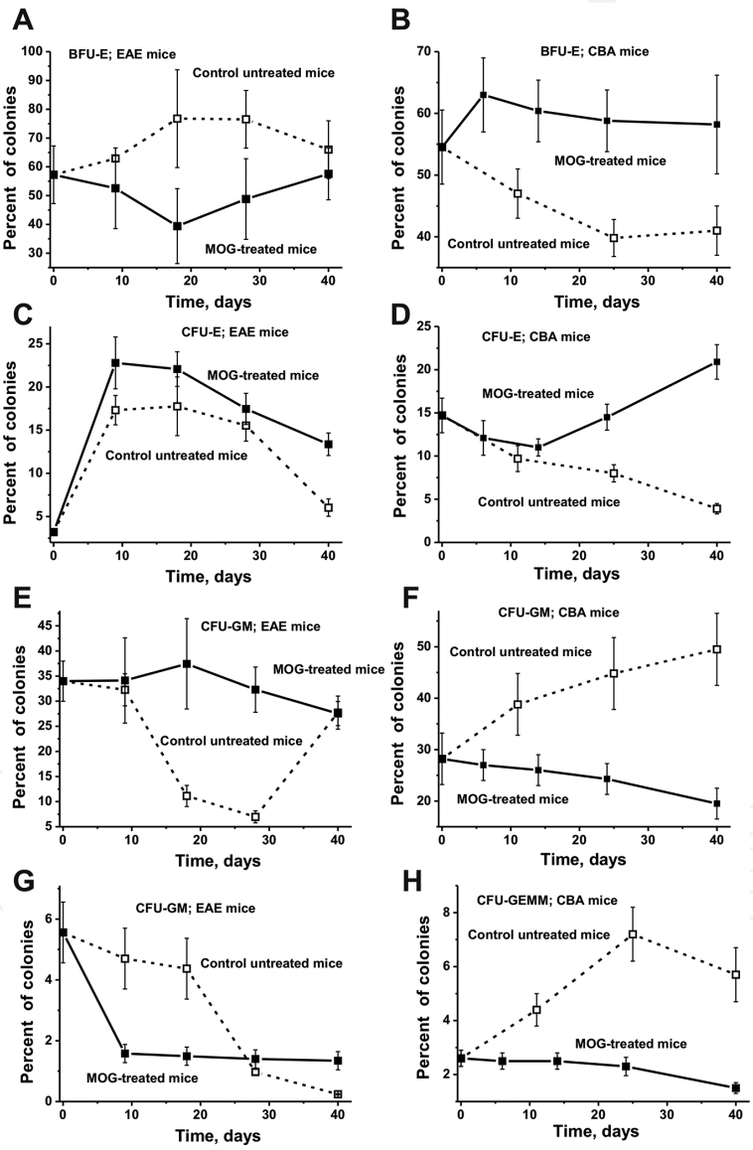

Figure 15. In time changes of an average relative content (%) of colony-forming units of bone marrow progenitor colonies of different type in the case of untreated and MOG-treated mice; average percent of different colonies (BFU-E + CFU-E + CFU-GM + CFU-GEMM) corresponding to 7 mice of each group of autoimmune C57B2/6 and non-autoimmune CBA mice are given; types of progenitor colonies and mice analyzed are shown on panels A–H.

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Cuprizone-induced demyelination is a widely used experimental model to study processes of de- and remyelination in the central nervous system [120–123]. We used EAE C57BL/6 mice to assess effects of cuprizone-induced demyelination on different parameters associated with autoimmune inflammation [124]. It was shown that treatment of mice with cuprizone (0–40 days) leads to a significant decrease in the size of the brain corpus callosum when compared with untreated mice. In addition, cuprizone treatment leads to a significant decrease of several indexes, which characterize spontaneous and MOG-induced EAE: increased levels of proteinuria, titters of anti-DNA and anti-MOG antibodies, the generation of Abzshydrolyzing DNA, myelin basic protein (MBP), and MOG. As it mentioned above, spontaneous and MOG-induced EAE is associated with a specific reorganization of the immune system resulting in changes in the profile and level of proliferation of mice HSCs. At day 40 of the experiment, cuprizone decreases the summed proliferation of HSCs (BFU-E, CFU-E, CFU-GM and CFU-GEMM) colony units ~1.2-fold in comparison with untreated mice, but does not significantly change the differentiation profile of BFU-E, CFU-GM, and CFU-GEMM cells. Our data indicate that cuprizone treatment is associated with demyelination, but not with stimulation of autoimmune processes.

It was shown that cuprizone has selective specificity against oligodendrocytes [120, 121]. Acute demyelination following treatment with cuprizone is associated with apoptosis of oligodendrocytes, activation of microglia, and phagocytosis of myelin sheaths [122, 123]. Cuprizone causes demyelination processes all over the brain, with the corpus callosum being the most affected structure. Also distinct foci of demyelination are found in the hippocampus, cerebellum, putamen, and the ventral part of caudal nuclei.

According to our data, cuprizone-induced demyelination is likely not associated with the development of typical autoimmune processes observed after spontaneous or MOG-induced EAE. It looks more like a specific poisoning of mice with cuprizone, leading to the manifestation of some symptoms seen in EAE. The effect of a cuprizone leading to the suppression of cell proliferation and activation of apoptosis [120–123] is consistent with our data on the decrease in the level of lymphocyte proliferation and inhibition of profile differentiation changes of stem cells in the bone marrow. A maximal decrease in the RAs of all Abzs after EAE mice treatment with cuprizone correlates with twofold decrease in the proliferation only CFU-E cells, while its effect on proliferation of other cells was negligible. The relative number of CFU-E colonies over time is significantly decreased in untreated CBA mice (**Figure 15D**), which Abs are completely inactive in the hydrolysis of MBP, MOG, and DNA (**Figure 12**). In addition, maximal activities of Abzs (**Figure 12**) correlate with maximal increase of CFU-E cells in treated CBA mice (**Figure 15**). Moreover, maximal increase in the activities of Abzs in MOG-treated EAE mice at 10–20 days (**Figure 12**) correlates with a sharp increase in the relative amount of these cells at this time (**Figure 15C**). The totality of our data suggests that it is the CFU-E cells may be early progenitors of cells producing catalytic antibodies.

On the one hand, in the debut of various autoimmune diseases usually occurs production of auto-antibodies to different autoantigens and abzyme with different catalytic activities. On the other hand, the repertoires of abzymes with different enzymatic activities and autoantibodies

to various autoantigens are constantly expanding. This is indicative of the fact that the basis of all autoimmune diseases may be to some extent similar.

7. Comparison of stem cell differentiation in SLE and EAE mice

C57BL/6 and MRL-lpr–lpr are two various mouse models spontaneously developing two different autoimmune diseases, EAE and SLE, respectively. Stimulation of EAE pathology development usually occurs after immunization of C57BL/6 mice with MOG [112, 113], while SLE after treatment of MRL-lpr–lpr mice with DNA [22–24]. It was interesting to understand a possible difference or similarity in the changing of the differentiation profiles of stem cells of the bone marrow in the case of these models comparing with those for the control CBA mice before and after their treatment with MOG and with DNA. **Figure 16** demonstrates the relative levels BFU-E, CFU-E, CFU-GM, and CFU-GEMM units (%) at the beginning (zero time), spontaneous changes and after mice treatment with MOG (57BL/6) and DNA (MRL-lpr–lpr) at 40 days of the experiments. One can see that the relative content of BFU-E colonies (%) constantly decrease in autoimmune EAE and SLE mice at transition from zero time to spontaneous development of these diseases (40 days) and acceleration of their development by treatment with MOG and DNA at 40 days (**Figure 16A**).

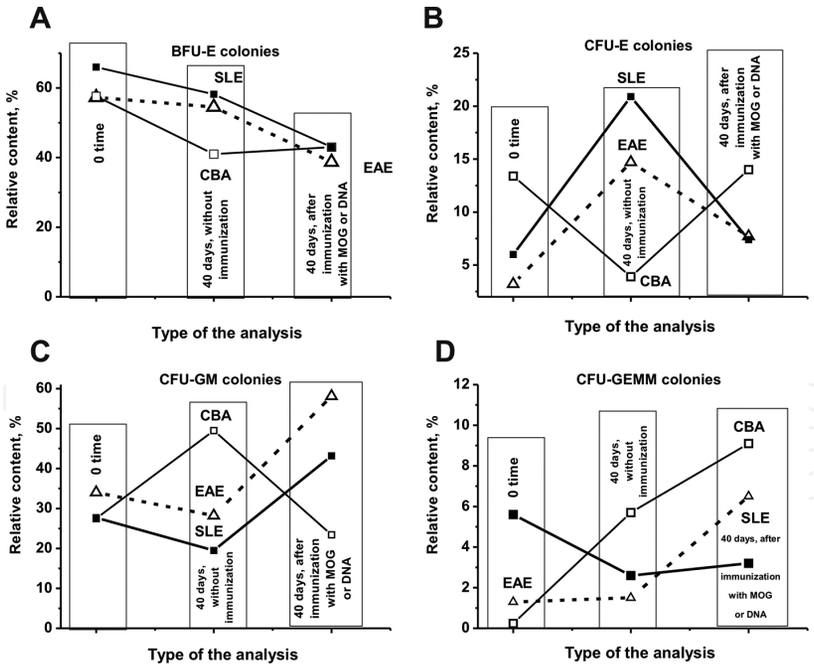

Figure 16. Change in the relative percent (sum of four types of colonies was taken for 100%) of BFU-E (A), CFU-E (B), CFU-GM (C), and BFU-GEMM (D) types of colonies in comparison with zero time of the experiments (first group of values) in the case of spontaneous development of EAE and SLE by respectively C57BL/6 and MRL-lpr–lpr mice after 40 days and changes of differentiation profile of HSCs in CBA mice after 40 days (second group of values); third group of values corresponds to relative amount of the colonies after 40 days in the case C57BL/6 and CBA mice treated with MOG [110, 111] and MRL-lpr–lpr mice immunized with DNA [22–24].

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Non-autoimmune CBA mice demonstrate at 40 days a decrease in the percent of BFU-E, when their treatment with MOG leads to remarkable increase in the relative number of these cells. Very similar regularities in the changes of the relative content of CFU-E (**Figure 16B**) and CFU-GM (**Figure 16C**) are observed for EAE and SLE mice, and they are directly opposite than that for the CBA mice. There is a remarkable but not essentially important difference in the curves corresponding relative number of CFU-GEMM colonies for EAE and SLE mice, but they are very different to that for CBA mice (**Figure 16D**). Thus, it is obvious that over time some changes in the profile of stem cell differentiation can occur in the case of non-autoimmune and autoimmune mice. However, these changes are very different or even opposite for non-autoimmune and autoimmune mice during their growth (CBA) or spontaneous development of ADs (SLE and EAE) as well as after immunization of mice with different specific stimulators of autoimmune processes. Thus, one can suppose that SLE and EAE pathologies in two different autoimmune lines of mice on overall demonstrate very similar regularities of change in differentiation profiles of bone marrow stem cells. Interestingly, at 40 days corresponding to spontaneous development of SLE in MRL-lpr/lpr and EAE in 57BL/6 mice demonstrating high activities of different Abzs, there is a strong increase in CFU-E colonies (**Figure 16B**). At the same time, very strong reduction in the relative number of only CFU-E colonies is observed in the case of CBA mice showing catalytically inactive antibodies (**Figure 16B**). This again is indicative of the fact that CFU-E can be precursors of cells producing Abzs in different organs. In this connection, it should be mentioned that IgGs from CSF of patients with MS 30- to 50-fold more active in the hydrolysis of MBP, DNA, and oligosaccharides comparing with Abs from sera of the same patients [110, 111]. It means, that even CSFs of patients with MS contain specific cells producing these catalytically active Abs.

8. Conclusion

It is obvious that in MS patients auto-Abs directed to nucleic acids, proteins, and polysaccharides with different catalytic functions may be induced by primary antigens. During the development of spontaneous and MOG-induced EAE in C57BL/6 mice, a specific reorganization of the immune system of mice takes place. This causes the generation of harmful catalytically active IgGs-hydrolyzing MOG, MBP, and DNA, as well as changes in differentiation of HSCs and to the increase in proliferation of lymphocytes and apoptosis in different organs [110]. Treatment of control non-autoimmune CBA mice with MOG led to different differentiation and proliferation of HSCs comparing with EAE C57BL/6 mice [111].

It was shown that DNA-, RNA-, oligosaccharide-, and MBP-hydrolyzing IgGs and/or IgAs and IgMs from patients with Ms are catalytically very heterogeneous; these Abzs can contain kappa- and lambda-types of light chains, demonstrate different affinity for substrates, different pH optima, may be metal-dependent or independent, and catalyze the hydrolysis of MBP as serine-like or metalloproteases. IgGs of four subclasses (IgG1–IgG4) are catalytically active in the hydrolysis of DNA and MBP, with their different contribution to the total activity of these Abzs in the hydrolysis of these substrates.

MS patients demonstrate some similarity with SLE patients in the development of the same medical, biochemical, immunological indexes including specific plaques in the brain, which appear on late stages of these diseases. Anti-DNA Abs is known as the main important diagnostic index for SLE, but these Abs were also identified as a major component of the intrathecal IgGs in brain and CNS cells of MS patients [40].

It is known that demyelinating plaques appear at relatively late stages in the development of MS, and their presence detected by brain MRI are essential for the diagnosis according to Mc Donald's criteria. At the same time, the detection of Abzs with DNase- and MBP-hydrolyzing activities was shown to be the earliest indicator of development of MS. Catalytic activities of nuclease and protease Abzs are usually very easily detectable at the onset of MS and other autoimmune diseases when the total concentrations of Abs to DNA, MBP, or other auto-antigens are still low and correspond to their ranges in healthy donors. Although Abzs with low activity can sometimes be detected in conventionally healthy people, the RAs of Abzs from MS and SLE patients are usually 1–3 orders of magnitude higher. Therefore, an appearance of some Abzs or a 10- to 100-fold increase in the activity of others over the average Abzs indices for healthy donors may be used as the earliest markers of autoimmune reactions in patients with MS and other autoimmune diseases.

Recognition and degradation of MBP peptides by serum auto-Abs was stated as a novel biomarker for MS [82]. But IgGs from SLE patients also efficiently hydrolyze MBP and oligopeptides corresponding to different AGDs of MBP [84, 85]. Thus, it is clear that early diagnostics of MS requires the use of all known independent methods to exclude SLE and probably other possible diseases leading to a formation of DNA- and MBP-hydrolyzing Abzs. Nevertheless, even revealing of DNase and RNase Abzs on early stages of MS may be very useful. For example, in the case of three patients, we have suggested the possibility of initial stages of MS, but the symptoms did not meet all Poser's and Mc Donald´s criteria. However, Abs found in the sera of these patients demonstrated a high DNase activity speaking in favor of a possibility of an early stage of MS. One and a half years later, these patients met Poser's and Mc Donald´ criteria, and after 2–3 consecutive years, brain plaques were also found in these patients.

The immune systems of individual MS patients generate Abzs, which can attack MBP of myelin-proteolipid shell of axons, while an established MS therapeutic Copaxone inhibits specific MBP-hydrolyzing activity of Abzs [82]. It means that the development of MS and probably SLE or other diseases associated with demyelination can be suppressed by specific inhibitors of MBP-hydrolyzing IgGs, IgAs, and IgMs.

All data indicative of the fact that CFU-E cells can be precursors of cells producing Abzs in different organs. In this connection, it should be mentioned that IgGs from CSF of patients with MS 30- to 50-fold more active in the hydrolysis of MBP, DNA, and oligosaccharides, then Abs from sera of the same patients [110, 111]. It means that even CSFs of patients with MS contain specific cells producing these catalytically active Abs. Moreover, DNase Abzs form SLE and MS patients are cytotoxic and induce apoptotic cell death. SLE and MS Abzs efficiently hydrolyzed polysaccharides. Therefore, it is very possible that abzymes with DNase, amylase,

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and MBP-hydrolyzing activities may in addition to other different factors cooperatively promote important neuropathological mechanisms in MS and SLE pathogenesis development.

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Abbreviations

Abs, antibodies; Abzs, abzymes, or catalytically active antibodies; AGDs, antigenic determinants; AI, autoimmune; AD, autoimmune disease; AIDS, human immunodeficiency syndrome; a/u, arbitrary units; BSA, bovine serum albumin; CC, correlation coefficient; HSCs, hematopoietic stem cells; CBA, (CBAxC57BL)F1 mice; MBP, human myelin basic protein; OP-17, OP-19, OP-21, and OP-25—17-25mer oligopeptides corresponding to four known MBP cleavage sites; MS, multiple sclerosis; m-BSA, methylated BSA; nat-DNA and den-DNA, native and denatured DNA, respectively; ODN, oligonucleotide, OP, oligopeptide; SLE- systemic lupus erythematosus, SDS-PAGE, SDS-polyacrylamide gel electrophoresis; RA, relative activity.

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