Chapter 3

Catalytic Antibodies in Norm and Systemic Lupus Erythematosus

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67790

Abstract

Systemic lupus erythematosus (SLE) is known as a systemic polyethiologic diffuse autoimmune disease characterized by connective tissue disorganization and the paramount damage of skin and visceral capillaries. Usually, SLE symptoms include high fever, hair loss, mouth ulcers, chest pain, swollen lymph nodes, painful and swollen joints, increased fatigue, and appearance of red rash more often on the face. The exact reason of SLE appearance is not really clear. Detection of catalytic Abs (abzymes) was shown to be the earliest indicator of different AI disease development. Some abzymes are cytotoxic and can play a dangerous negative role in the pathogenesis of AI diseases. SLE is characterized by the appearance of abzymes with several different catalytic functions including hydrolysis of peptides and proteins, DNA, RNA, and oligosaccharides. In addition, monoclonal SLE abzymes are characterized by extraordinary diversity in the affinity to the substrates, physicochemical and catalytic characteristics, optimal conditions of catalysis, cytotoxicity, etc. Production of abzymes in SLE mice is associated with changes in the differentiation of hematopoietic stem cells of bone marrow, increase in lymphocyte proliferation, and significant suppression of cell apoptosis in different organs. In this chapter, abzymes with different catalytic activities in SLE are described.

Keywords: systemic lupus erythematosus, catalytic antibodies, hydrolysis of RNA, DNA myelin basic protein, and oligosaccharides, apoptosis, cytotoxicity, diversity of monoclonal antibodies

1. Introduction

According to classical conception, antibodies (Abs) are specific proteins produced by the immune systems with exclusive function of antigen binding. Antibodies can act similarly to specific enzymes, but in contrast to enzymes, they cannot catalyze chemical conversions of

their ligands. For most part of antibodies, this observation is correct. At the same time during the last 30 years, it was shown that Abs against chemically stable analogues modeling the transition states of chemical reaction can catalyze many different reactions [1–8]. Such artificial catalytic antibodies were called abzymes (derived from antibody enzymes). Abzymes (Abzs) can catalyze more than 200 different chemical reactions and are new biological catalysts attracting great interest during recent years and are well described (for review see [1–8] and refs therein).

First, natural Abzs were found in patients with bronchial asthma; they hydrolyze vasoactive intestinal peptide [9]. Then IgGs hydrolyzing DNA were revealed in the blood of patients with systemic lupus erythematosus (SLE) [10]. The third natural Abzs were SLE IgGs with RNase activity [11]. To date, many catalytic Abs (IgGs and/or IgAs, IgMs) catalyzing hydrolysis of different RNA, DNA, nucleotides, oligopeptides, proteins, lipids, and oligosaccharides were revealed in the sera of patients with various autoimmune and viral diseases (for review, see Refs. [8, 12–22] and refs therein).

Some idiotypic Abzs against foreign antigens and auto-Abzs to self-antigens having different catalytic activities may be spontaneously induced by primary antigens simulating in varying degrees the transition states of chemical reactions [8, 12–22]. At the same time, some antiidiotypic Abs against active centers of many enzymes are also catalytically active [8, 12–22, 23–28]. Healthy humans do more often not demonstrate catalytic Abs or their activities are extremely low. It was shown that detection of Abzs is the earliest indicator of different autoimmune diseases (ADs) development [8, 12–22]. At the outset and early stages of ADs analyzed, the repertoire of abzymes is more often relatively narrow, but it is expanding very much with the progress of AI diseases; the generation of diverse Abzs with many various activities and functions may be observed [8, 12–22]. Some abzymes are cytotoxic and dangerous for people; they can play a very important negative role in the different AD pathogenesis [8, 12–22]. However, specific positive roles have been also proposed for several abzymes. Increase in Abzs activities is associated with a specific reorganization of the immune system including change in differentiation profile and level of proliferation of hematopoietic stem cells of bone marrow as well as lymphocyte proliferation in different organs of SLE and experimental autoimmune encephalomyelitis (EAE) in mice [29–32]. Different mechanisms of abzymes production were revealed in healthy animals after external immunization and in autoimmune mice during their spontaneous or antigen-induced development of autoimmune processes [29–32].

Catalysis of different reactions by abzymes is potentially important for many different fields; specific reaction for a synthesis of new drugs, which may be useful for therapy, estimation of abzyme's possible role in innate and adaptive immunity, as well as for understanding of destructive responses and self-tolerance in ADs [33–37].

In this chapter, Abzs with various catalytic activities in SLE are described and compared with abzymes in case of other autoimmune pathologies. In addition, a possible role of different defects of immune systems resulting in changes of differentiation profile of hematopoietic stem cells (HSCs) of mice bone marrow as well as an increase in lymphocyte proliferation in

http://dx.doi.org/10.5772/67790

thymus, bone marrow, spleen, and a significant suppression in these organs of cell apoptosis associated with the abzymes production is discussed.

2. Features of the immune status of patients with systemic lupus erythematosus

As mentioned above, SLE is systemic polyethiologic diffuse autoimmune disease, symptoms of which for different patients vary significantly and may be from mild to severe. The exact reason and mechanisms of SLE development till yet is not clear [38]. Genetic, environmental, hormonal, and immune factors may play an important role for the development of SLE. Many different autoimmune diseases (ADs) including SLE are characterized by spontaneous generation of primary antibodies to nucleotides, nucleic acids and their complexes, proteins, polypeptides, polysaccharides, etc. [8, 16–22, 36, 38–42]. Anti-DNA auto-Abs without catalytic activities are detectable even in the sera of healthy humans and their relative titres vary from individual to individual significantly [43, 44].

SLE is usually considered to be associated with autoimmunization of patients with DNA, since sera of such patients usually contain anti-DNA Abs and DNA in increased concentrations comparing with that for healthy volunteers [38]. However, comparing to healthy donors, anti-DNA antibody concentrations are higher not only in patients with SLE (36% of patients) [43, 45], but also in Hashimoto's thyroiditis (23%) [43], multiple sclerosis (17–18%) [43, 46, 47], rheumatoid arthritis (7%), myasthenia gravis (6%), and Sjogren's syndrome (18%) [43]. In addition, from the cloning of the IgG repertoire from directly active plaques and periplaque regions of brain and from B-cells of the cerebrospinal fluid of MS patient, new keys to the understanding of this pathology were proposed [48]. High affinity anti-DNA Abs were shown to be the major components of the intrathecal IgG response. In addition, monoclonal anti-DNA Abs of multiple sclerosis (MS) patients and Abs specific to DNA derived from SLE patients interact efficiently with the surface of neuronal cells and oligodendrocytes [48]. Recognition of cell-surface by these Abs was DNA-dependent. The data indicate that Abs against DNA may be important for autoimmune and neuropathological mechanisms in chronic SLE and MS [48]. Interestingly, SLE and MS patients show some similarity in the same medical, biochemical, and immunological indexes including anti-DNA and other auto-Abs [13–22].

Anti-DNA and anti-RNA Abs with DNase and RNase activities were for the first time detected in sera of SLE patients [10, 11] and then with other ADs [13–22]. The origin of natural Abzs is very complex. First, similar to artificial abzymes, they may originate against analogues of transition states of chemical reactions or against enzyme substrates acting as haptens [8–22]. Many antigens can change their conformation after association with various proteins, and in such complexes, their structure could mimic that of a transition state of chemical reaction substrate. For example, DNA is a bad antigen and immunization of animals with pure DNA or RNA leads to the production of abzymes with very low DNase and RNase activities [49, 50].

Many anti-DNA auto-Abs in SLE are directed against DNA-histone nucleosomal complexes, resulting from internucleosomal cleavage during apoptosis [42]. Apoptotic cells and their different components are the primary antigens as well as immunogens in SLE and are important for the recognition, processing, perception, and/or apoptotic autoantigen presentation by antigen-presenting cells during development of autoimmune processes [42]. Therefore, immunization of mice with complex of DNA and histones or positively charged methylated bovine albumin, simulating positively charged histones, results in production of anti-DNA Abs and DNase abzymes with high activity [29–31, 49, 50]. It was shown that abzymes with different activities may be obtained with a significantly higher incidence in autoimmune mouse strains comparing to conventionally used control nonautoimmune mice [51, 52]. Immunization of autoimmune-prone MRL-lpr/lpr mice with DNA-protein complexes also results in significantly higher production of anti-DNA Abs and abzymes with DNase activity comparing with nonautoimmune CBA and BALB/c healthy mice [29–31]. At the same time, artificial antiidiotypic abzymes can be induced by immunization of animals with different enzymes [23–28]. It was first suggested that natural SLE DNase Abzs may be antiidiotypic abzymes to topoisomerase I [53]. Immunization of rabbits with DNase I led to the production of Abzs with DNase activity of antiidiotypic nature [54]. Idiotypic Abs first were obtained by immunization of animals with DNase I and then they were used to elicit a polyclonal antiidiotypic Abs hydrolyzing DNA; it indicates for the existence of internal Abs structure mimicking active centre of DNase I [54]. We have suggested that polyclonal DNase Abzs in autoimmune patients may be a cocktail of abzymes against complexes of proteins with DNA and RNA and antiidiotypic abzymes to different DNA-hydrolyzing enzymes. Therefore, we have immunized rabbits with DNase II, DNase I, pancreatic RNase A, DNA, and RNA [49, 50, 55–57]. In all cases, abzymes with intrinsic DNase and RNase activities were revealed. IgGs against DNase I with DNase activity also have an antiidiotypic nature [55]. Interestingly, 74–85% of the total polyclonal IgGs against RNase A possessing RNase and DNase activities belong to antiidiotypic Abs, while 15–26% of the Abzs cannot interact with affinity sorbent-bearing Abs against RNase A; they bind with DNA- and RNA-Sepharoses and may be antibodies to nucleic acids bound to RNase [56]. In addition, only ~10% of the polyclonal total IgGs demonstrating DNase and RNase activities from sera of rabbits immunized with DNase II have antiidiotypic nature, while the remaining 90% of Abzs did not interact with Sepharose-bearing Abs against DNase II, they may also be Abs to nucleic acids bound to DNase II [57]. The relatively low amount of antiidiotypic abzymes against DNase II hydrolyzing DNA and RNA may be a consequence of low immunogenicity of DNase II active site comparing with other antigenic determinants of this nuclease. Antibodies against DNA and RNA complexes with proteins and other antinuclear components were found in the blood sera of patients with several multisystem connective tissue diseases including SLE [58]. Interestingly, abzymes agains DNA and RNA bound with proteins are usually significantly more active in the hydrolysis of these substrates than antiidiotypic Abzs against enzyme active centres [17–22, 49, 50, 55–57]. Thus, RNase A, DNase I, DNase II, and other DNA- and RNA-dependent enzymes can themselves be antigens producing not only antiidiotypic abzymes with corresponding active sites, but these enzymes can interact with RNA and DNA and induce formation of anti-RNA and or anti-DNA abzymes possessing no affinity for these enzymes, but having higher catalytic

activities than antiidiotypic Abzs. In addition, various proteins interacting with DNA and RNA can differ in their ability to produce antiidiotypic Abzs and the formation of abzymes against bound nucleic acids. Overall, it is clear that abzymes of patients with various autoimmune diseases can be very different cocktails of idiotypic antibodies directly against DNA, RNA, and against complexes of these antigens with different enzymes or proteins as well as antiidiotypic Abs against many DNA-dependent enzymes [17–22, 49, 50, 55–57].

3. Catalytic activities of SLE prone mice antibodies

It was shown that DNase Abzs of patients with SLE [59], MS [16], and DNA-hydrolyzing Bence-Jones proteins of patients with multiple myeloma [60] are cytotoxic, able to penetrate cell nucleus and cause fragmentation of nuclear DNA leading to cell apoptosis. A significant decrease in cell apoptosis in the case of ADs may be a very important factor providing the increase in the level of specific lymphocytes producing auto-Abs and abzymes, which are usually eliminated in different organs of healthy mammals [61, 62]. The cell apoptosis caused by Abzs with DNase activity leads to increase in the concentration of histones complexes with DNA fragments in the blood of mammals and, consequently, to production of antibodies against DNA and DNA-hydrolyzing abzymes. Thus, the appearance Abzs with DNase activity in the blood of mammals may be a very important factor in the strengthening of the autoimmune reactions [13–22]. The abzymes with DNase activity should be considered as very dangerous since they can stimulate development of autoimmune reactions. The overall level of autoimmune reactions may depend on the ratio of cytotoxic (harmful) and beneficial to organisms auto-Abs. Therefore, it was very interesting to elucidate what factors underlie in the AI processes development and how possible mechanisms of autoimmunity are associated with the production of abzymes. Some data suggest that various ADs can originate from defects in the hematopoietic stem cells (HSCs) [63]. Therefore, it was reasonable to analyze what defects or changes may be revealed in the HSCs during spontaneous and DNA-induced development of SLE in autoimmune prone MRL-lpr/lpr mice.

It is known that after spontaneous development of SLE, MRL-lpr/lpr mice are characterized by visual symptoms of autoimmune pathology (baldness of head and parts of the back, pink spots, general health deterioration, etc.). Appearance of pronounced visual symptoms usually well correlate with high proteinuria (≥3 mg/ml of protein concentration in urine) [64, 65]. It was shown previously that sera of spontaneously diseased MRL-lpr/lpr mice contain Abzs with high DNase activity correlating with high proteinuria and visual symptoms usually at age of 5–12 months [64, 65], which is a typical period of signs of deep SLE pathology of MRL-lpr/lpr mice [66]. Obviously, that the state of "health" in the case of autoimmune-prone mice should be considered quite conventional, the development autoimmune pathology nevertheless is spontaneous, and AI processes leading to deep pathology increase gradually. To distinguish different levels of SLE development, MRL-lpr/lpr mice without typical autoimmune symptoms and demonstrating no abzyme activities (similar to nonautoimmune healthy control mice) were independently of age tentatively designated as healthy mice, while the

animals having no visual or biochemical SLE symptoms but demonstrating detectable activities of abzymes were provisionally named as prediseased MRL-lpr/lpr mice. Mice demonstrating all visual symptoms and biochemical indexes of SLE were designated as diseased animals. We have compared healthy (2–3 months of age) and spontaneously diseased MRL-lpr/lpr mice with all visible symptoms no older than 7 months [29–31]. For a more precise characterization of the various states of these mice, we have evaluated a variety of medical, biochemical, and immunological characteristics of their status including the relative levels of Abs to various autoantigens of abzymes demonstrating different catalytic activities.

The average anti-DNA Abs concentration in the case of (CBAxC57BL)F1 and BALB/c nonautoimmune control mice was estimated to be approximately 0.03–0.04 A_{450} units and was comparable with that for healthy autoimmune-prone MRL-lpr/lpr mice (0.032 A_{450} units) [29–31]. After spontaneous development of SLE in MRL-lpr/lpr mice (depending of individual mice during 4–7 months), it increases to 0.2 A_{450} units, but there were no remarkable change in the anti-DNA Abs titers in the case of control nonautoimmune mice during 7–8 months of the experiment [29–31]. After MRL-lpr/lpr mice immunization with complex of methylated-BSA with DNA (further marked as DNA), the average concentration of anti-DNA Abs increased to approximately 0.6 A_{450} units [29–31]. It should be mentioned that IgG antibodies from the sera of control 2–7 month-old nonautoimmune CBA and BALB mice and conditionally healthy 2–3 months old MRL-lpr/lpr mice were shown to be catalytically inactive [29–31]. At the same time, during spontaneous development of SLE and especially after MRL-lpr/lpr mice immunization with DNA the relative catalytic DNase activity was significantly increased. **Figure 1(A)** demonstrates hydrolysis of supercoiled (sc) plasmid DNA by IgGs from various mice after 2 h of incubation. To quantify the DNase activity, a concentration of each electrophoretically and immunologically homogeneous IgG preparation (containing no any canonical enzymes) converting scDNA to relaxed DNA during 0.2–4 h of incubation without formation of linear or fragmented DNA was used (for example, lanes 1–3, **Figure 1A**). The relative efficiency of DNA hydrolysis was estimated from the relative percentage of DNA in the band of sc and relaxed DNA; the relative amount of DNA in these two bands for DNA incubated without IgGs or with Abs from healthy mice was taken into account. The measured relative activities (RAs) were normalized to standard conditions (0.1 mg/ml Abs, 2 h) and a complete hydrolysis of scDNA giving hydrolyzed form was taken for 100% of DNase activity. The RAs of IgGs in the hydrolysis of ATP (**Figure 1B**) and maltoheptaose (MHO; **Figure 1C**) were also estimated using the same approach as in the case of DNase activity.

All data obtained are given in **Table 1**. One can see, that at 7 months of age before development of visible pathology markers (similar to mice of 1–3 months of age before deep MRL-lpr/lpr mice spontaneous pathology) MRL-lpr/lpr mice demonstrate no proteinuria (urine proteins <3.0 mg/ml). In addition, they are characterized by a relatively weak increase in the concentrations of Abs to native and denatured DNA. Moreover, the values of these parameters for some individual prediseased mice are comparable with the values observed for healthy mice. Interestingly, IgGs from sera of healthy MRL-lpr/lpr and control CBA, BALB mice possess well-determined amylase activity. This activity increased in the case of prediseased MRL-lpr/lpr mice, but the observed difference with healthy animals was not statistically significant. The changes in this parameter become statistically significant only for mice

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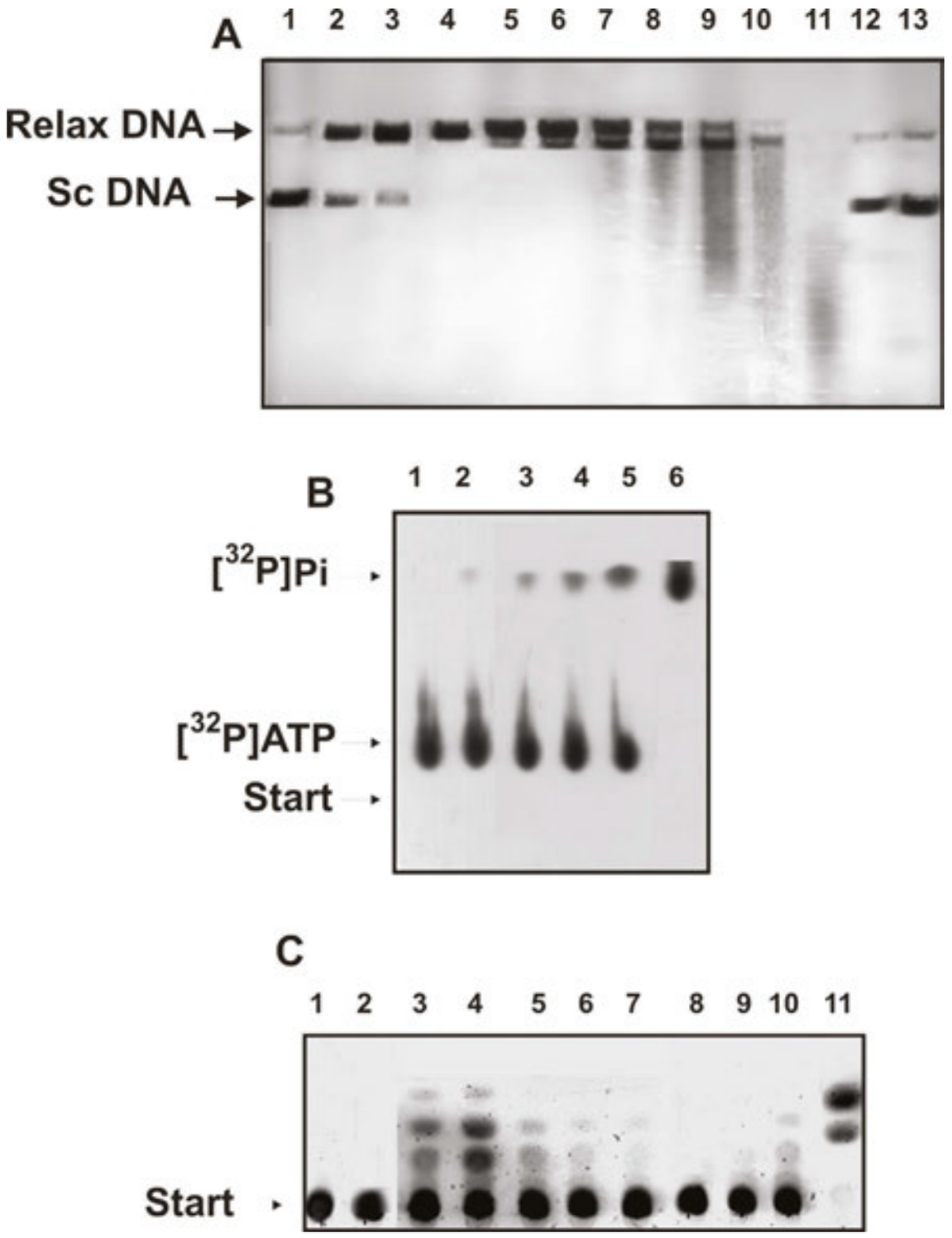

Figure 1. Determination of relative DNase (A), ATPase (B), and amylase (C) activities of catalytic IgGs (0.1 mg/ml) from different mice [29–31]. Analysis of DNA hydrolysis was performed using electrophoresis in 0.8% agarose gels. Before electrophoresis supercoiled pBluescript DNA (A) was incubated for 2 h at 30°C with IgGs from 11 different mice (lanes 1–11); lane 12, DNA incubated with Abs of healthy mouse; lane 13, DNA incubated alone. Hydrolysis of [γ-^{32}P]ATP (B) and maltoheptaose (C) was analyzed respectively by thin-layer chromatography on PEI cellulose and on Kieselgel plates. Reaction mixtures containing 0.2 mM ATP were incubated for 2 h at 30°C; lanes 2–6 correspond to IgGs from 5 different mice; lane 1 to ATP incubated alone. Standard reaction mixtures containing 0.15 mM maltoheptaose were incubated at 30°C for 12 h: lanes 2–11 correspond to IgGs from 10 different mice, lane 1, the substrate incubated alone.

Group description	Number of mice	Urine protein, mg/ml**	Abs to native DNA, A_{450}*	Abs to denatured DNA, A_{450}*	DNase activity, %*	ATPase activity, %*	Amylase activity, %*
	Control males and females						
(CBA × C57BL) F1 (3–7 mo.)	8 (4 f + 4 m)	0.12 ± 0.07	0.04 ± 0.01	0.02 ± 0.01	0***	0***	1.0 ± 0.5***
BALB/c (3–7 mo.)	8 (4 f + 4 m)	0.1 ± 0.08	0.03 ± 0.01	0.017 ± 0.004	0	0	1.1 ± 0.5
	MRL-lpr/lpr males						
Healthy (2–3 mo.)	5	0.38 ± 0.02	0.032 ± 0.01	0.09 ± 0.07	0	0	1.9 ± 1.2
Healthy, pre-diseased (7 mo.)	5	0.8 ± 0.3	0.11 ± 0.02	0.16 ± 0.05	**3.0 ± 1.0**ξ	**0.4 ± 0.25**	3.1 ± 1.4
Diseased (7 mo)	8	8.0 ± 3.1	0.18 ± 0 .08	0.23 ± 0.11	**22.0 ± 24.0**	**68.3 ± 9.8**	**3.7 ± 1.0**
Immunized	6	9.5 ± 1.7	0.6 ± 0.17	1.1 ± 0.16	**360.0 ± 230.0**	**1333 ± 530**	**17.6 ± 7.5**
	MRL-lpr/lpr females						
Healthy (2–3 mo)	5	0.31 ± 0.03	0.08 ± 0.03	0.12 ± 0.06	0	0	1.8 ± 1.1
Healthy, pre-diseased (7 mo)	5	0.9 ± 0.2	0.20 ± 0.06	0.08 ± 0.04	**6.1 ± 2.8**	**2.4 ± 1.7**	3.6 ± 1.4
Diseased (7 mo)	5	5.0 ± 3.8	0.16 ± 0.12	0.21 ± 0.12	**20.0 ± 21.0**	**65.0 ± 93.0**	**9.2 ± 5.4**

*For each mouse, the mean of three repeats is used.

**Proteinuria corresponds to ≥3 mg of total protein/ml of urine.

***100% relative activity corresponds to a complete transition of the substrate to its products after the hydrolysis in the presence of 0.1 mg/ml IgGs.

ξStatistically significant changes in parameters are given in bold.

Table 1. Autoimmune characteristics of AI-prone MRL-lpr/lpr and control non-autoimmune mice [31].

with deep pathology (**Table 1**). It is necessary to emphasize that IgGs of healthy MRL-lpr/lpr mice do not possess any DNase and ATPase activities and only the increase in these activities at a predisease stage should be considered as statistically significant indicator of the outset of spontaneous autoimmune disease of mice. After developing of deep SLE pathology, the RAs of DNase activity in the case of male and female mice increases 3.3- and 7.3-fold, respectively, comparing to prediseased mice, while increase in ATPase activity is significantly greater, 27- and 171-fold, respectively (**Table 1**). Thus, only these activities are the most important indicators of predisease state and deep pathology of MRL-lpr/lpr mice.

4. Possible role of brain stem cells and lymphocytes in development of SLE

4.1. Differentiation of bone marrow stem cells in SLE prone mice

The relationship between the relative activities of abzymes and the formation of following hematopoietic progenitors colonies has been analyzed: CFU-GM, granulocytic-macrophagic colony-forming unit; CFU-E, erythroid burst-forming unit (late erythroid colonies);

CFU-GEMM, granulocytic-erythroid-megacaryocytic-macrophagic colony-forming unit; and BFU-E, erythroid burst-forming unit (early erythroid colonies) [29–31]. In the bone marrow of healthy MRL-lpr/lpr males and females (3 months of age), normal distribution of committed progenitors was observed, and the blood serum IgGs in these mice as well as control CBA mice show no detectable DNase and ATPase activities (**Table 2**). In MRL-lpr/lpr males and females (7 months old) having no proteinuria and SLE clinical manifestations but demonstrating detectable activities of abzymes, the relative number of BFU-E and CFU-GEMM colonies increased ~2- and ~16.4–28.4-fold, respectively. For spontaneously deep diseased males and females showing high RAs of DNase and ATPase activities, the profile of HSC differentiation was changed significantly comparing with prediseased mice: BFU-E colonies number increased approximately two times, while the number of CFU-GEMM and CFU-GM colonies decreased by factors of 2.4–3.4 and ~2.6–4.0, respectively. After the development of SLE induced by the mice immunization with DNA, the highest rise in anti-DNA Abs, Abz activities, and proteinuria was observed [29–31]. In addition, a very specific differentiation profile of HSC was revealed (**Table 2**). The numbers of CFU-GEMM and BFU-E colonies were 4.3- and 3.6-fold lower than for spontaneously diseased mice, while the number of CFU-GM colonies was comparable. Interestingly, the profiles of bone marrow HSC differentiation for immunized mice and healthy mice were not much different (**Table 2**). The data of **Tables 1** and **2** are summarized in **Figure 2**.

One can see that in the condition of predisease a strong increase in the relative number of CFU-GM colonies is observed, and at this time, there is a reliable and statistically significant appearance of DNase and ATPase activities of IgG antibodies. At transition from predisease

Group description	Visual symptoms	Number of mice	Number of colonies*		
			BFU-E	CFU-GM	CFU-GEMM
CBA (3–7 mo)	No	8	3.0 ± 0.5**	7.3 ± 1.0**	0.25 ± 0.05**
	MRL-lpr/lpr males				
Healthy (2–3 mo.)	No	5	6.5 ± 1.5	7.0 ± 1.0	0.5 ± 0.1
Healthy, pre-diseased (7 mo)	No	5	12.7 ± 1.4	30.0 ± 1.3	9.2 ± 1.9
Diseased (7 mo)	Yes	5	25.3 ± 9.8	7.4 ± 0.4	3.9 ± 2.0
Immunized (3 mo)	yes, weak	5	7.0 ± 2.1	6.0 ± 2.6	0.9 ± 0.7
	MRL-lpr/lpr females				
Healthy (2–3 mo)	No	5	5.5 ± 0.5	11 ± 2.5	0.5 ± 0.2
Healthy, pre-diseased (7 mo)	No	5	11.5 ± 2.0	23.0 ± 3.0	8.2 ± 3.0
Diseased (7 mo)	Yes	5	22.1 ± 8.0	9.0 ± 3.9	2.4 ± 1.8

*For each mouse, the mean of four repeats is used.

**Mean ± confidence interval.

Table 2. Formation of bone marrow progenitor colonies in from control nonautoimmune and MRL-lpr/lpr mice [31].

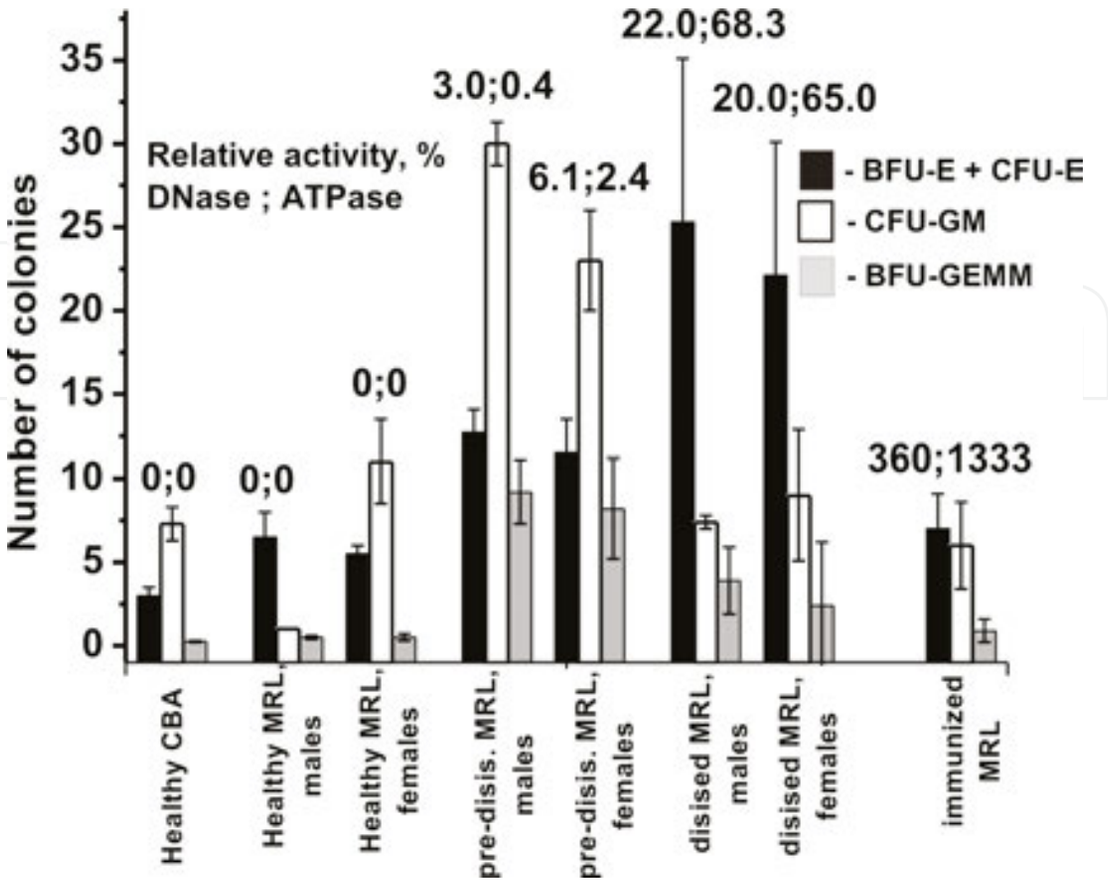

Figure 2. The relative profile of differentiation of bone marrow progenitors. Relative number of total erythroid cells (BFU-E+ CFU-E), CFU-GM, and CFU-GEMM colonies in the case of healthy CBA, conditionally healthy MRL-lpr/lpr mice at 3 months of age, after MRL-lpr/lpr mice development of pre-disease and deep SLE, as well as after mice immunization with DNA is shown [29–31]. The numbers above the bars show using a semicolon the relative average activity of mice serum IgGs in the hydrolysis of DNA and ATP, respectively.

condition to deep SLE pathology, there is additional change in profile differentiation of bone marrow HSC; the number of CFU-GM colonies is significantly decreased, but at the same time, a significant increase in BFU-E cells is observed. Such change in differentiation profile of bone marrow HSC is associated with significant increase in DNase and ATPase activities of IgGs. Healthy MRL-lpr/lpr mice 3 months of age treated with DNA show a very strong increase in DNase and ATPase activities, but differentiation profile of bone marrow HSC is almost same as for healthy male and female MRL-lpr/lpr mice. This could indicate that appearance of abzymes in healthy mice after their immunization with DNA is not associated with the change of differentiation profile of bone marrow HSC, and there may be some other ways of this phenomenon realization. Taking this into account, we analyzed lymphocyte proliferation in different organs of MRL-lpr/lpr mice [29–31].

4.2. Lymphocyte proliferation in SLE prone mice

Spontaneous development of SLE results in remarkable average increase in lymphocyte proliferation in all analyzed organs of males and females comparing with healthy control mice (**Figure 3**) [29–31].

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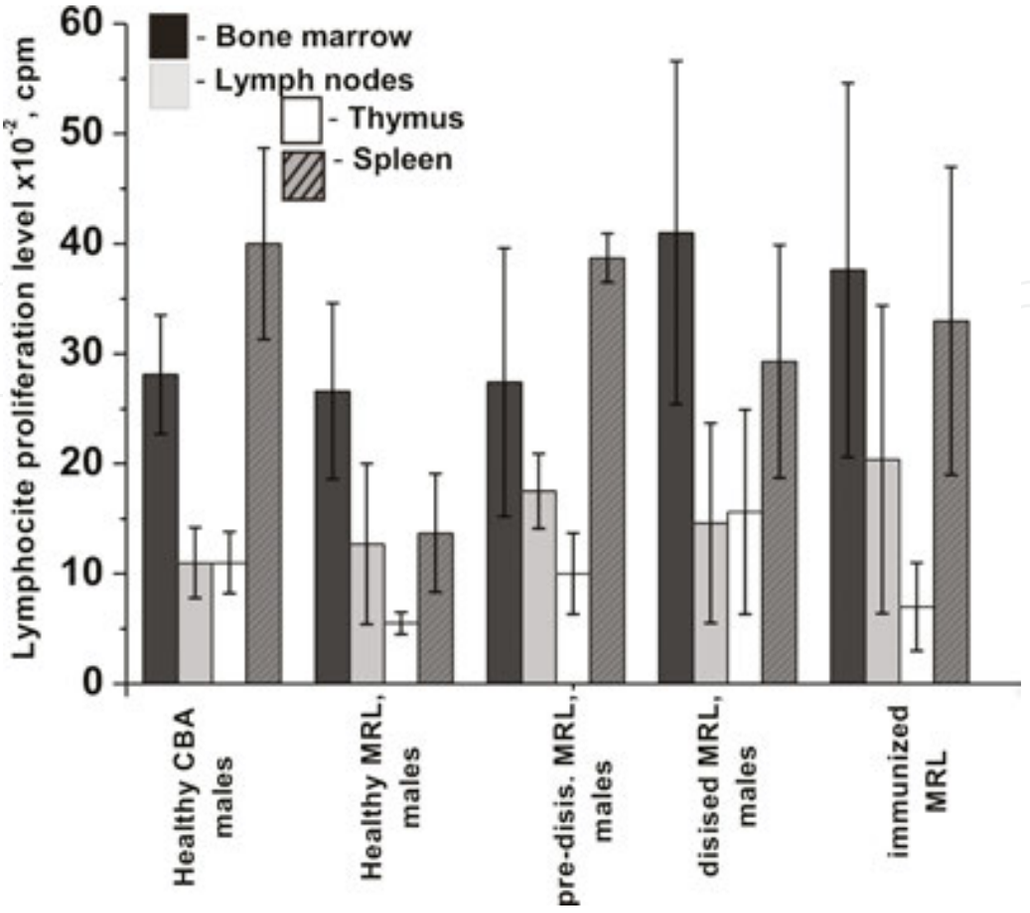

Figure 3. The relative level of lymphocyte proliferation in different organs of male healthy CBA, conditionally healthy MRL-lpr/lpr mice at 3 months of age, after development of pre-disease and deep SLE pathology MRL-lpr/lpr in mice, as well as after mice immunization with DNA is shown [29–31]. The designation of the various mouse organs is indicated in the figure.

Interestingly, the relative level of lymphocyte proliferation in the spleen of healthy control CBA mice is approximately threefold higher than that for healthy MRL-lpr/lpr mice (**Figure 3**). Transition from healthy to spontaneously prediseased mice leads to the increase in lymphocyte proliferation in spleen by ~2.8-fold in parallel with increase of average level of the proliferation 1.4- and 1.8-fold in lymph nodes and thymus, respectively [29–31]. While there is no remarkable difference in lymphocyte proliferation in bone marrow of healthy CBA, healthy and prediseased MRL-lpr/lpr mice, the diseased animals demonstrate increase in this parameter by a factor of ~1.5 (**Figure 3**). The spontaneous pathology of MRL-lpr/lpr mice develops slowly and most of the mice are showing signs of the deep disease only from 5 to 9 months of life. The average values of the lymphocyte proliferation of diseased MRL-lpr/lpr mice are significantly increased in all organs compared to healthy mice. Interestingly, immunization of healthy 3 months old MRL-lpr/lpr mice with DNA leads to the increase of lymphocyte proliferation in all organs except thymus at 20 days after their treatment (**Figure 3**). Thus, a significant increase in the relative level of lymphocyte proliferation in mice may be an important factor causing the development of abzymes with very high activity after their immunization with DNA. As mentioned above, mice immunized with DNA do not show

significant changes in bone marrow HSC differentiation profile. However, DNA-treated mice demonstrate high level of bone marrow lymphocyte proliferation (**Figure 3**). This may be due to the fact that DNA has little effect on the profile of stem cells differentiation but effectively stimulates increase in the proliferation and changes the profile of mouse bone marrow lymphocyte differentiation.

In this regard, data comparing relative Abz activities from serum and cerebrospinal fluid (CSF) of the same MS patients with multiple sclerosis should be noted [67–69]. It was shown that IgGs from sera and cerebrospinal fluid of MS patients are active in the hydrolysis of DNA, MBP, and oligosaccharides. In addition, the specific RAs of these abzymes from the CSF of MS patients are dependently on their different activities were approximately 30- to 60-fold higher comparing to serum Abzs from the same patients [67–69]. It means that during spontaneous or induced development of ADs as a result of specific differentiation of lymphocytes in cerebrospinal fluid there may be formation of cells producing different catalytic antibodies directly in cerebrospinal fluid. Thus, one cannot exclude that the increased level of Abz activities in MRL-lpr/lpr mice immunized with DNA may be a consequence of specific additional differentiation of naive lymphocytes not only in different organs but also in the cerebrospinal fluid. Another important factor reducing the relative number of lymphocytes producing abzyme may be cell apoptosis.

4.3. Cell apoptosis in SLE prone mice

It is known that in norm (healthy mammals) harmful cells including lymphocytes are eliminated by apoptosis [61, 62]. The decrease in the lymphocyte apoptosis, producing Abzs harmful to mammals, can lead to increase in autoimmune reactions and acceleration of ADs development. The relative level of lymphocyte apoptosis in different organs and tissues of MRL-lpr/lpr mice was analyzed (**Figure 4**) [31].

In control CBA and healthy MRL-lpr/lpr mice, the cell apoptosis level in different organs on average was comparable (**Figure 4**). The prediseased mice demonstrated relatively low decrease in lymphocyte apoptosis in bone marrow, but its remarkable increase in spleen. Transition from predisease state to deep SLE led to a significant decrease in the cell apoptosis level in all organs comparing with healthy and prediseased mice and maximal decrease was observed for bone marrow lymphocytes (**Figure 4**). However, the statistically significant two- to threefold maximal decrease in the apoptosis level was observed for bone marrow, thymus lymph nodes, and spleen of the mice immunized with DNA, which correlates with a very strong increase in the specific Abz activities of treated mice comparing to the spontaneously diseased animals (**Figure 4**). Therefore, it should be assumed that in the case of mice predisposed to ADs, introduction of foreign antigen can inhibit the elimination of harmful lymphocytes including ones producing dangerous abzymes by apoptosis and stimulate the proliferation of such cells. Overall, immunization of healthy MRL-lpr/lpr mice with DNA leads to the production of abzymes with very high activities, but it is not associated with noticeable change in profile of HSC differentiation but mainly caused by increase in lymphocyte proliferation and specific suppression of lymphocyte apoptosis in different organs [35]. In this regard, it should be mentioned that these regularities may to some extent be common in

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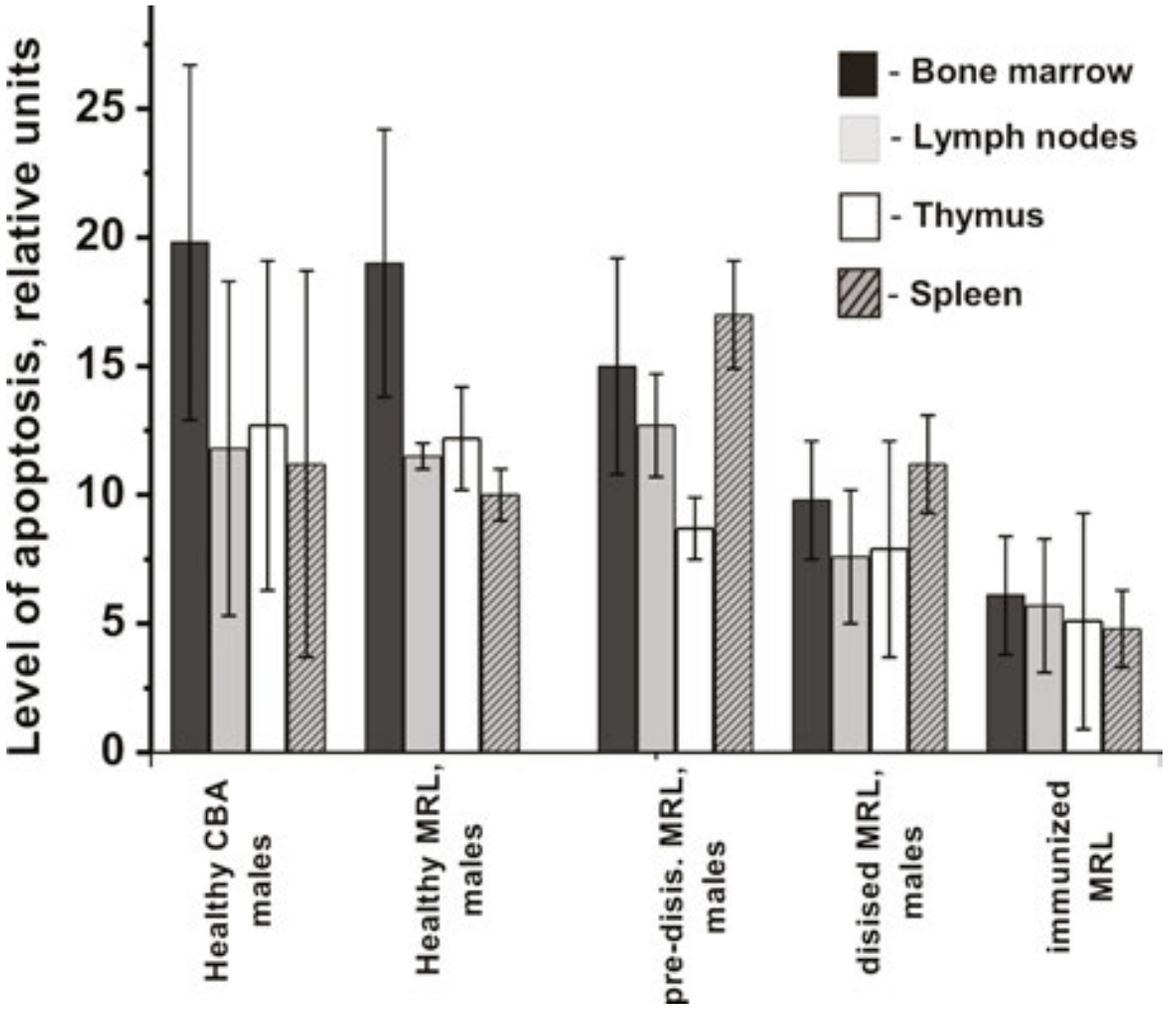

Figure 4. The relative level of lymphocyte apoptosis in different organs of male healthy CBA, conditionally healthy MRL-lpr/lpr mice at 3 months of age, after MRL-lpr/lpr mice development of pre-disease, and deep SLE pathology, as well as after mice immunization with DNA [29–31]. The designation of the various organs is indicated in the figure.

the development of various autoimmune diseases. For example, using experimental C57BL/6 autoimmune encephalomyelitis (EAE) mice (a model mimicking human MS), it was recently shown that spontaneous EAE development leads to the production of Abs to myelin basic protein (MBP) and DNA and to Abzs efficiently hydrolyzing these substrates, which associated with significant changes of the differentiation profile and level of lymphocytes proliferation of mice bone marrow HSC [32]. Immunization of these mice with MOG35 results in a very strong acceleration in the development of EAE and a very strong increase of the relative activities of abzymes hydrolyzing MOG, MBP, and DNA. The relative percent contents of total erythroid cells, CFU-GEMM and CFU-GM colonies at 3 months of age in healthy, nonautoimmune CBA, conditionally healthy, MRL-lpr/lpr and EAE C57BL/6 mice before and after development of these autoimmune pathologies were compared (**Figure 5**).

One can see that the relative content (percent of total number of different cells) of various bone marrow colonies after spontaneous achievement of deep pathologies by MRL-lpr/lpr and EAE mice is nearly the same. In addition, the level of lymphocyte proliferation as well

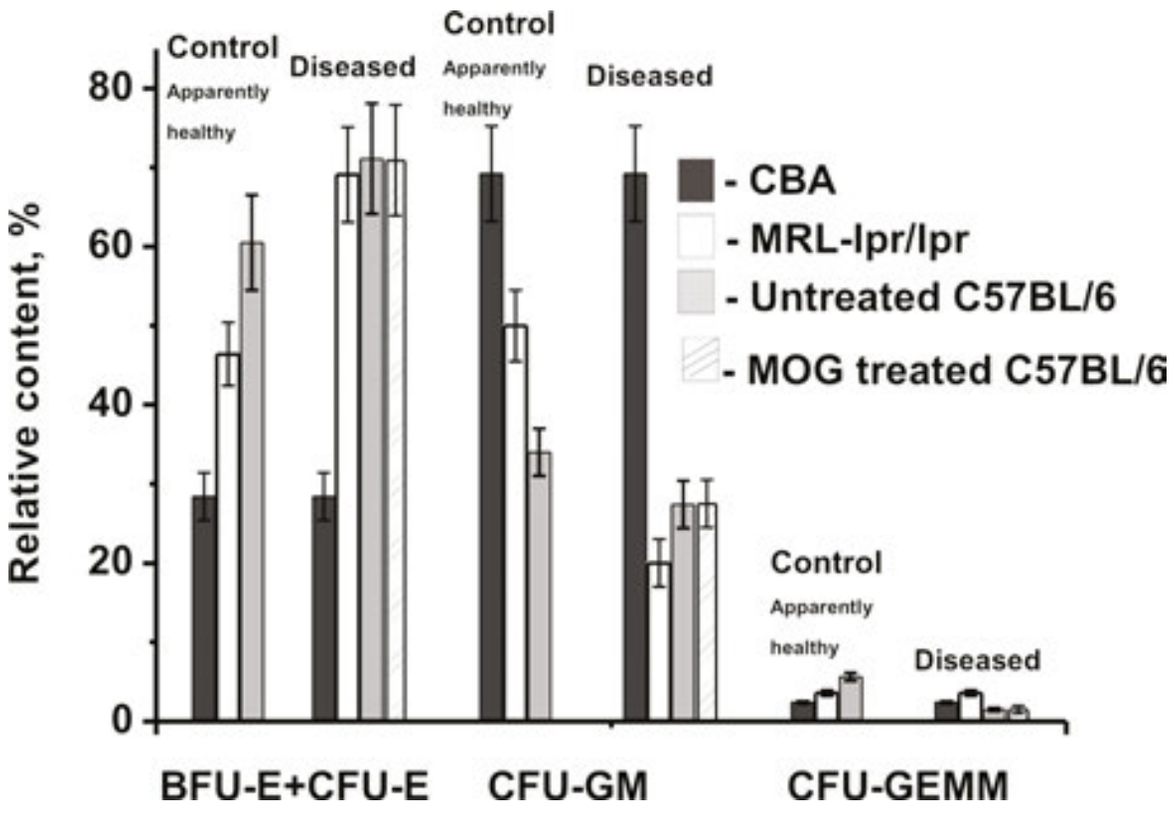

Figure 5. The relative content (%) of total erythroid cells (BFU-E+ CFU-E), CFU-GM, and CFU-GEMM colonies in the case of healthy CBA, conditionally healthy MRL-lpr/lpr and C57BL/6 mice at 3 months of age, and after development of, respectively, EAE and SLE is shown [32]. For C57BL/6 mice, the relative contents of progenitor colonies after spontaneous and MOG-stimulated development of EAE are given.

as cell apoptosis in different organs including bone marrow of healthy MRL-lpr/lpr and EAE mice, at stages of their prediseases and deep pathologies were also comparable [32]. In addition, it was shown that abzymes hydrolyzing MBP are formed in the early stages of human MS, unlike in later stages of SLE, while the reverse situation was observed for abzymes with DNase activity [70–87]. One gets the impression that SLE and MS differ greatly in their initial stages but become to some extent more similar at the later stages of these diseases. The blood of patients with SLE and MS after a long illness contains Abs to a variety of autoantigens and abzymes hydrolyzing nucleotides, oligosaccharides, lipids, DNA, RNA, MBP, and many other proteins [13–21, 70–93].

Clinically definite MS diagnosis is more often based on tomographic detection of brain-specific plaques appearing on late stages of this disease. But, similar brain plaques were also detected on the late stages of SLE [38, 41]. MS is a central nervous system disease resulting in the manifestation of different psychiatric and nervous disturbances. Neuropsychiatric disturbances occur also in about 50% of patients with SLE and carries a poor prognosis (reviewed in [41]). SLE affects mainly on the central nervous system and it supposedly more than any other inflammatory systemic disease causes various psychiatric disorders [41]. Peripheral nervous system involvement seems to be much less. Neural cell injury and rheological disturbances mediated by auto-Abs may be due to two of the main possible mechanism of tissue damage [41]. Interplay between these processes is determined by genetic factors, and may be modulated by hormones, complicated by a many of secondary factors, may explain the wide

spectrum of features revealed in SLE [41]. Thus, not only specific changes in the profile of differentiation of bone marrow stem cells, increased levels of lymphocyte proliferation and suppression of apoptosis of harmful cells in different organs leading to the production of dangerous abzymes with various activities, but also some other indicators of different psychiatric and nervous disturbances in varying degrees, are common for some patients with SLE and MS.

5. Application of rigid criteria for analysis of antibodies catalytic activities

The sera of healthy humans and mammals contain usually autoantibodies to many different antigens, including RNA, DNA, proteins, and other antigens [13–22, 43, 44]. Natural abzymes from the sera of patients with different AI diseases are products of different immuno-competent cells and usually polyclonal in origin ([13–22] and refs cited here). Purification of natural abzymes containing no canonical enzymes is a very important task in their study; peculiarities of such antibodies isolation were discussed in detail in reviews [13, 19]. Electrophoretically and immunologically homogeneous IgG fractions with or without different catalytic activities from the sera of healthy donors and autoimmune patients described in this chapter were first purified using Protein G-Sepharose, while IgAs and IgMs by affinity chromatography on Protein A-Sepharose under conditions removing nonspecifically bound proteins. Then IgAs were separated from IgMs and IgGs from possible admixtures of canonical enzymes by FPLC gel filtration in acidic conditions (pH 2.6) destroying immuno-complexes [29–32, 49, 50, 55–57, 64–103]. Overall, ~900 kDa IgMs, 170 kDa IgAs, and 150 kDa IgGs did not contain possible contaminating proteins detected by acrylamide gel silver staining under reducing and nonreducing conditions.

The application of rigid criteria allowed the authors of the first article describing natural abzymes hydrolyzing vasoactive intestinal peptide to obtain irrefutable evidence that this activity is an intrinsic property of IgGs from sera of patients with asthma [9]. Later several additional rigid criteria were proposed (reviewed in Refs. [13, 19]). We applied a set of these strict criteria [9, 13–22] for the analysis of DNase and RNase [11, 31, 64, 65, 70–75], MBP-hydrolyzing [76–79], ATPase [30], and amylase [29, 88–91] activities as intrinsic properties of IgG and/or IgM and IgA antibodies from sera of SLE patients and mice. Several more important of them may be summarized as follows: (1) all Abs were electrophoretically homogeneous; (2) FPLC gel filtration of these Abs under conditions destroying strong noncovalent complexes (acidic buffer, pH 2.6) did not abolish these activities, and activities peaks exactly coincided with peaks of intact Abs; (3) immobilized mouse IgGs against the light chains of human Abs absorbed completely these activities; these activity's peaks coincided with the peaks of Abs eluted using acidic buffer; and (4) F(ab) and F(ab)2 fragments of Abs showed to some extent comparable levels of the activities comparing with intact Abzs. To exclude possible artifacts causing by hypothetical traces of canonical enzymes, Abs from sera of SLE patients were subjected to SDS-PAGE in a gel copolymerized with polymeric DNA or RNA and their nuclease activities were detected *in situ* by gel incubating in the standard reaction buffer. Staining of the gels after the electrophoresis with ethidium bromide (after refolding of Abs) showed sharp dark bands against a fluorescent background of nucleic acids. After incubation with DTT only light chains of SLE Abs demonstrated nuclease activities. After SDS-PAGE amylase, ATPase and MBP-hydrolyzing activities of SLE Abs were analyzed using extracts of

2- to 3-mm fragments of a longitudinal gel slice. Since SDS destroys all noncovalent protein complexes, the revealing of the analyzed activities in the gel zones of only intact IgGs and their separated light chains together with the absence of any other bands of the activities or proteins gave direct evidence that Abs from sera of SLE patients possess analyzed enzymatic activities. The fulfilment of these criteria was observed for SLE IgG, IgA, and IgM abzymes with all activities mentioned above. Some typical examples of rigid criteria application in the case of DNase, RNase, and MBP-hydrolyzing activities of SLE IgGs are given in **Figures 6** and **7**.

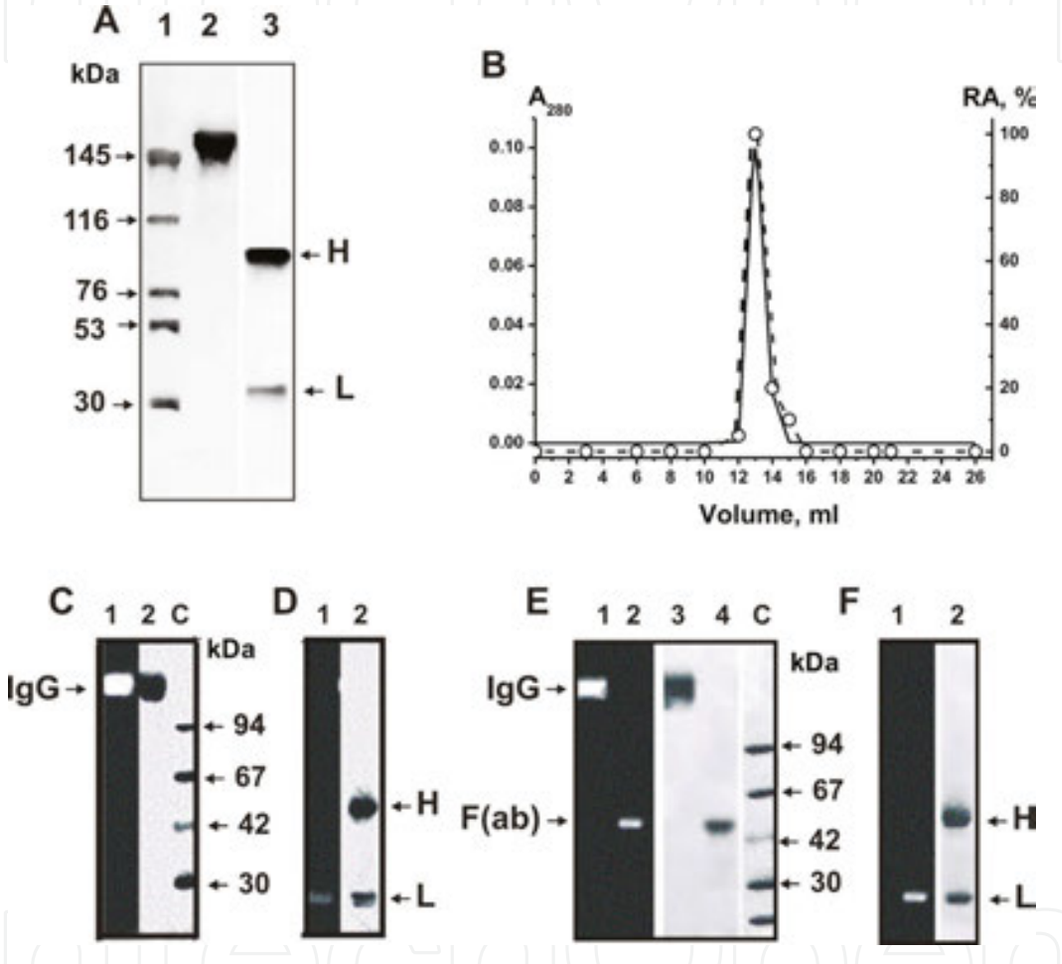

Figure 6. Analysis of the implementation of strict criteria of intrinsic enzymatic activities of IgGs from sera of SLE patients and mice. SDS-PAGE analysis of homogeneity of IgGmix (mixture of equal amounts of Abs from sera of 10 SLE patients) before (lane 2) and after (lane 3) Abs boiling with DTT; lane 1 shows positions of protein markers (A) [71]. FPLC gel filtration of IgGmix (mixture of 15 preparations) corresponding to diseased MRL-lpr/lpr mice on a Superdex 200 column in the acidic buffer (pH 2.6) destroying different immunocomplexes after IgGs incubation in the same buffer (B) [31]. *In situ* gel assay of DNase (C and D) and RNase (E and F) activities of IgGmix (10 preparations) corresponding to SLE patients using respectively gels containing polymeric DNA and RNA [71]. DNase and RNase activities were revealed by ethidium bromide staining as dark bands on the fluorescent background; lanes 1 (C and E) correspond to IgGs before, while lines 1 (D and F) after Abs mild treatment with DTT, lane 2 (E) to F(ab) fragments of IgG (negatives are given). Lanes 2 (C and D), 3 (E) and 2 (F) intact IgGs or their separated L and H chains, while lane 4 (E) corresponds to F(ab) fragments, positions of which were revealed by treatment of the gels with Coomassie R250. Lanes C (C and E) correspond to proteins with known molecular masses [71].

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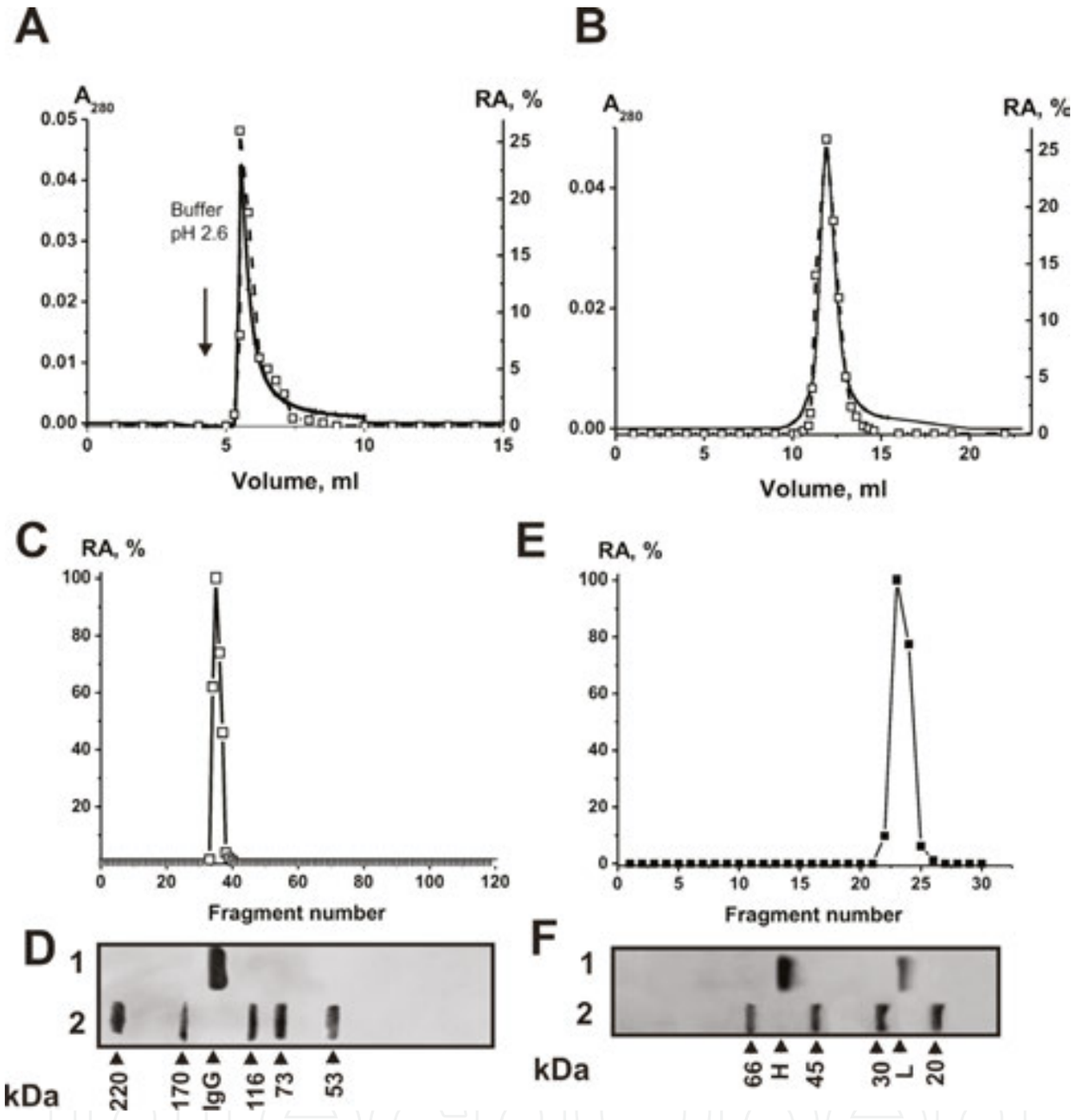

Figure 7. Application of the strict criteria to show that MBP-hydrolyzing activity is intrinsic property of IgGs from sera of SLE patients [76, 77]. Affinity chromatography of the IgGmix (Abs of 10 patients) using Sepharose bearing mouse Abs against human IgGs (A) and FPLC gel filtration IgGmix on column with Superdex 200 in acidic buffer (pH 2.6) after incubation of Abs in the same buffer (B): (—), absorbance at 280 nm (A280); (□), relative activity (RA) of IgGmix in the hydrolysis of human MBP (A and B). A complete hydrolysis of MBP (0.5 mg/ml) for 24 h at 37°C was taken for 100%. Analysis of MBP hydrolysis by IgGmix and its separated L and H chains after SDS-PAGE (C and E). After SDS-PAGE of IgGmix using nonreducing (C) and reducing (E) conditions, the gel was incubated under conditions for renaturation of Abs. The relative MBP-hydrolyzing activity (RA, %) was estimated using the extracts of 2- to 3-mm many gel fragments (C and E) of first longitudinal slices. The RA of IgGmix corresponding to a complete hydrolysis of MBP (0.5 mg/ml) after 24 h of standard mixture (30 μl) incubation with 20 μl of extracts was taken as 100%. The second control longitudinal slices corresponding to the same gels were treated with Coomassie R250 (lanes 1; D and F). Lane 2 (D and F) demonstrates position of protein molecular mass markers.

Similar rigid criteria were used by us for evidence of the catalytic activity belonging to the antibodies, hydrolyzing nucleotides, DNA, RNA, various peptides, proteins, oligosaccharides from blood with different autoimmune diseases, and animals immunized with different antigens [11, 13–22, 32, 49–57, 64–65, 67–103].

6. SLE patient's abzymes with DNase and RNase activities

6.1. Polyclonal DNase and RNase abzymes

Healthy humans do not demonstrate antibodies with detectable DNase and RNase activities, their levels are more often on the borderline of sensitivity of the detection methods [13–22]. The RAs of DNase and RNase abzymes from the sera of patients with SLE vary markedly from patient to patient [13–22].

We analyzed the possible heterogeneity of catalytic properties of polyclonal DNase and RNase IgGs from SLE patients and observed an extreme heterogeneity in kinetic and thermodynamic parameters, relative specific activities and substrate specificities, which are different very much from patient to patient. Chromatography on DNA-cellulose showed that only 10–30% of the total electrophoretically homogeneous IgGs and IgMs dependently on patient may be bound to the affinity sorbent. Interestingly, when Abs were eluted from DNA-cellulose by a NaCl gradient (0–3 M) and then acidic buffer (pH 2.6) the Abs and their DNase and RNase activities were distributed all over the chromatography profile (for example, **Figure 8A**) [70, 71]. The same situation was observed for Abs with nuclease activities from sera of MS patients [8, 80–82], and rabbits immunized with DNA, RNA, DNase I, DNase II, and pancreatic RNase [49, 50, 55–57]. The affinity of Abs fractions for these substrates was increased gradually with the increase in eluting salts concentrations. When IgGs eluted from DNA-cellulose were fractionated on Sepharose bearing immobilized monoclonal mouse Abs against anti-kappa or anti-lambda human IgGs, 60–70% of IgGs were adsorbed by Abs against lambda- and 30–40% by Abs against kappa-Abs (**Figure 8B**) [70, 71].

The fractions corresponding IgGs with kappa-light chain were about 30- to 50-fold more active in hydrolysis of both RNA and DNA than lambda-IgGs. SLE IgGs and IgAs with DNase activity [70, 71] similarly to Abs with nuclease activity from sera of patients (and mice) with other autoimmune diseases [80–82, 95, 99–103] efficiently hydrolyzed all single- and double-stranded DNA of different sequences and length. The substrate specificity of SLE IgGs with RNase activity, however, was unique within certain limits for Abs from every individual SLE patient [11]. In contrast to human RNases, SLE IgGs effectively hydrolyze the most resistant poly(A) substrate for all known human RNases [104–109]. SLE IgGs demonstrated a very slow hydrolysis of poly(C) [11], which is the best substrate for all mammalian RNases [104–106]. Therefore, for more detail analysis of IgGs of SLE patients ribo$(pA)_{13}$ was used. **Figure 8(C)** demonstrates pH dependence of [5′-^{32}P]$(pA)_{13}$ hydrolysis by three of six SLE IgGs analyzed and by two human blood RNases. All six dependences showed individual features of pH dependencies. In contrast to all SLE abzymes, RNases

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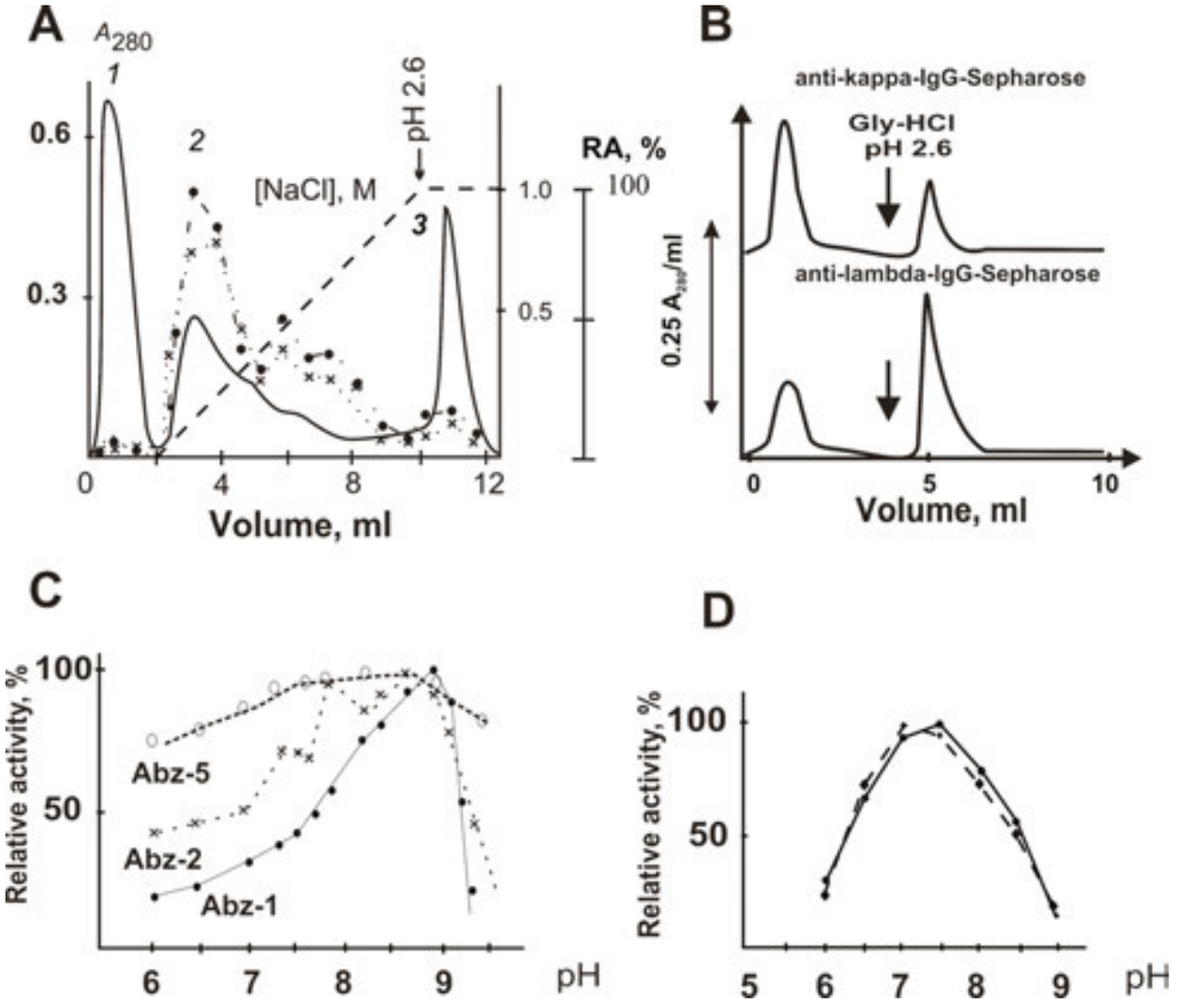

Figure 8. Separation of SLE IgMs having affinity to DNA by affinity chromatography on DNA-cellulose (A) [70]: (—) absorption at 280 nm, (•) and (x) RA of Abs in hydrolysis of respectively [5′-^{32}P](pA)$_{13}$ and [5′-^{32}P]d(pA)$_{13}$, 1–5 μl of each fraction was added to 100 μl of reaction mixture and maximal RNase and DNase activity was taken for 100%. Separation of anti-DNA IgGs containing light chains of kappa and lambda type by affinity chromatography on Sepharoses bearing immobilized Abs against human kappa- and lambda-IgGs [71]: (—) absorption at 280 nm (B). pH dependencies of the relative RNase activity of three SLE IgGs (C) and human serum RNases (RNase 3 (•) and RNase 4 (•)) (D) in the hydrolysis of [5′-^{32}P](pA)$_{13}$ [71].

have only one pH optimum (6.8–7.5) for hydrolysis of poly(A) [104–106] (**Figure 8D**). Polyclonal SLE abzymes more often shows high activity at pH from 6.0 to 9.5. For example, preparation Abz-1 demonstrates maximal activity at pH 8.8; Abz-2 shows three marked pH optima at pH 8.5, 7.7, and 7.2, while Abz-5 hydrolyzes RNA with comparable efficiency at pH values from 6.0 up to 9.5 (**Figure 8C**). Abz-3 and Abz-4 also demonstrated several pronounced optima at pH 6.0–9.5 similar with that for Abz-2, while Abz-6 showed no optimum, like Abz-5.

Interestingly, even at fixed pH 7.5 initial rates corresponding to increase in oligonucleotide concentrations were consistent with Michaelis-Menten kinetics only for all human RNases

and Abz-1, for which was only one interception of curves in coordinates of Cornish-Bowden (**Figure 9A**) [71]. Three IgGs (Abz-2–Abz-4) demonstrated several apparent values of both K_m and V_{max} (for example, **Figure 9B**). The apparent K_m and k_{cat} values for Abz-5 and Abz-6 having comparable activity at pH from 6.0 to 9.0 demonstrated fan-like Cornish-Bowden dependencies showing smooth changes of the apparent K_m and V_{max} values with increase in substrate concentration, and there were no evident intersection points (**Figure 9C**). It means that Abz-5 contains a lot of monoclonal abzymes with comparable and different K_m and V_{max} values in their wide range. The data obtained are summarized in **Table 3** [71]. Similar pronounced heterogeneity was observed for SLE polyclonal DNase and RNase IgGs and IgAs [70, 71].

It should be mentioned that the same preparations of polyclonal Abzs hydrolyzed RNA approximately 10- to 300-fold faster than DNA [70, 71]. In addition, several monoclonal IgGs against B-DNA of different sequences (from SLE mice) efficiently hydrolyze single- and double-stranded RNA and DNA in a sequence-independent manner, the RNase activity was by a factor of 30–100 higher than of DNA [107]. Our findings indicate that a variety of anti-DNA and anti-RNA abzymes are able to hydrolyze both DNA and RNA [13–22]. In this respect, it should be mentioned that after immunization of rabbits with DNA, RNA, DNase I, DNase II, and pancreatic RNase I, antinuclease IgGs with different affinity to DNA were separated for several fractions by chromatography on DNA-cellulose [49, 50, 55–57]. IgGs of all fractions demonstrated DNase and RNase activity and RNase activity was 10- to 50-fold higher than DNase one. Only one small fraction in the case of abzymes obtained after immunization of rabbits with RNA demonstrated only DNase activity and was not able to hydrolyze RNA [50]. The data obtained testify in favor of the formation of abzymes with chimeric structure of the active centers, which are mostly able to hydrolyze both DNA and RNA.

Canonical RNases are usually specific for sequences (for example, RNase T1 is specific for guanosines, while RNase A for Py-A sequences) or for structural features (nuclease S1, for example, hydrolyzes only single-stranded domains of RNA). Abzymes of SLE and other autoimmune patients demonstrate novel RNase activities. Some of them may be stimulated by Mg^{2+}; they are not sequence-specific but sensitive to subtle and/or drastic folding changes of structurally well-characterized tRNA [72–75]. Two $tRNA^{Lys}$ [72, 74], one corresponding to human mitochondria, while the second $tRNA^{Lys}$ is a mutant revealed in patients with myoclonic epilepsy, in which A nucleotide at position 50 is changed for G nucleotide. Different canonical RNases including RNase A showed no difference in cleavage patterns of these $tRNA^{Lys}$ [72]. However, in the presence of Mg^{2+} RNase SLE abzymes produced new cleavage sites; the mutant $tRNA^{Lys}$ showed a significantly different sensitivity for abzymes in the substrate mutated region, and the hydrolysis was detected at new positions, showing local structural or conformational changes of $tRNA^{Lys}$.

Most of Mg^{2+}-dependent abzymes display usually no sequence specificity; they are more sensitive to structural features of different tRNAs specific for Gln, Phe, Asp, and Lys [72–75]. Abzymes of some AI patients demonstrate RNase A-type as a major specificity showing minor differences (preference for CpA and UpA sequences). However, some abzymes

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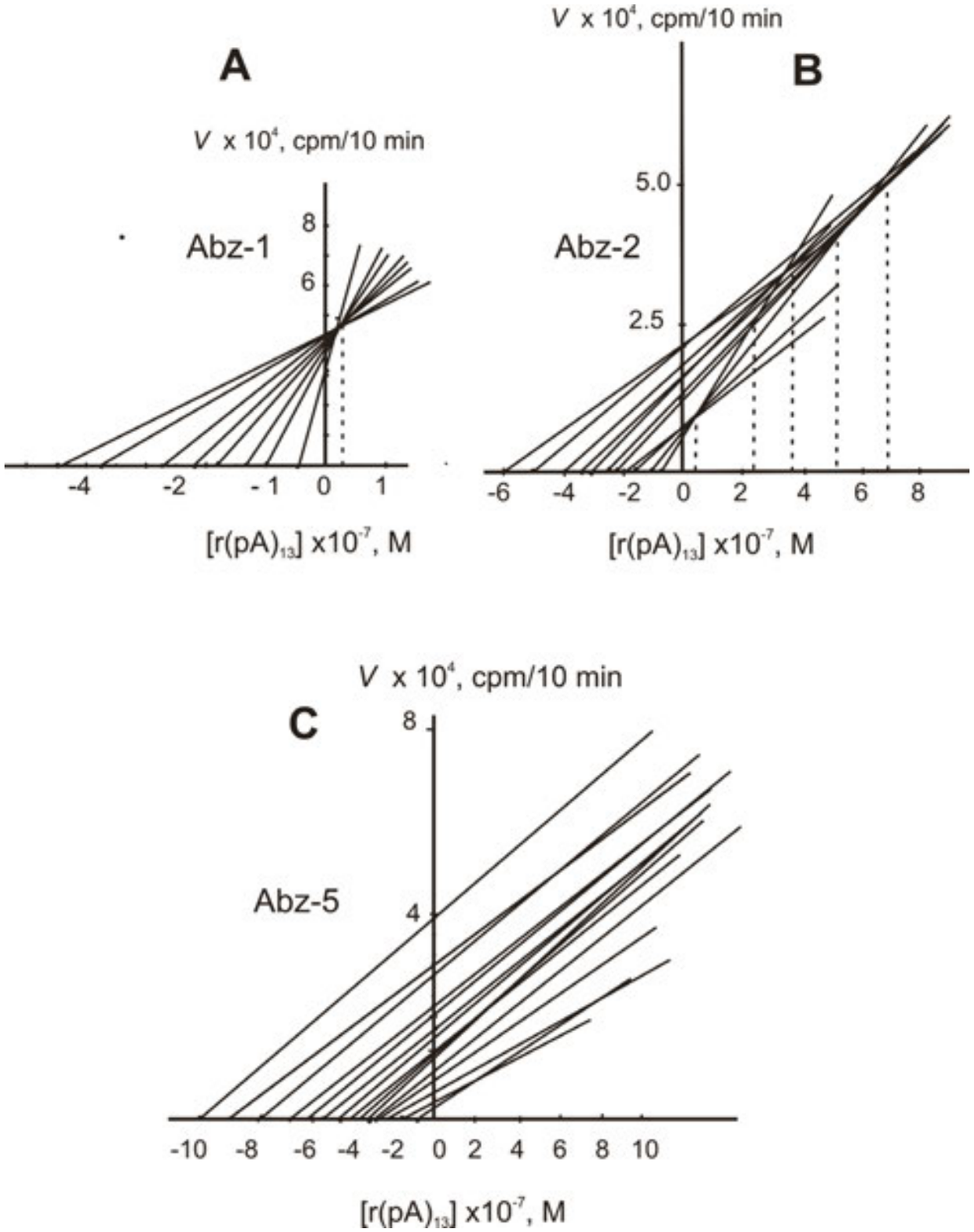

Figure 9. Initial rates of [5′-^{32}P](pA)$_{13}$ hydrolysis as a function of oligonucleotide concentration for three SLE IgGs [71]. The K_m and V_{max} values were determined using the Cornish-Bowden coordinates (A–C). The common intersection points give K_m and V_{max} values characterizing different abzymes; the absence of intersection points indicates that many different monoclonal abzymes with similar and different kinetic parameters catalyze the oligonucleotide hydrolysis.

Substrate	Preparation	K_m[α] (M)	k_{cat} (min^{-1})
Antibodies			
$d(pA)_{13}$	Abz-1	7×10^{-8}	2.0×10^{-2}
$(pA)_{13}$		4×10^{-8}	1.4
$d(pA)_{13}$	Abz-2	4.7×10^{-8} to 3.0×10^{-7}	2.0×10^{-3} to 7.1×10^{-2}
$d(pA)_{13}$			
$(pA)_{13}$		5.1×10^{-8} to 4.4×10^{-7}	0.12–0.84
$p(U)_{10}$		$9.0 \times 10^{-8} - 4.1 \times 10^{-7}$	$3.2 \times 10^{-3} - 1.3 \times 10^{-2}$
$(pA)_{13}$	Abz-3–Abz-4	1×10^{-8} to $2 \times 1\ 0^{-6}$	1.0×10^{-2} to 2.5
RNases			
$p(A)_{13}$	RNase A	3.4×10^{-6}	2.2×10^{-2}
$p(A)_{13}$	RNase 3	4.9×10^{-6}	1.7×10^{-2}
$p(U)_{10}$		2.1×10^{-6}	37
$p(A)_{13}$	RNase 4	7.2×10^{-6}	5.2×10^{-2}
$p(U)_{10}$		5.6×10^{-6}	26

[α]The errors of the values determination were within ± 10–30%.

Table 3. Kinetic parameters for hydrolysis of different oligonucleotides by catalytic SLE-IgGs, human serum RNases and pancreatic ribonuclease A [71].

contain a major subfraction demonstrating T1-type of RNase specificity. The Mg^{2+}-stimulated RNase IgGs had more often a cobra venom RNase V1-like specificity in cleavage of $tRNA^{Phe}$ with a unique Mg^{2+}-dependent specificity toward double-stranded regions [72, 74]. In spite of some similarities, SLE abzymes show specificities quite different from that of RNase V1, but this specificity remarkably differs from patient to patient. Overall, monoclonal SLE abzymes entering to total pool of Abzs can discriminate between subtle or large structural changes and nucleotide sequences. Interesting that IgGs from patients with different AI and viral diseases can demonstrate different patterns of various tRNAs cleavage [72–75].

Abzs from the sera of patients with MS [80–82], viral hepatitis [100], HIV-infected patients [95], with tick-borne encephalitis [101], Hashimoto's thyroiditis [102], and schizophrenia [103] also demonstrated DNase activity. All these diseases are characterized by different levels of the relative activity of abzymes, and DNase activity increases approximately in the following order: diabetes ≤ viral hepatitis ≈ tick borne encephalitis < polyarthritis ≤ Hashimoto's thyroiditis ≤ schizophrenia < AIDS ≤ MS < SLE [13–22, 80–82, 95, 101–103]. Overall, DNase and RNase polyclonal Abzs from sera of patient with SLE, MS, and other AI and several viral diseases may be characterized by a relatively small or extremely large content of polyclonal nuclease abzymes containing different relative amounts of kappa- and lambda-Abzs, demonstrating from one to several pH optima, having a different net

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charge, may be dependent or not on different metal ions, and demonstrate different substrate specificities.

6.2. Monoclonal nuclease SLE abzymes

The active centres of DNase, RNase, protease, and oligosaccharide-hydrolyzing abzymes from SLE, MS, and patients with other diseases are usually located on the light chains of these Abs [13–22]. The heavy chain is mainly responsible for specific antigen recognition and significantly increased antigen affinity for antibodies. The isolated light chains of IgGs cleavage vasoactive intestinal peptide with the activity 32-fold higher than Fab fragments [9]. Isolated by SDS-PAGE light chains of different abzymes were more active than the intact ones [13–22]. But, only multiple myeloma Bence-Jones proteins should be considered as natural human monoclonal abzymes [60].

A phagemid library of immunoglobulin kappa light chains derived from lymphocytes of peripheral blood of three SLE patients (106 variants) was cloned in pCANTAB5His6 vector. For amplification of the phage library *Escherichia coli* TG1 presenting monoclonal light chains (MLChs) on the surface of phage particles was used [111, 112]. Phage particles containing a pool of various MLChs having different affinity for DNA were separated by chromatography on DNA-cellulose (**Figure 10A)**.

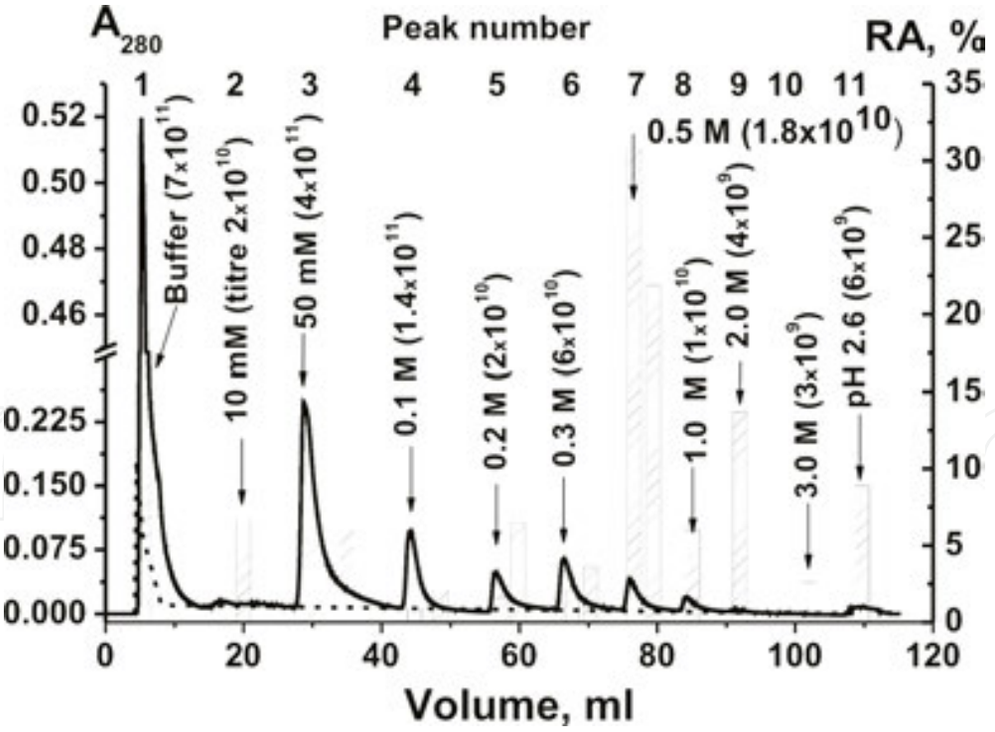

Figure 10. Affinity chromatography of phage particles on DNA-cellulose: (—) and (– – –), A280 values correspond respectively to the material of phage particles containing or not cDNA of kappa light chains [111]. The bars show the RAs of 16 fractions of 11 peaks eluted with various concentrations of NaCl and acidic buffer (pH 2.6). The titres of the particles of different peaks are given in the parenthesis.

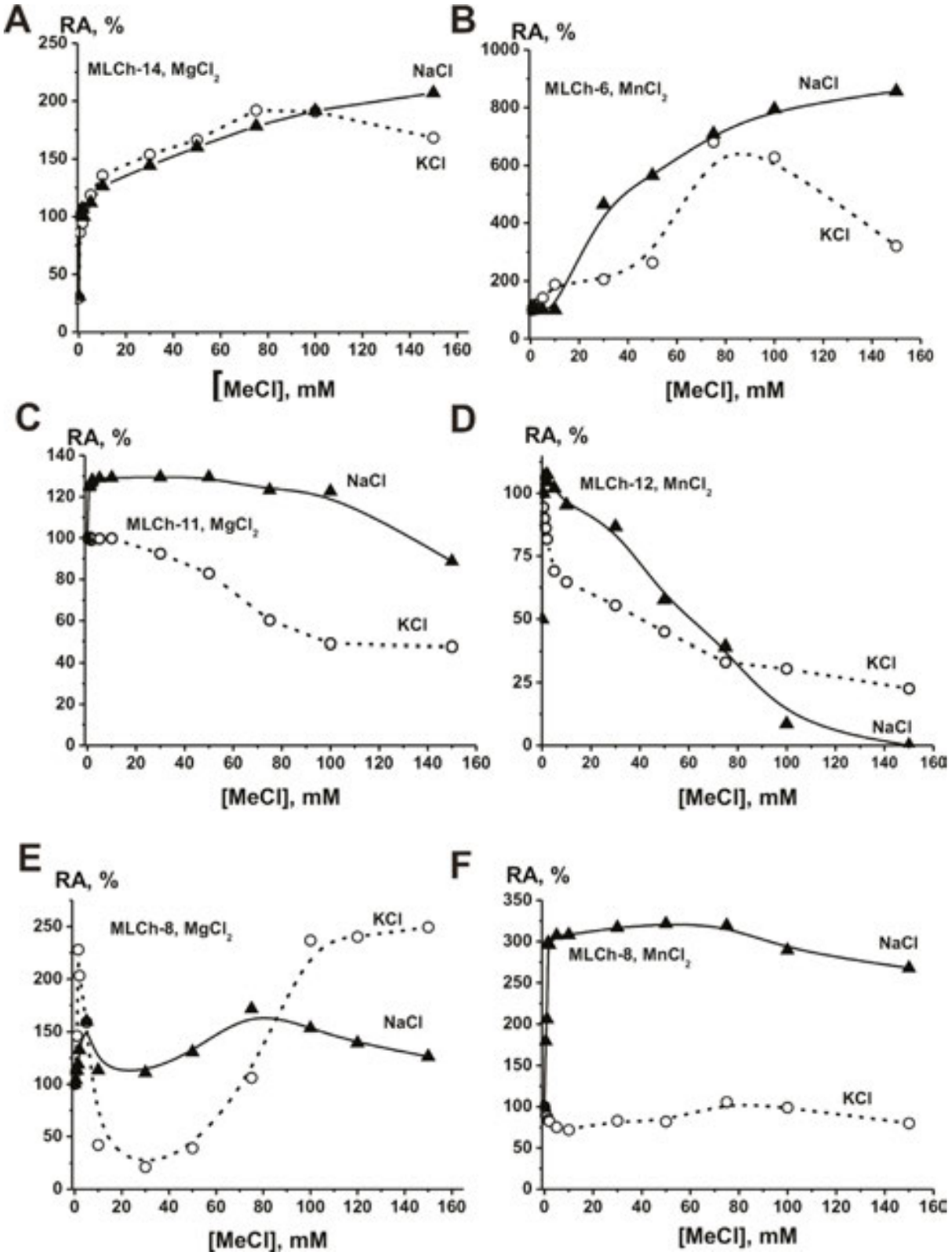

Figure 11. Dependences of the RAs of DNase activity for several MLChs upon the concentration of KCl and NaCl at 2 mM concentrations of $MgCl_2$ and $MnCl_2$ (A–F) [111]. The RAs in the absence of KCl and NaCl were taken for 100%. Numbers of MLChs (15 nM) and the used $MnCl_2$ or $MgCl_2$ are given on panels A–F.

The phage particles were distributed between 16 fractions of 11 peaks and all fractions corresponding to new small pools of anti-DNA MLChs demonstrated DNase activity (**Figure 10**). Thus, all small pools of MLChs of 16 fractions with different affinity of phage particles for DNA contained not only light chains without, but also with DNase activity. For preparation of individual colonies, *E. coli* HB2151 and phage particles eluted from DNA-cellulose with 0.5 M NaCl (peak 7) demonstrating high DNase activity were used. For study of DNase activity, 45 of 451 individual colonies from two Petri dishes were chosen in a random way; 15 of 45 single colonies (~33%) were capable to hydrolyze DNA.

MLChs of 15 single colonies were used for purification of individual MLChs using chromatography on Ni^{2+}-charged HiTrap chelating Sepharose and by following FPLC gel filtration [111]. The preparations of ~28-kDa MLChs were electrophoretically homogeneous, showed positive answer with mouse IgGs against human Abs light chains at Western blotting and positive ELISA answer using plates containing immobilized DNA; *in situ* analysis demonstrated DNase activity in the gel zone corresponding only to MLCh [111].

The dependences of DNase RAs for various MLCh preparations on the concentration of different metal ions were analyzed. It was shown that $MgCl_2$ and $MnCl_2$ are good activators of all 15 MLChs. Since K^+ and Na^+ ions can influence on the spatial structures of different enzymes, antibodies, and nucleic acids, we have analyzed dependencies of the RAs upon concentration of these ions at fixed concentrations of $MgCl_2$ and $MnCl_2$ (2 mM). All MLChs demonstrated very specific dependencies and optimal concentrations of NaCl and KCl (for example, **Figure 11**). Optimal concentrations of NaCl and KCl for 15 MLChs are given in **Table 4**. It was interesting to see whether optimal concentrations of $MgCl_2$ and $MnCl_2$ can depend on NaCl and KCl concentrations. **Figure 12** shows that for all MLChs in the presence of KCl and NaCl in different concentrations the dependencies reach plateau at 1.5–2.0 mM concentration of $MgCl_2$ and $MnCl_2$. Several MLChs demonstrated shape-bell dependencies; but the inhibition was usually observed at Mn^{2+} and Mg^{2+} concentrations higher than 2–4 mM (**Figure 12**) [111].

Various canonical DNases demonstrate usually different pH optima, but all of them have only one pH optimum [108, 109]. In contrast to known canonical DNases, polyclonal DNase Abzs from the sera of patients with SLE and other diseases can contain from one to many monoclonal abzymes demonstrating from 1 to 2–8 well pronounced pH optima in range from 5 to 10 [13–22, 72, 73, 82–84, 110]. pH optima of 15 MLChs in the presence of 2 mM $MgCl_2$ as well as for DNase II and DNase I were analyzed and several typical dependencies are given in **Figure 13**. DNase I has only one optimum at pH 7.0–7.2, while DNase II demonstrates one optimum at pH 4.9–5.0 (**Figure 13A**). Optimal pH for all 15 MLChs is given in **Table 5** [111].

Eight of 15 MLChs demonstrated only one pH optimum, while seven preparations shows two different pH optima (**Figure 13**). It was shown that Mn^{2+}, Co^{2+}, Ni^{2+}, and Ca^{2+} ions activate DNase I in significantly smaller degree than Mg^{2+} ions [108, 109]. The RAs of 15 MLChs in the presence of 6 various metal ions (2 mM) using optimal NaCl and KCl concentrations and optimal pH were estimated (**Table 6**). The maximal activity for various

Number of MLCh preparation	$MnCl_2$		$MgCl_2$	
	[KCl], mM	[NaCl], mM	[KCl], mM	[NaCl], mM
1	0–0.5; > 0.5 inhib.[a]	0–0.5; > 0.5 inhib.	30.0; > plateau[b]	100.0; >120.0 inhib.[a]
2	100; >120 inhib.	100; > 120 inhib.	75.0; >100 inhib.	15.0; >100.0 inhib.
3	5.0; > 10 inhib.	5.0; > 10.0 inhib.	0.0; > 0.0 inhib.	0.0; > 0.0 inhib.
4	2.5; > 4.0 inhib.	7.5; > 10.0 inhib.	5.0 > 10.0 inhib.	8.5; > 15.0 inhib.
5	5.0; > 10 inhib.	5.0; > 10.0 inhib.	0.0 > 0.0 inhib.	0.0; > 0.0 inhib.
6	1–2 and 75; >75 inhib.[c]	1–2 and 150	1.5–30 plateau; >40.0 inhib.	1–3 and 50.0; >100.0 inhib.
7	0.5; >1.0 inhib.	2.5; >3.0 inhib.	3.0; >4.0 inhib.	8.0; >10.0 inhib.
8	0–10 inhib.; 75; >80 inhib.	5–150 plateau	5.0 and 75.0; >80.0 inhib.	1.5; 2.0–75 inhib.; 100; >plateau[c]
9	20–25; >30 inhib.	2.0; >3.0 inhib.	10.0; >12.0 inhib.	5.0 and 75.0; >80.0 inhib.
10	2.5; plateau[b]	10.0; >15.0 inhib.	2.5; >5.0 inhib.	5.0; >7.0 inhib.
11	1–10 plateau; >10 inhib.	0.5–2.0 plateau; >2.0 inhib.	0–10.0 plateau; >10.0 inhib.	2–50 plateau; >60.0 inhib.
12	0.0; >0.0 inhib.	2.0 and 5–10; >10.0 inhib.	1–5; >10.0 inhib.	1–2 and 5–10; >10.0 inhib.
13	0.0; >0.0 inhib.	0.0 and 10.0; >10.0 inhib.	0.1–0.3; >0.7 inhib.	0–0.1; >0.1 inhib.
14	100–150 plateau	100–150 plateau	75.0; >100 inhib.	150.0
15	1.5; >2 inhib.	50.0; >50.0 inhib.	0.0 and 50.0; >50.0 inhib.	0–10 and 50.0; >50.0 inhib.

[a]For each value, a mean of two measurements is reported, optimal concentrations are given in bold; the mark (>value inhib.) means that the dependence demonstrates bell-shaped character and that at higher concentrations of the salt the inhibition of the reaction is observed.

[b]The mark (30 (or any other value in bold); >plateau) means that optimal concentration corresponds to 30 mM and there is no remarkable inhibition up to maximal concentration (100–150 mM) of NaCl or KCl used.

[c]The mark (1.5; 2.0–75 inhib.; 100; >plateau (or other similar values)) means that there are two optimal concentrations at 1.5 and 100 mM salt and a significant decrease in the activity of analyzed MLCh at concentrations in the region 2.0–75 mM.

Table 4. The optimal concentrations of KCl and NaCl in the case of individual recombinant MLChs in the presence of 2 mM $MgCl_2$ or 2 mM $MnCl_2$ [111].

MLChs was observed in the presence of different $MeCl_2$ salts, but in average the activity decreased in the following order: $MnCl_2$ > $CoCl_2$ > $MgCl_2$ > $NiCl_2$ ≈ $CaCl_2$ (**Table 6**). For all 15 MLChs, apparent k_{cat} values were estimated (**Table 6**) [111]. Overall, all 15 MLChs showed enzymatic properties very different from canonical DNases and each MLCh preparation demonstrated a very specific ratio in the RAs in the presence of different metal ions used (**Table 6**).

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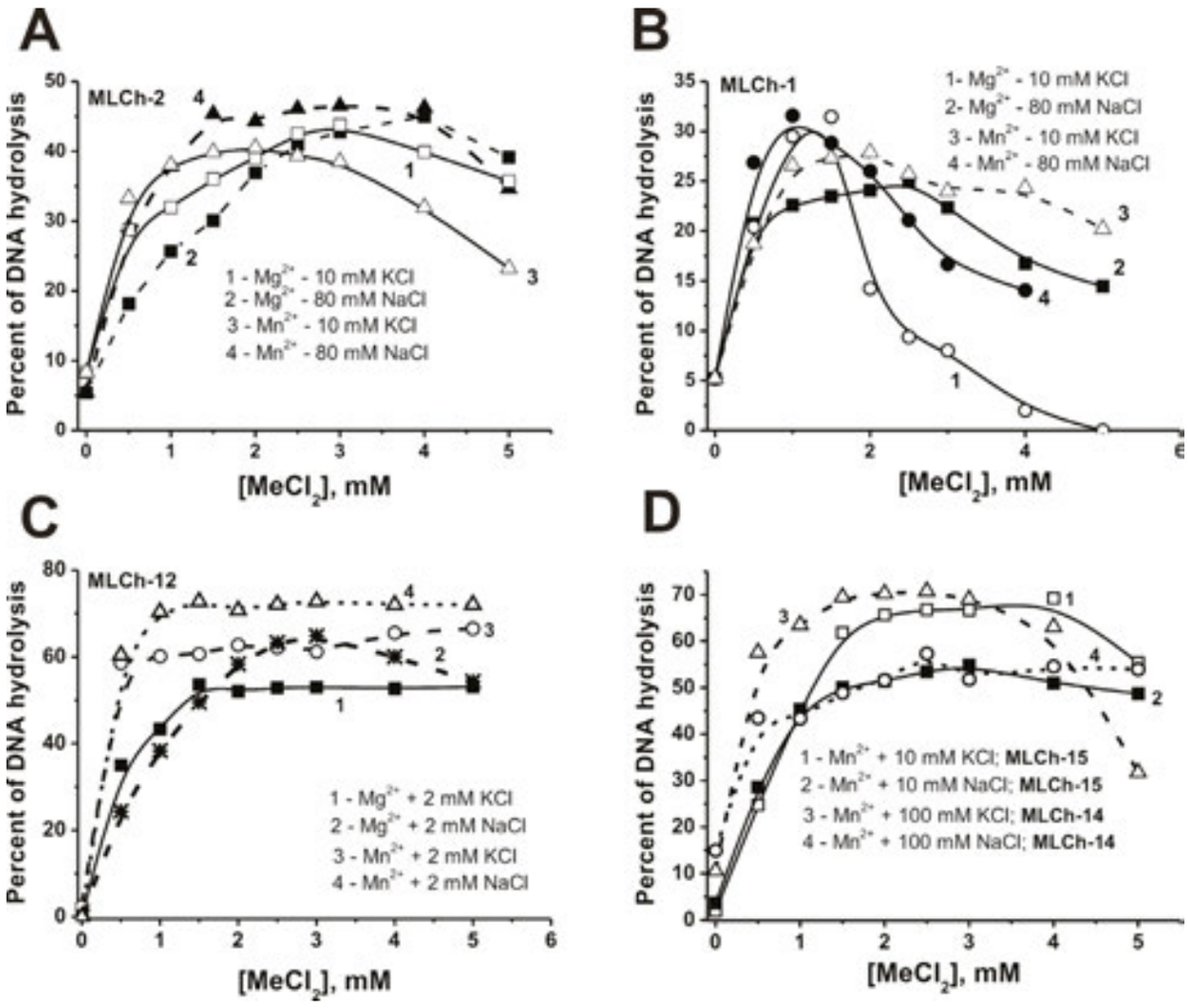

Figure 12. Dependences of the RAs of DNase activity for several MLChs (20 nM) on the concentrations of $MnCl_2$ and $MgCl_2$ at different fixed KCl and NaCl concentrations (A–D) [111]. Numbers of MLChs and concentrations of KCl and NaCl used are given on panels A–D. A complete hydrolysis of plasmid DNA was taken for 100%.

Our previous findings demonstrated that polyclonal abzymes from sera of patients with SLE, MS, and other autoimmune and/or viral diseases can contain many monoclonal DNase and RNase abzymes showing very different enzymatic properties [13–22]. At the same time, estimation of possible number of monoclonal abzymes in their total pools was very difficult, since they can have comparable or different affinity for DNA, significantly different optimal pHs, various k_{cat} values, and different dependencies on various Me^{2+} and Me^{+} ions.

In our study DNase activity only for 45 of 451 single colonies corresponding to only one (eluted with 0.5 M NaCl) of 16 fractions with different affinity for DNA-cellulose was analyzed, while MLChs of all these fractions effectively hydrolyze DNA [111]. Fifteen of 45 individual MLChs (~33%) were active in the hydrolysis of DNA. Taking into account the fact that only 45 of 451 colonies were analyzed, it should be assumed that even this fraction

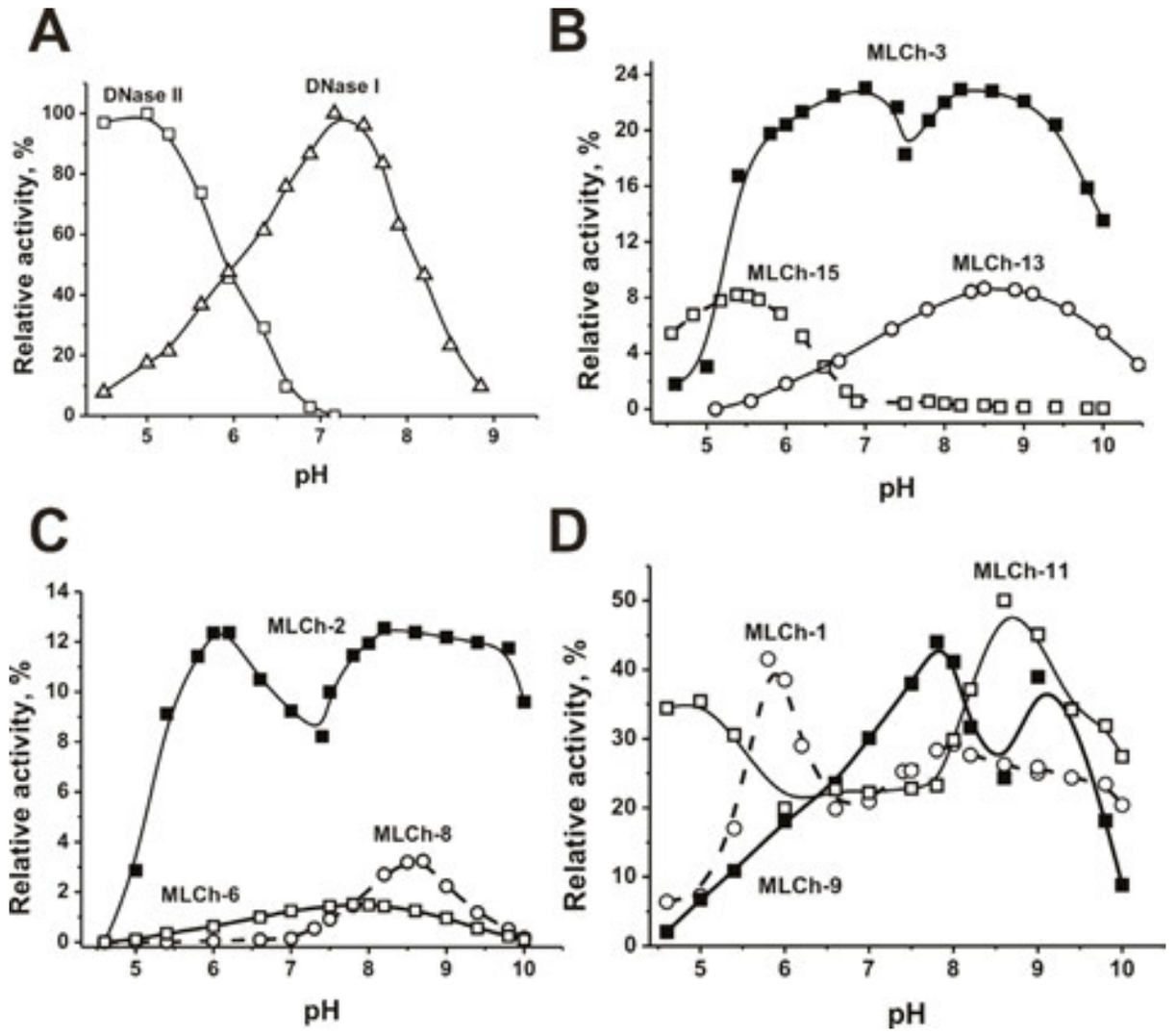

Figure 13. Dependences of the RAs of DNA-hydrolyzing activity of human DNase I and DNase II (A) and of nine MLChs (B–D) on pH of reaction mixtures [111]. The RAs for DNase II were estimated in the absence, while for DNase I, in the presence of $MgCl_2$ (10 mM). The RAs of nine MLChs were estimated using optimal $MeCl_2$ (2 mM), optimal concentration of KCl or NaCl (see **Table 6**). Reaction mixtures were incubated at 30°C for 0.4–5.0 h and then the data obtained were normalized to standard conditions; complete hydrolysis of scDNA for 30 min in the presence of various MLChs (10 nM) was taken as 100%.

contains much greater number of monoclonal abzymes with DNase activity. In this regard it should be mentioned the data of other article [112]. After separation of phage particles on DNA-cellulose, the fraction eluted by an acidic buffer (pH 2.6) was used for obtaining of MLChs (~28 kDa) with DNase activity. In this case, 33 of 687 individual colonies were chosen randomly for study of MLChs. Nineteen of 33 clones (58%) demonstrated DNase activity [112]. Detection of DNase activity *in situ* after SDS-PAGE of purified MLChs using gel containing DNA showed that they are not contaminated by canonical DNases. MLChs demonstrated from one to two pH optima. MLChs were inactive after the dialysis

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DNase or number of MLCh preparation	Optimal pH	
	pH_1	pH_2
DNase I	7.0–7.2	No second optimum
DNAse II	4.9–5.0	No second optimum
1	5.7–5.9	7.9–8.1[b]
2	6.0–6.2	8.2–8.3
3	6.9–7.0	8.2–8.5
4	7.5–7.6	No second optimum
5	6.9–7.0	8.2–8.5
6	7.8–8.0	No second optimum
7	6.2–6.4	No second optimum
8	8.5–8.6	No second optimum
9	7.8–8.0	8.9–9.1
10	6.1–6.3	8.5–8.7
11	4.8–5.0	8.6–8.7
12	7.7–7.9	No second optimum
13	8.5–8.7	No second optimum
14	7.9–8.1	No second optimum
15	5.4–5.6	No second optimum

[a]For each value, a mean of two measurements is reported.

[b]For various MLChs one or two optimal pHs were revealed.

Table 5. The optimal pH values for DNase II, DNase I, and 15 different recombinant and individual MLChs[a] [111].

against EDTA but were activated by different metal ions; the ratio of RA in the presence of Mg^{2+}, Mn^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+}, and Co^{2+} was individual for each MLCh preparation. Na^+ and K^+ suppressed DNA-hydrolyzing activity of these MLChs at different concentrations [112]. Hydrolysis of DNA by all MLChs was consistent with Michaelis-Menten kinetics. These recombinant MLChs demonstrated high affinity for DNA (K_m = 3–9 nM) and high k_{cat} values (3.4–6.9 min^{-1}) [112]. Even if we assume that each of the above-mentioned 16 fractions can contain only about 20 MLChs with DNase activity, their total number may be close to 300, but it is obvious that this number is much larger and can be close from one to several thousands.

Number of MLChs	Mg^{2+}	Mn^{2+}	Zn^{2+}	Ni^{2+}	Co^{2+}	Cu^{2+}	Ca^{2+}	pH[c]	[NaCl] or [KCl], mM[d]	*App.* k_{cat}, min^{-1}[b]
1	92.7	**100**[a]	6.5	10.7	20.6	0.0	4.0	5.8[e]	100.0 Na^+	0.6 ± 0.04
	60.7	100.0	7.5	11.5	18.5	0.0	5.4	8.0[g]	100.0 Na^+	
2	69.6	100.0	55.4	83.8	73.0	0.0	10.1	6.1	80.0 Na^+	0.1 ± 0.01
	100.0	92.3	18.5	95.6	63.7	3.0	26.0	8.2	80.0 Na^+	
3	78.3	98.5	42.7	56.2	0.3	9.0	100.0	6.9	5.0 K^+	
	1.3	100.0	3.6	11.9	99.0	0.0	0.0	8.4	5.0 K^+	0.33 ± 0.03
4	51.0	100.0	80.0	56.0	24.0	5.0	26.0	7.5	7.5 Na^+	0.5 ± 0.04
5	78.3	98.5	42.7	56.2	0.0	9.0	100	6.9	5.0 K^+	0.3± 0.03
	1.3	100.0	3.6	11.9	99.0	0.0	0.0	8.4	5.0 K^+	0.34 ± 0.03
6	1.3	100.0	3.6	11.9	99.0	0.0	0.0	7.9	1.0 K^+	0.02 ± 0.003
7	75	70	80	85	100.0	5	21	6.3	2.5 Na^+	0.3 ± 0.04
8	58.5	100.0	18.3	19.7	0.0	0.0	16.2	8.5	5.0 Na^+	0.06 ± 0.07
9	100.0	85.2	15.9	0.0	85.4	0.0	75	7.8	1.5 Na^+	0.7 ± 0.08
10	25	20	15	100.0	17	2	39	8.6	10.0 Na^+	0.2 ± 0.02
11	80	85	43	24	100.0	0.0	8	8.6	5.0 K^+	0.7 ± 0.06
12	55.0	88.5	16.5	56.3	100.0	1.2	56.2	7.8	5.0 K^+	0.12 ± 0.01
13	34.5	25.0	16.9	83.3	100.0	4.8	41.9	8.6	0.1 K^+	0.13 ± 0.01
14	80.9	75.2	19.7	100.0	59.5	6.3	37.3	8.0	5.0 Na^+	0.11 ± 0.01
15	100.0	49.2	55.7	33.5	85.8	45.0	70.0	5.5	1.5 Na^+	0.12 ± 0.01

[a]The maximal RAs in the presence of one of seven metal ions used was taken as 100% and given in bold; the error of the values determination (two independent experiments) did not exceed 7–10%.

[b]The apparent k_{cat} values using optimal conditions were calculated as $k_{cat} = V_{max}$ (nM/min)/[MLCh] (nM).

[c]Optimal pHs were used for every of MLCh preparation.

[d]Optimal concentrations of KCl and NaCl in the case of different MLChs were used.

[e]The RAs for several MLChs were found in the case of two different pH optima.

Table 6. The RAs of different recombinant MLChs in the presence of various metal ions (2 mM) at optimal pHs and concentration of KCl and NaCl [111].

7. Abzymes with protease activity

7.1. Polyclonal abzymes hydrolyzing myelin basic protein

The increased level of antibodies to myelin basic protein and abzymes hydrolyzing MBP was revealed for the first time in the blood of patients with multiple sclerosis [83–87]. It was shown that Abs of healthy donors cannot hydrolyze MBP [13–22, 83–87]. The most widely accepted theory of multiple sclerosis pathogenesis assigns the major role in the destruction of myelin

including MBP to the inflammation related to autoimmune reactions [43, 113–115]. Increased levels of Abs and oligoclonal IgGs in the cerebrospinal fluid together with clonal B cell accumulation in the CSF and lesions of MS patients are among the main evidences of MS [115].

ELISA was used for comparison of the relative levels of Abs against MBP in the sera of 12 healthy donors and 14 patients with SLE [77]. For healthy donors the concentrations of auto-Abs were not zero and varied from 0.02 to 0.16 A_{450} units; in average 0.09 ± 0.04 A_{450} units; similar value (0.09 ± 0.04 A_{450} units) was previously revealed for other 10 healthy volunteers [83]. Relative concentrations of anti-MBP Abs of SLE patients were changed from 0.27 to 0.54 A_{450} units, in average 0.38 ± 0.08 A_{450} units [77]. Using the same test system, it was previously revealed that the indexes of anti-MBP Abs for 25 MS patients are changed from 0.67 to 0.98 A_{450} units, in average 0.8 ± 0.1 A_{450} units [83, 84]. Thus, all SLE patients demonstrated in average ~4.2-fold higher level of anti-MBP Abs then healthy donors, but by a factor of ~2.1 lower level than MS patients.

Electrophoretically and immunologically homogeneous polyclonal IgGs were purified from the sera of SLE patients by sequential chromatography of serum proteins on Protein-G Sepharose using conditions removing nonspecifically bound proteins, followed by FPLC gel filtration in condition destroying immune complexes [77, 78] similarly to obtaining of MS IgGs [84–87]. It was shown that 150 kDa SLE IgGs are electrophoretically homogeneous and in contrast to Abs from healthy donors are active in the hydrolysis of MBP [55, 77]. To prove that MBP-hydrolyzing activity of SLE IgGs is their intrinsic property, we have checked the fulfilment of several known strict criteria described above including analysis of Ab protease activity after SDS-PAGE (**Figure 7**) [77]. In addition, it was shown that in contrast to canonical proteases, the SLE polyclonal IgGs separated using MBP-Sepharose specifically hydrolyzed only MBP (**Figure 14A**) but not many other tested control proteins (**Figure 14B**) [77].

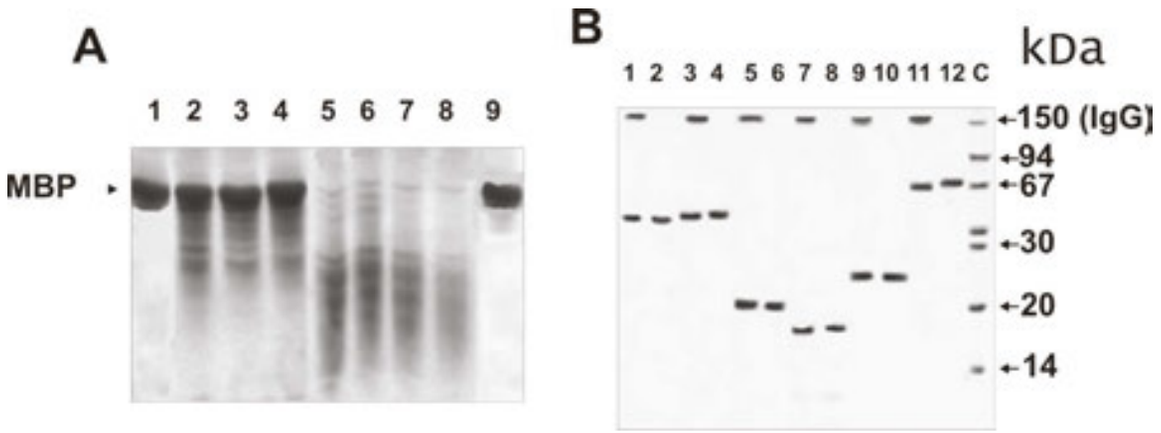

Figure 14. The hydrolysis of MBP (0.7 mg/ml) by 3 μg/ml IgGmix (mixture of IgGs of 12 SLE patients) separated by chromatography on MBP-Sepharose after incubation for 1 (lane 2), 2 (lane 3), 3 (lane 4), 14 (lane 5), 16 (lane 6), 18 (lane 7), and 24 h (lane 8) (A) [77]. Lanes 1 and 9 correspond respectively to MBP incubated during 24 h alone or in the presence of 0.2 mg/ml IgGmix from 12 healthy volunteers. SDS-PAGE analysis of the hydrolysis of different control proteins by the same IgGmix (B). Proteins were incubated for 24 h with 30 μg/ml IgGmix (odd numbers) or without antibodies (even numbers): BSA (lanes 1 and 2), human serum albumin (lanes 3 and 4), casein from human milk (lanes 5 and 6), lysozyme from hen eggs (lanes 7 and 8), bovine aldolase (lanes 9 and 10), and lactoferrin from human milk (lanes 11 and 12). Lane C corresponds to standard protein markers. SLE IgGs specifically hydrolyze only MBP (A), but not other proteins (B).

Protease IgGs from the sera of ~95–100% of patients with different autoimmune pathologies [9, 66, 116], human milk Abs hydrolyzing casein [96, 117], Abs from AIDS patients hydrolyzing HSA, casein, and HIV reverse transcriptase [95] are serine-like proteases, whose activity is strongly decreased after their preincubation with serine protease-specific inhibitors PMSF. In addition, a high metal-dependent MBP-hydrolyzing activity for MS IgGs [86] and casein-hydrolyzing of human milk sIgAs [96] were recently revealed. It was shown that antiintegrase IgGs and IgMs of HIV-infected patients can contain abzymes hydrolyzing viral integrase of four types, resembling serine, thiol, metal-dependent, and acidic proteases, the ratio of which may be individual for every AIDS patient [97, 98].

It was shown that preincubation of individual polyclonal SLE IgGs with specific inhibitor of thiol proteases iodoacetamide and of acidic proteases pepstatin A [77, 78] similarly to MS IgAs, IgGs, and IgMs [84–87] leads to a small effect (5–15%) on MBP hydrolysis by SLE IgGs. PMSF specifically inhibiting serine proteases and inhibitor of metalloproteases EDTA remarkably or significantly suppressed proteolytic activity of SLE IgGs (**Figure 15A**).

The inhibition of Abz activity by EDTA is significantly greater than by PMSF. Overall, all individual SLE abzymes possess specific ratio of RAs in the presence of PMSF and EDTA (**Figure 15A**). Interestingly, polyclonal SLE abzymes was more sensitive to EDTA than MS Abs [77]. Catalytic heterogeneity of polyclonal abzymes with several different activities from patients with various autoimmune diseases and animals was shown in many papers [65, 80–82, 92]. The above data also demonstrate extreme heterogeneity of SLE abzymes hydrolyzing MBP. In addition, polyclonal MBP-hydrolyzing abzymes of every patient are characterized with specific dependence of RAs upon pH; the pH profile of each IgG is unique (**Figure 15B** and **D**). The effect of several different metal ions on the MBP-hydrolyzing activities of dialyzed against EDTA individual 12 SLE polyclonal IgGs was analyzed (**Figure 16A** and **B**; B is a continuation of A).

All 12 IgGs demonstrated the individual ratios of RAs in the presence of eight various metal ions. To analyze the "average" effect of different metal ions on SLE and MS IgGs, we have used SLE IgGmix and MS IgGmix before (**Figure 16C**) and after (**Figure 16D**) their dialysis against EDTA. Ca^{2+} was shown to the best activator of SLE IgGmix the effect of different metals decrease in the following order: $Ca^{2+} > Co^{2+} \geq Ni^{2+} \geq Mg^{2+} \geq Mn^{2+} \geq Cu^{2+}$. Fe^{2+} did not activate SLE IgGmix, while Zn^{2+} inhibits its activity. MS IgGmix demonstrated a different order of the metal-dependent activity: $Mg^{2+} > Mn^{2+} \geq Cu^{2+} \geq Ni^{2+} \geq Co^{2+} \geq Ca^{2+}$, while Fe^{2+} and Zn^{2+} slightly inhibit MBP hydrolysis (**Figure 16C** and **D**).

In addition, the mixture of electrophoretically homogeneous IgGmix was separated to fractions of IgG1–IgG4 subclasses and to fractions of IgGs containing lambda- and kappa-type of light [76]. The immunological purity of IgGs of all types was revealed by ELISA; the preparations of IgG1, IgG2, IgG3, and IgG4 did not contain IgGs of other subclasses. The lambda - and kappa-IgGs and IgG1–IgG4 were active in the hydrolysis of MBP and their RAs and k_{cat} values are given in **Table 7**. Kappa-IgGs demonstrated 1.2-fold lower apparent k_{cat} (2.4×10^{-2} min^{-1}) than lambda-IgGs (2.8×10^{-2} min^{-1}). The apparent k_{cat} values of different subclasses IgG abzymes in the hydrolysis of MBP increased in the following order (min^{-1}): IgG4 (1.7) < IgG2 (2.7) < IgG3 (2.9) < IgG1 (3.0) (**Table 7**). The relative content of kappa-IgGs and lambda-IgGs

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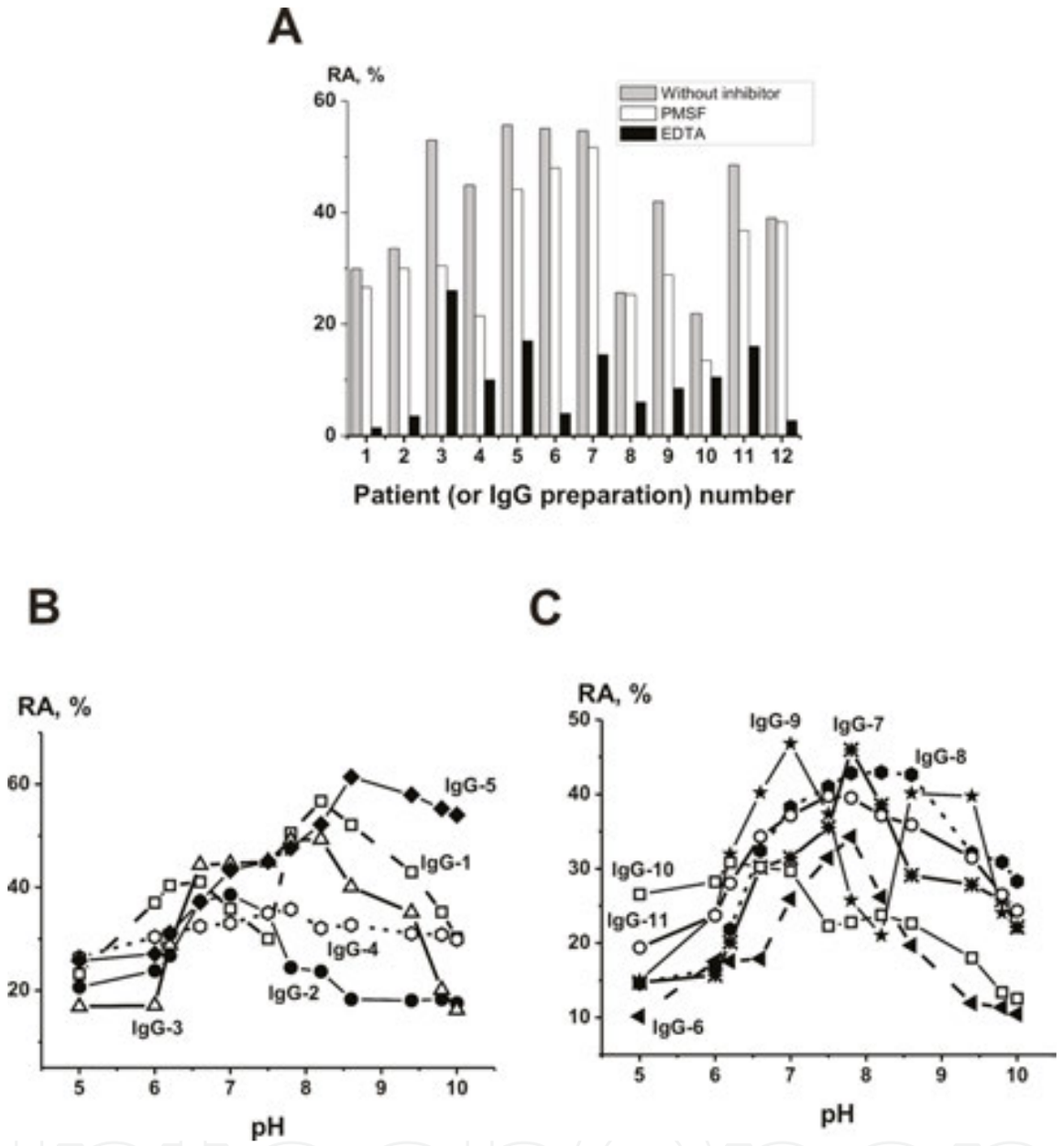


Figure 15. The relative proteolytic activities of 12 different individual SLE IgGs in the hydrolysis of MBP (0.5 mg/ml) before (gray columns), after their preincubation with PMSF (white columns) or 35 mM EDTA (black columns) (A) [77]. The pH dependences of the RAs of MBP-hydrolyzing activity of 11 individual SLE IgGs (B and C). The complete hydrolysis of MBP (0.5 mg/ml) was taken for 100%.

as well as IgG1–IgG4 in nonfractionated IgGmix was estimated (**Table 7**). Taking this content into account, the relative contribution of kappa-IgGs and lambda-IgGs into the total MBP-hydrolyzing activity of IgGmix was estimated as 48.4 ± 4.0% and 55.5 ± 4.3%, respectively (**Table 7**). The relative contribution of SLE IgGs of different subclasses to the total proteolytic activity of IgGmix was estimated in a similar way: IgG1 (73.0 ± 3.4%) > IgG2 (19.1 ± 1.8%) > IgG3 (6.7 ± 0.3%) > IgG4 (1.2 ± 0.2%). These data provided the first evidence that SLE IgGs of all types possess MBP-hydrolyzing activity, but they differ in the relative contribution into the total activity of proteolytic activity of polyclonal abzymes [76]. Kappa-IgGs and lambda-IgGs

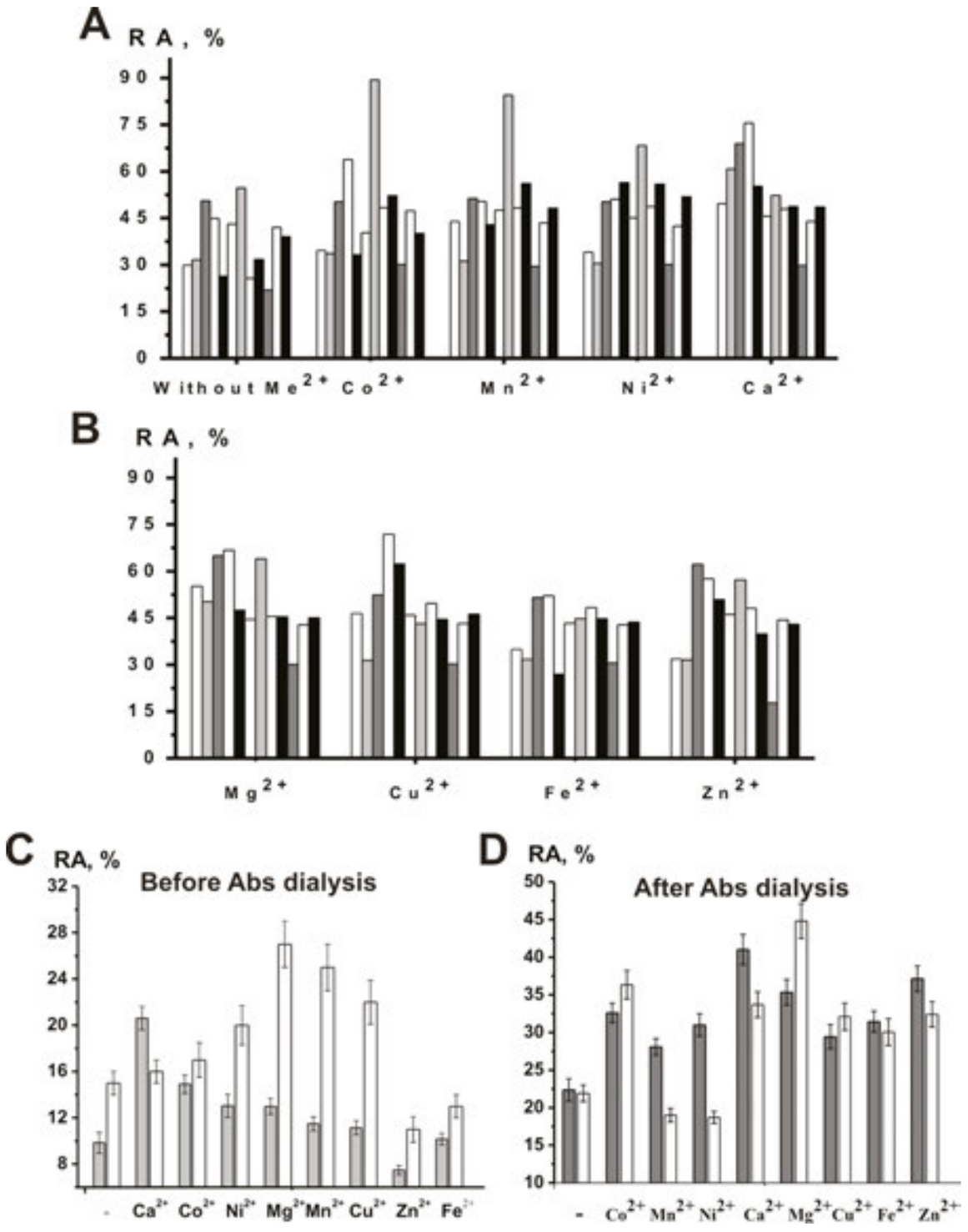


Figure 16. The RAs of 12 SLE IgGs dialyzed against EDTA in the hydrolysis of MBP [77]; 12 columns of different color of the first set (A) correspond to 12 individual Abs in the absence of external metal ions, while 8 other columns sets to the same 12 IgGs in the presence of 8 different metal ions marked on panels A and B. An average error did not exceed 10% (A and B). The RAs of SLE IgGmix (gray columns) and MS IgGmix (white columns) before (C) and after (D) these Abs dialysis against EDTA in the cleavage of MBP in the presence of different metal ions (2 mM). A complete hydrolysis of MBP (0.5 mg/ml) for 1 h in the presence of 0.01 mg/ml Abs was taken for 100%. Various metal ions are shown in panels A–D.

IgG	Content, %	RAs (mg MBP/1 h) / mg of IgGs**	Apparent k_{cat}, $\times 10^2$ (min^{-1})§	Contribution to the total activity, %#
IgG, nonfractionated	100	1.95 ± 0.05	2.6 ± 0.07	100
IgGs containing lambda- and kappa-types of light chains				
kappa-IgG	44.6 ± 4.0	2.1 ± 0.16*	2.8 ± 0.21	48.4 ± 4.0
lambda-IgG	55.4 ± 5.0	1.8 ± 0.15	2.4 ± 0.20	55.5 ± 4.3
IgGs of different subclasses				
IgG1	70.8 ± 2.0	2.3 ± 0.11	3.0 ± 0.14	73.0 ± 3.4
IgG2	20.6 ± 3.0	2.0 ± 0.2	2.7 ± 0.25	19.1 ± 1.8
IgG3	6.7 ± 1.5	2.2 ± 0.08	2.9 ± 0.10	6.7 ± 0.3
IgG4	1.9 ± 1.0	1.3 ± 0.07	1.7 ± 0.09	1.2 ± 0.2

*For each fraction, a mean of two repeats is used.

**Relative activities at fixed 0.75 mg/ml concentration of MBP were estimated.

§Apparent k_{cat} values were calculated as $k_{cat} = V$ (M/min)/[IgG] (M).

#Contribution of different IgGs to the total activity of nonfractionated Abs was calculated taking into account the relative content of these IgGs within polyclonal IgG_{mix} and their RAs in the hydrolysis of MBP.

Table 7. Relative specific MBP-hydrolyzing activities (RAs) of IgGs of different types and their relative contributions to the total activity of polyclonal IgG_{mix} [76].

hydrolyzed MBP within a wide range of pH values (5.3–9.5) and showed comparable pH dependencies, while the pH profiles for IgG1–IgG4 were unique (**Figure 17**).

These results clearly demonstrate that IgGs of all four subclasses are very heterogeneous and can consist of different sets of catalytic subfractions of polyclonal IgG having quite distinct pH dependencies. **Figure 17** shows the relative influence of PMSF and EDTA on the MBP-hydrolyzing activity of different IgGs. The nonfractionated IgGs and lambda-IgGs demonstrated lower inhibition by PMSF than that for EDTA (**Figure 17**). The inhibition of serine-like and metal-dependent activities of kappa-IgGs were comparable. PMSF suppressed MBP-hydrolyzing activity of IgG3, IgG2, and IgG1 by 13–17%, while the decrease of this activity by EDTA was significantly greater, 30–45%. There was no noticeable PMSF effect on the IgG4 activity, while EDTA decreased its activity by ~65% (**Figure 17**). Thus, IgG1–IgG4, kappa-IgGs, and lambda-IgGs are characterized by specific ratios of metal-dependent and serine-like proteolytic activities.

The cleavage site specificity of different IgG preparations in the case of four oligopeptides corresponding to four antigenic determinants of MBP was analyzed [76]. Overall, kappa-IgGs and lambda-IgGs, as well as IgG1–IgG4 demonstrated either different patterns of four oligopeptides cleavage, or at least stimulate the accumulation of the same products of the hydrolysis with different efficiency.

The dialysis of IgGs caused a more pronounced decrease in the activity of kappa-IgGs than of lambda-IgGs [76]. Addition to the reaction mixtures of Ca^{2+} + Mg^{2+} or Ca^{2+} + Co^{2+} led to

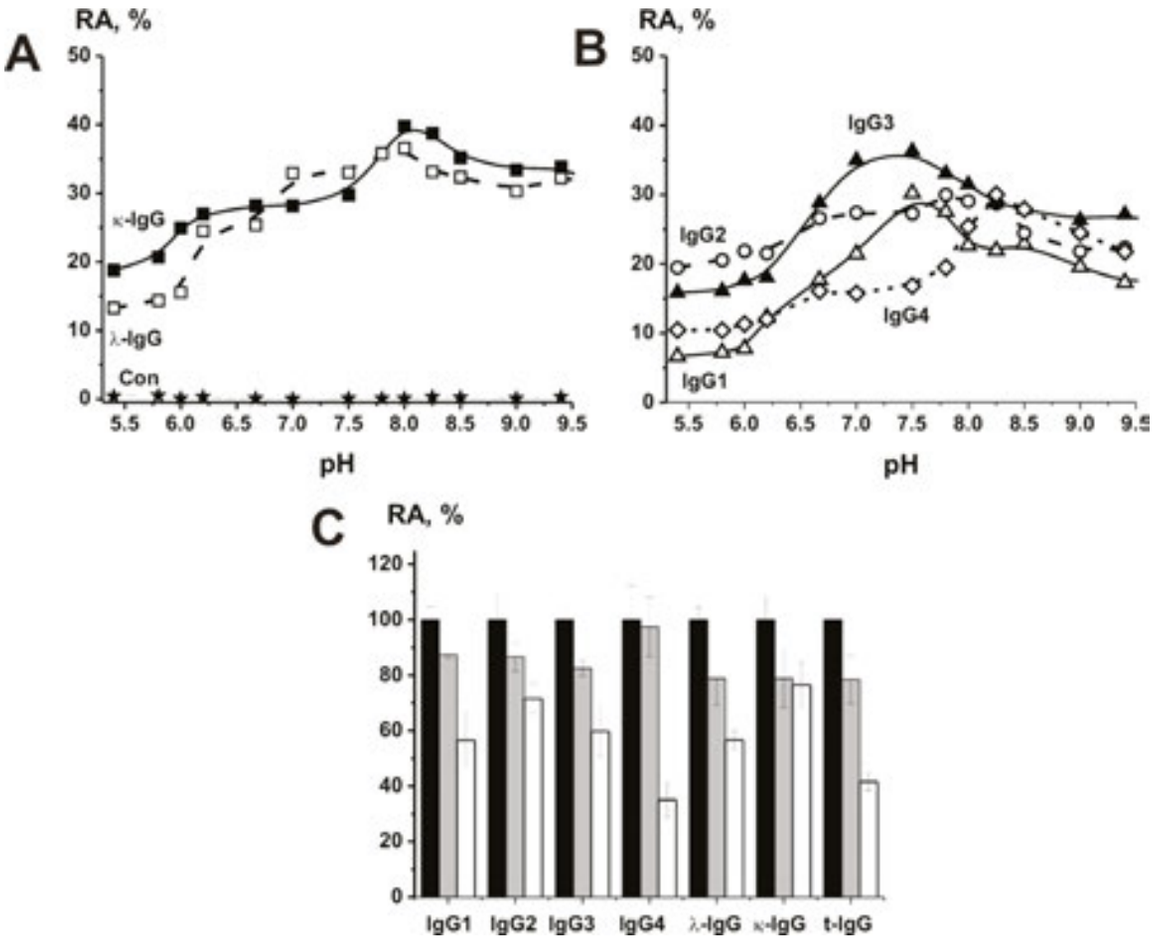


Figure 17. The pH dependence of RAs of MBP-hydrolyzing SLE kappa-and lambda-IgGs (A), as well IgG1, IgG2, IgG3, and IgG4 (B) [76]. Hydrolysis of MBP incubated without IgGs was used as control ("Con," A). The RAs of MBP-hydrolyzing activity of SLE IgG1, IgG2, IgG3, IgG4, and total IgGmix (t-IgG) (C). The RAs were determined before (black columns) and after IgGs preincubation with PMSF (gray columns) and EDTA (white columns). The RAs of all IgGs in the absence of the inhibitors were taken as 100%.

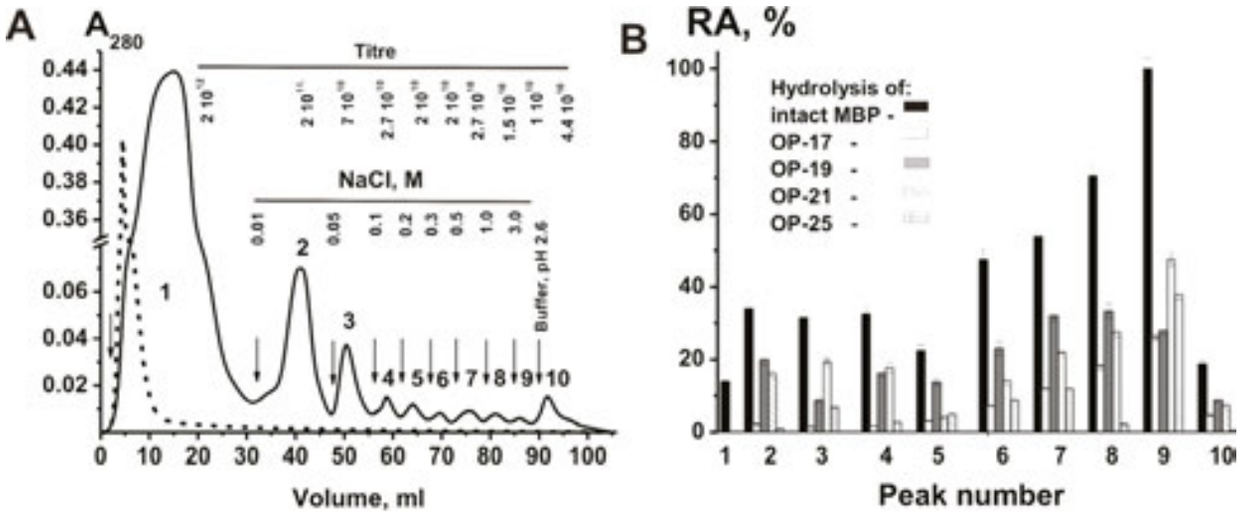
approximately comparable increase in the RAs of dialyzed lambda-IgG (1.6- to 1.7-fold), kappa-IgG (2.0- to 2.3-fold), and nonfractionated IgGs (1.7- to 1.8-fold). Ca^{2+}+Co^{2+} together cannot activate IgG1, while in the presence of Ca^{2+} + Mg^{2+} its activity increased by a factor of 1.6. Ca^{2+} + Co^{2+} increased the activity of IgG2 (~2.9-fold), IgG3 (~6.4-fold), and IgG4 (~6.0-fold). A significant increases in the RAs were revealed for Ca^{2+} + Mg^{2+} in the case of IgG3 (~3.5-fold), IgG4 (~4.4-fold), and IgG2 (~5.7-fold). While the Ca^{2+} + Mg^{2+} combination was the best for the activation of IgG2 and IgG1, IgG4, and IgG3 showed the highest activity in the presence of Ca^{2+} + Co^{2+}. The ratios of RAs of all IgG preparations before and after their dialysis against EDTA, as well as in the presence of different metal ions, were individual for every preparation analyzed. These data indicate for an extreme Me^{2+}-dependence diversity of different subclasses SLE IgGs hydrolyzing MBP.

The extraordinary diversity of polyclonal abzymes with DNase, RNase, and proteolytic activities was shown not only in the case of SLE, but also other diseases [13–22]. Very unexpected enzyme properties have been discovered in the case of monoclonal abzymes of patients with SLE.

7.2. Monoclonal SLE abzymes hydrolyzing myelin basic protein

For analysis of MBP-hydrolyzing activity of Abs, we have used the same phagemid library of kappa light chains [118–120] as for analysis of MLChs with DNase activity [111, 112]. The phage particles containing MLChs with different for MBP were separated by affinity chromatography on MBP-Sepharose (**Figure 18A**).

The pool of phage particles was distributed between 10 peaks eluted from the sorbent and all MLChs of fractions of 10 new small pools efficiently hydrolyzed MBP and four oligopeptides (OPs) corresponding to four immunodominant MBP sequences containing cleavage sites (**Figure 18B**). However, there were no any detectable particles peaks having considerable affinity for MBP after similar chromatography of phage particles with pCANTAB plasmid containing no cDNA of light chains (**Figure 18A**). Thus, the MLChs pools of all 10 phage particles fractions having different affinity to MBP contain both inactive and catalytically active light chains hydrolyzing MBP. Similar distribution all over the chromatography profiles was



C

OP-21

AAQKRPSQRSK***YLASASTMDHARHGFLPR***RHRDTGILDSLGRFFGSDRGAPK

OP-17

RGSGKDGHHAARTTHYGSLPQKAQGHRPQD***ENPVVHFFKNIVTPRTP***PPSQG

OP-19

KGRGLS***LSRFSWGAEGQKPGFGYGG***RASDYKSAHKGLKGHD

OP-25

AOGTLSKIFKLGGRDSRSGSPMARR

Figure 18. Affinity chromatography on MBP-Sepharose of phage particles: (– –) and (—) absorbance at 280 nm of particles corresponding plasmid respectively without and with kappa light chains cDNA (A) [118]. Relative titres of phage particle and NaCl concentrations corresponding to various peaks are shown on panel A. The bars (B) indicate the RAs of 10 phage particles small pools of peaks 1–10 eluted from the MBP-Sepharose with different NaCl concentrations and acidic buffer (pH 2.6) (A); the reaction mixtures containing MBP (0.7 mg/ml) were incubated at 30°C for 12 h or different 1 mM OPs: OP17, OP19, OP21, and OP25 (see panel C) were incubated with 109 plaque-forming units/ml phage particles for 6 h and a complete hydrolysis of the substrates was taken as 100%. Complete MBP protein sequence and positions of four OPs sequences containing the protein cleavage sites (C).

observed for polyclonal IgGs from SLE and MS patients in the case of their chromatography on MBP-Sepharose [76, 77, 86, 87].

Phage particles eluted from MBP-Sepharose with 0.5 M NaCl (peak 7, **Figure 18A**) were used for preparation of individual colonies. Overall, 72 of 440 individual colonies choosing in a random way were used for study of MBP-hydrolyzing activity. MLChs of 22 of 72 single colonies (~30%) possess MBP-hydrolyzing activity. All 22 recombinant catalytically active MLChs containing a sequence of 6 histidine residues interacting with Ni^{2+} ions and 5 MLChs without activity were purified by chromatography on charged with Ni^{2+} ions HiTrap chelating Sepharose and by following FPLC gel filtration. Then a mixture of equal amounts of 22 catalytically active monoclonal MLChs (act-MLChmix) and second mixture of five preparations without activity (inact-MLChmix) were prepared. The electrophoretical homogeneity of ~26- to 27-kDa inact-MLChmix and act-MLChmix was shown by SDS-PAGE with silver staining (**Figure 19A**, lane 1).

MLChmix was subjected to SDS-PAGE; its proteolytic activity was revealed after extraction of proteins from the separated gel slices only in the band corresponding to the MLCh (**Figure 19A** and **B**). Act-MLChmix demonstrated activity in the hydrolysis of MBP (**Figure 19C**, lane 4), while inact-MLChmix had no activity (**Figure 19C**, lane 2). Moreover, in contrast to canonical proteases cleaving all proteins, act-MLChmix hydrolyzes only MBP (**Figure 19C**, lane 4) but no other control proteins (**Figure 19C**, lanes 5–8). All 22 act-MLChs and 5 inact-MLChs showed positive answer with mouse Abs (conjugated with horseradish peroxidase) against light chains of human Abs at Western blotting and positive ELISA response using plates with immobilized MBP.

The RAs in the hydrolysis of four different OPs were analyzed by TLC. **Figure 19(D)** and **(E)** demonstrates several typical examples of the OP19 and OP21 hydrolysis by different MLChs [118]. Initially, we have assumed that every of 22 MLChs corresponds to IgGs to one of four known specific MBP immunodominant sequences and that each MLCh can bind and hydrolyze only one of four OPs. At the same time, unexpected results were obtained. The RAs for 22 MLCh are summarized in **Figure 19(F)**. All 22 MLChs hydrolyzed only three or four OPs and with significantly different efficiency in the case of every OP. Hydrolysis of OP17 MBP was very weak (~1–1.5%) except seven MLChs: 15 ≥ 10 ≥ 12 ≥ 1 ≥ 16 ≥ 20 ≥ 8 (1.6–7.1%) (**Figure 19F**). All MLChs except MLCh-22 hydrolyzed efficiently OP21 and several other OPs, while six other MLChs (8, 9, 10, 12, 13, and 14) demonstrated high activity only in the cleavage of OP21. Several MLChs (1–7, and 11) efficiently hydrolyzed OP19 and OP21, while MLCh-18 and 20 cleaved OP21 and OP25. Four recombinant MLChs (15, 17, 19, and 21) cleaved three OPs with relatively high efficiency, while MLCh-16 hydrolyzed all four OPs (**Figure 19E**). The ratios of the RAs in the hydrolysis of four OPs were specific for every MLCh (**Figure 19E**). OP21 and OP19 were shown to be the best substrates for most MLChs, while 15–22 MLChs better hydrolyzed OP25.

In contrast to MS IgGs [76, 77, 84–87], SLE polyclonal abzymes with MBP-hydrolyzing activity are less sensitive to PMSF than to EDTA. The effect of PMSF and EDTA on the RAs of 22 different MLChs was analyzed [118]. **Figure 20(A)** shows that the 12 MLChs (1, 2, 3, 5, 7, 8, 12, 13, 15, 16, 17, and 19) are metal-dependent proteases; they cannot not remarkably decrease

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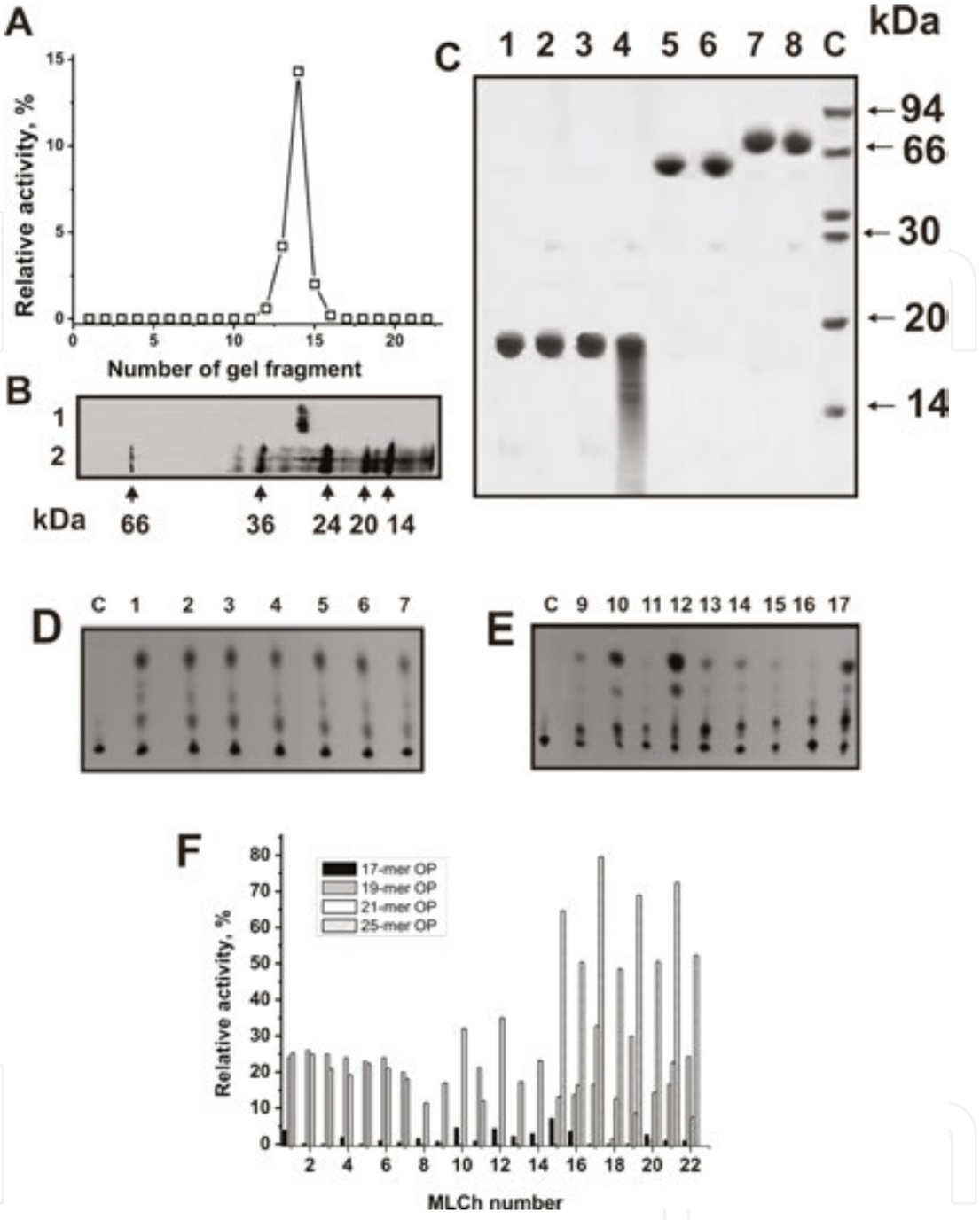

Figure 19. SDS-PAGE analysis of proteolytic activity (A) and homogeneity of act-MLChmix (7 μg) (B, lane 1) using a 5–16% gradient gel with following silver staining; the arrows indicate the positions of protein markers (B, lane 2) [118]. After electrophoresis the gel was incubated using special conditions for renaturation of act-MLChmix. The RAs in the hydrolysis of MBP (%) was determined using the extracts of 2- to 3-mm 22 gel fragments (A). The complete hydrolysis of MBP (0.7 mg/ml) after 24 h of mixture (20 μl) incubation with 15 μl of extracts was taken for 100%. SDS-PAGE analysis of MBP hydrolysis by 30 μg/ml inact-MLChmix (lane 2) and by act-MLChmix (lane 4) for 4 h; MBP incubated alone (lanes 1 and 3). The absence of detectable hydrolysis of control 0.7 mg/ml proteins by act-MLChmix is shown: human serum albumin (lane 6), human milk lactoferrin (lane 8); lanes 5 and 7 correspond to the proteins incubated alone. Lane C corresponds to standard protein markers. TLC analysis of OP19 (D) and OP21 (E) hydrolysis by different MLChs. The 1 mM OPs were incubated at 30°C for 24 h without MLChs (lanes C) or in the presence of 50 μg/ml of various MLChs (MLChs numbers are given on top of the panels) demonstrating relative activities in the hydrolysis of OP19 and OP21. Panel F shows the RAs of 22 various MLChs in the hydrolysis of OP25, OP21, OP19, and OP17.

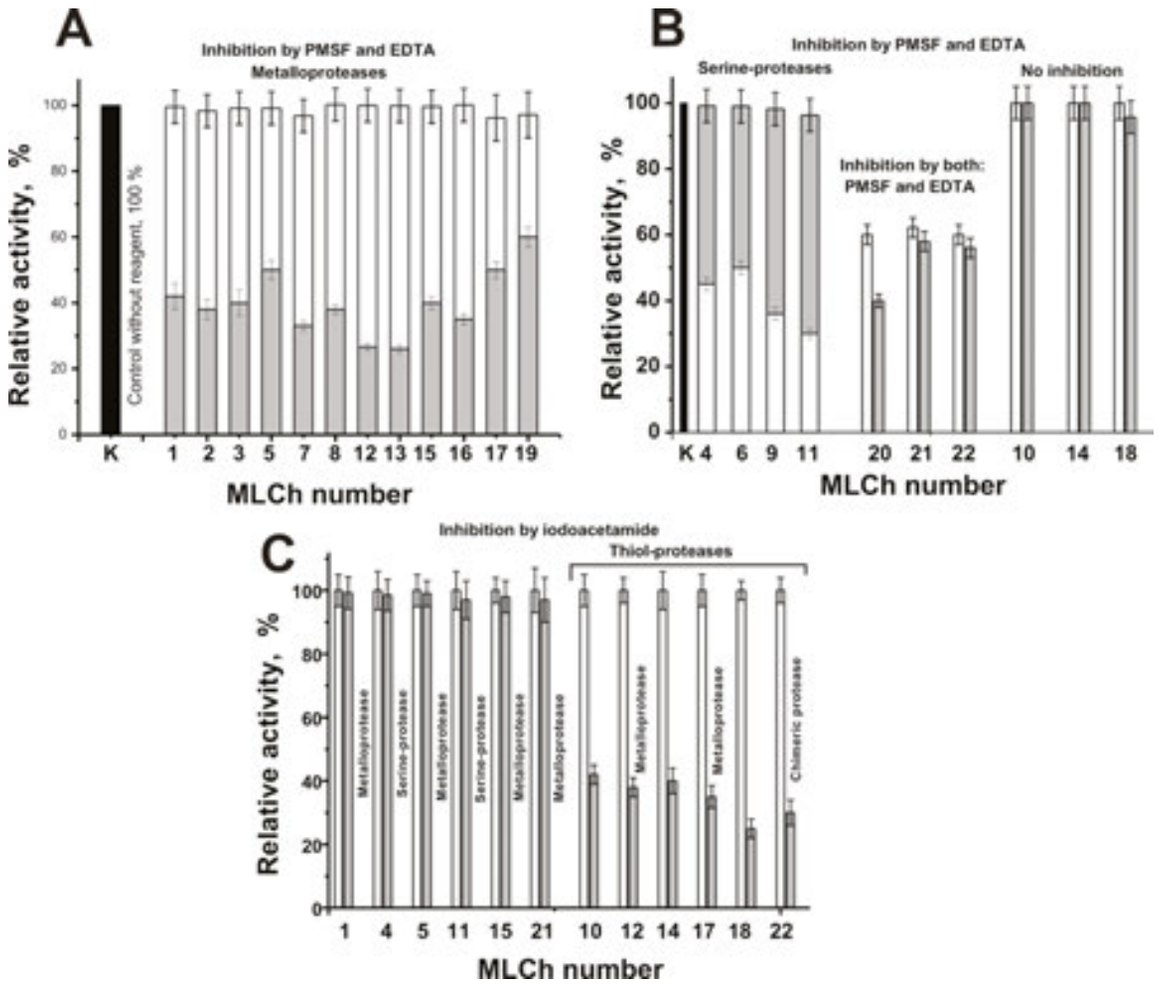

Figure 20. The RAs of 22 MLChs in hydrolysis of MBP after Abzs preincubation with specific inhibitors of proteases of different type. MLChs (0.1 mg/ml) were preincubated without of other components (black bars), with 50 mM EDTA (gray bars) or with 1 mM PMSF (white bars); then aliquots of these mixtures were added to standard reaction mixtures (A and B) [118]. Several examples (C) of the RAs of MLChs having metal-dependent activity (1, 5, 12, 15, and 21) and serine-protease-like activity (4 and 11), demonstrating no iodoacetamide-dependent activity; three MLChs (10, 14, and 18) showing negative response to EDTA and PMSF. MLCh-22 demonstrating positive effects of EDTA and PMSF, but significantly decreasing its activity after preincubation with iodoacetamide. White and gray bars show, respectively, the activity before (control) and after MLChs treatment with iodoacetamide (panel C). The RAs of all 22 MLChs before their treatment with different inhibitors were taken as 100%.

their proteolytic activity after incubation with PMSF, while EDTA significantly suppresses their MBP-hydrolyzing activity.

Four MLChs (4, 6, 9, and 11) demonstrate serine-like proteolytic activity; PMSF suppressed their activity, but there was no noticeable effect of EDTA (**Figure 20B**). PMSF suppressed protease activity of three MLChs (20, 21, and 22) by ~40%, and their inhibition by EDTA was to some extent comparable, 40–60% (**Figure 20B**). Thus, three MLChs (20–22) are characterized to some extent comparable ratios of metal-dependent and serine-like protease activities. A very intriguing situation was observed for three MLChs (18, 14, and 10); EDTA and PMSF do not remarkably decreased their proteolytic activity (**Figure 20B**). No significant suppression (5–15%) of MS and SLE polyclonal MBP-hydrolyzing abzymes by specific

inhibitors of thiol proteases was revealed previously [76, 77, 84–87]. However, iodoacetamide inhibited integrase hydrolyzing activities of all polyclonal IgG and IgM preparations from HIV/AIDS patients by 12–99% [97, 98]. Proteolytic activities of three MLChs (18, 14, and 10) not inhibited by EDTA and PMSF were significantly suppressed by iodoacetamide, while there was no effect on the most of MLChs with metal-dependent and serine-like activities (for example, **Figure 20C**). Thus, these three MLChs (18, 14, and 10) are thiol proteases. Interestingly, but iodoacetamide significantly suppressed the activities of MLChs 17 and 12 (**Figure 20C**), which were also significantly inhibited by EDTA (**Figure 20A**). One can suppose that MLChs 17 and 12 may be MLChs, the active sites of which contain amino acid residues corresponding to metal-dependent and thiol proteases. A very surprising data were obtained for MLCh-22; its activity was significantly suppressed not only by EDTA and PMSF (**Figure 20B**), by also iodoacetamide (**Figure 20C**). The relative number of MLChs, which activity depend on iodoacetamide is only approximately 27% of all 22 MLChs, while at the same time, several of them possess metal-dependent and serine-like activities. Therefore, the relative contribution of thiol-like protease activity to a total MBP-hydrolyzing activity of polyclonal SLE and MS abzymes may be significantly lower than of Abzs with metal-dependent and serine-like proteolytic activities and, therefore, depending on the patient a relative contribution of thiol-like protease to the total activity may be about 5–15%, as found previously for polyclonal Abzs [76, 77, 84–87]. The effects of various metal ions on the protease activities of 22 MLChs were compared (**Figure 21A** and **B**; B is a continuation of A).

Seven different metal ions did not effect on the activity of MLChs with serine-like (9, 6, and 4) and thiol-dependent (18, 14, and 10) activities. Five MLChs (19, 17, 13, 8, and 2) were only slightly activated by several Me^{2+} ions, while Ca^{2+} was the best activator. Two MLCh preparations (5 and 3) were Co^{2+} dependent, but preparation 15 was better stimulated by Ni^{2+}, MLCh-16 and MLCh-20 were respectively Mn^{2+}- and Zn^{2+}-dependent (**Figure 21**). MLChs 22 and 12 were activated by two different metal ions, Zn^{2+} and Ca^{2+}. Two MLChs were activated by three different Me2 ions: MLCh-7 ($Ca^{2+} > Zn^{2+} > Co^{2+}$) and MLCh-1 ($Ca^{2+} > Ni^{2+} > Mg^{2+}$). In addition, MLCh-21 was activated by four ($Cu^{2+} > Ca^{2+} > Co^{2+} > Zn^{2+}$) metal ions. These data show the extreme Me^{2+}-dependence diversity of IgGs from SLE patients and their light chains in the hydrolysis of MBP [118].

All 22 MLCh preparations hydrolyzed efficiently MBP within a wide range of pHs (5.0–10), but in contrast to polyclonal SLE IgGs, they show mainly only one pH optimum [118]. Only the pH profile for preparation 4 demonstrates optimal pH at 5.7–5.9 and pronounced shoulder at pHs 6.5–7.5 (**Figure 21C**). The apparent k_{cat} values under optimal conditions for every MLCh were estimated. The data on several characteristics of 22 various MLCh preparations are summarized in **Table 8** [118]. One can see that all MLChs demonstrate very different physicochemical and enzymatic properties.

On the next step we analyzed in more detail three additional MLChs (numbers 23–25) corresponding to peak 7 eluted from MBP-Sepharose with 0.5 M NaCl (**Figure 18A**) [119, 120]. These three MLChs were purified and characterized in detail exactly similar to above described 22 preparations [118]. The DNA sequence of NGTA1-Me-pro demonstrated high

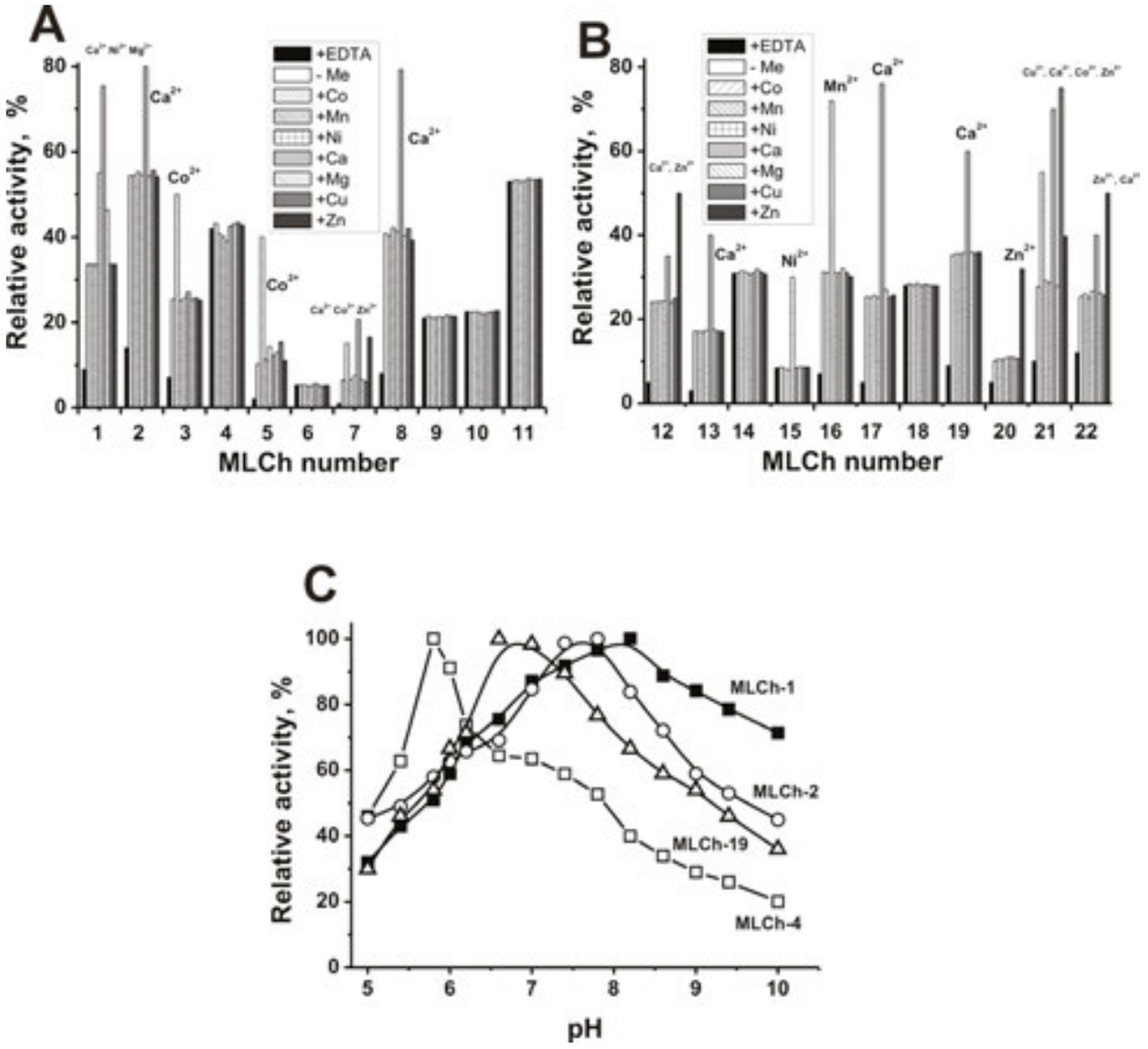

Figure 21. Effect of various metal ions on the RAs of 22 MLChs in the hydrolysis of MBP (A and B) [118]. Black first bars correspond to the RAs in the presence of EDTA, while white bars to MLChs without external metal ions. The MLChs numbers of and type of Me^{2+} ions, as well as best activators of various MLChs are shown on panels A and B. Typical examples of the dependences of four MLChs in MBP hydrolysis on pH of reaction mixtures are given (C).

identity to germline VL genes of IgLV8-61*02, IgLV8-61*01, and IgLV8-61*03IGKV1 (90% of identity) [120]. DNA sequence of NGTA2-Me-pro-Tr indicated high identity with germline VL gene IGKJ1*01 (100%), IGKJ4*01 (95.7%), IGKJ4*02 (91.2%), IGKV1-5*03 (87.9%), IGKV1-5*01 (86.2% of identity), and IGKV1-5*02 (85.6%) [119]. DNA sequence of NGTA3-pro-DNase has similarity with germline DNA sequence of light chains of several IgGs: IGKJ1*01 (100% of identity), IGKJ4*01 (95.7%), IGKJ4*02 (91.2%); IGKV1-5*03 (79.8% of identity), IGKV1-5*02 (78.4%), and IGKV1-5*01 (78.4%) [Timofeeva, Nevinsky, personal communication]. Thus, all three MLChs were shown to be typical light chain of Abs [119, 120, personal communication].

NGTA1-Me-pro was shown to be a specific metalloprotease; only EDTA efficiently inhibits its activity, while specific inhibitors of thiol-, serine-, and acidic-like proteases did not suppress its MBP-hydrolyzing activity (**Figure 22A**) [120].

MLCh number	Optimal pH[a]	Optimal Me^{2+} cofactor	Apparent k_{cat}, min^{-1a}
1	8.0–8.2	**Ca^{2+}**(Ni^{2+},Mg^{2+})[c]	0.14 ± 0.01[b]
2	7.6–7.8	**Ca^{2+}**	0.12 ± 0.01
3	7.6–7.8	**Co^{2+}**	0.09 ± 0.007
4	5.7–5.9	Me^{2+}-independent	0.12 ± 0.008
5	7.5–7.7	Co^{2+}	0.24 ± 0.02
6	7.0–7.2	Me^{2+}-independent	0.01 ± 0.001
7	7.2–7.4	**Ca^{2+}**(Zn^{2+},Co^{2+})	0.07 ± 0.004
8	7.4–7.6	**Ca^{2+}**	0.17 ± 0.003
9	7.2–7.4	Me^{2+}-independent	0.03 ± 0.001
10	8.1–8.3	Me^{2+}-independent	0.05 ± 0.002
11	7.7–7.8	Me^{2+}-independent	0.09 ± 0.006
12	7.8–8.0	**Zn^{2+}**(Ca^{2+})	0.19 ± 0.001
13	6.1–6.3	**Ca^{2+}**	0.16 ± 0.001
14	7.0–7.2	Me^{2+}-independent	0.06 ± 0.0003
15	7.0–7.2	**Ni^{2+}**	0.17 ± 0.007
16	6.9–7.1	**Mn^{2+}**	0.12 ± 0.001
17	8.2–8.4	**Ca^{2+}**	0.17 ± 0.001
18	6.2–6.4	Me^{2+}-independent	0.05 ± 0.0007
19	6.7–6.9	**Ca^{2+}**	0.1 ± 0.01
20	8.2–8.4	**Zn^{2+}**	0.09 ± 0.006
21	6.9–7.1	**Cu** (Ca^{2+}, Co^{2+}, Zn^{2+})	0.18 ± 0.007
22	7.8–8.0	**Zn^{2+}**(Ca^{2+})	0.11 ± 0.006

[a]For each value, a mean ± S.E. of two/three measurements is reported.

[b]Optimal pH of reaction mixtures and optimal metal cofactor (given in bold) were used for every of MLCh preparations; the apparent k_{cat} values under optimal conditions were calculated as $k_{cat} = V_{max}$ (M/min)/[MLCh] (M). MLChs were used in different concentrations (0.05–0.5 M) depending of their relative activity.

[c]The best metal activator is given in bold, while alternative cofactors demonstrating relatively high activation are given in parenthesis.

Table 8. The optimal pH values, optimal metal cofactors, and apparent k_{cat} values for 22 recombinant individual MLChs in the hydrolysis of MBP [118].

Seven various metal ions increase NGTA1-Me-pro activity in the following order: $Ca^{2+} > Mg^{2+} > Ni^{2+} \geq Zn^{2+} \geq Co^{2+} \geq Mn^{2+} > Cu^{2+}$ (**Figure 22B**). NGTA1-Me-pro demonstrated two different very well expressed pH optima at pH 6.0 and 8.5 (**Figure 22C**). **Figure 22(D)** indicates that at pH 6.0 MLCh has optimum at ~6 mM, when at pH 8.5 at 1 mM $CaCl_2$. The apparent values of K_m and k_{cat} for MBP in the presence of optimal $CaCl_2$ concentration at pH 6.0 (20 ± 2 μM;

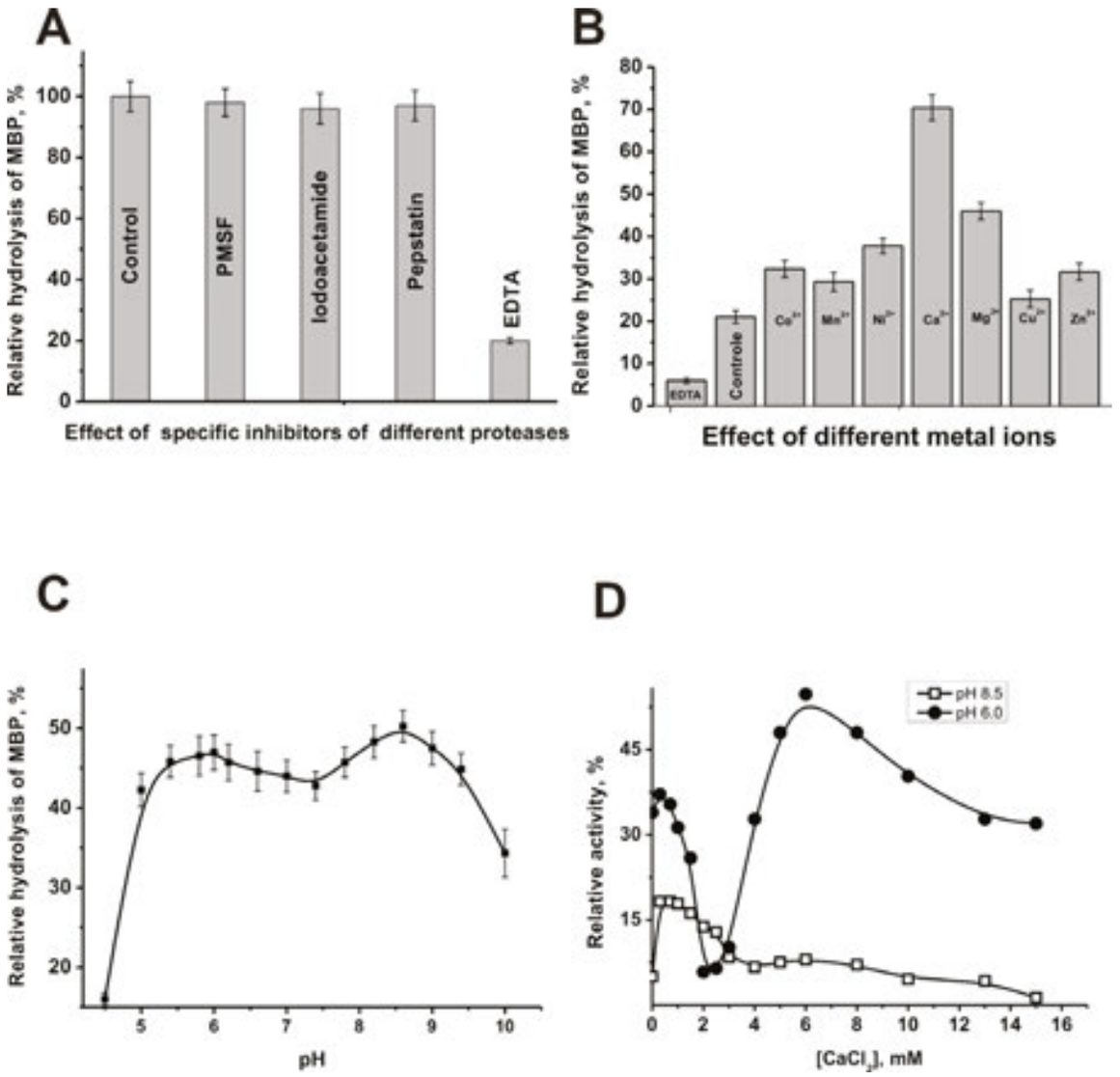

Figure 22. The RAs of NGTA1-Me-pro in the hydrolysis of MBP before and after its preincubation with specific inhibitors of various type proteases [120]. MLCh (0.1 mg/ml) was preincubated without other components (control), or the presence of EDTA, PMSF, pepstatin, and iodoacetamide; 1.0 μl of the mixtures were added to 29 μl of MBP-containing standard reaction mixtures (A). The RA before NGTA1-Me-pro preincubation with various inhibitors was taken as 100%. Effects of different Me^{2+} ions (2 mM) and EDTA on the RAs of MLCh are shown (B). Dependence of the RA upon pH of reaction mixture is shown (C). Dependence of NGTA1-Me-pro activity on CaCl2 concentration at pHs 6.0 and 8.5 (D).

0.22 ± 0.02 min^{-1}; 6.0 mM $CaCl_2$) and pH 8.5 (40 ± 3 μM; 0.07 ± 0.005 min^{-1}; 0.7 mM $CaCl_2$) were different. All data obtained unequivocally testified that NGTA1-Me-pro has two independent metal-dependent active centers [120].

MLCh NGTA2-Me-pro-Tr demonstrated two different activities: trypsin-like and metalloprotease. **Figure 23(A)** shows that NGTA2-Me-pro-Tr is not sensitive to pepstatin and iodoacetamide [119]. Preincubation of this MLCh with specific inhibitor of serine-like proteases results in a decrease of its activity for 42 ± 4%.

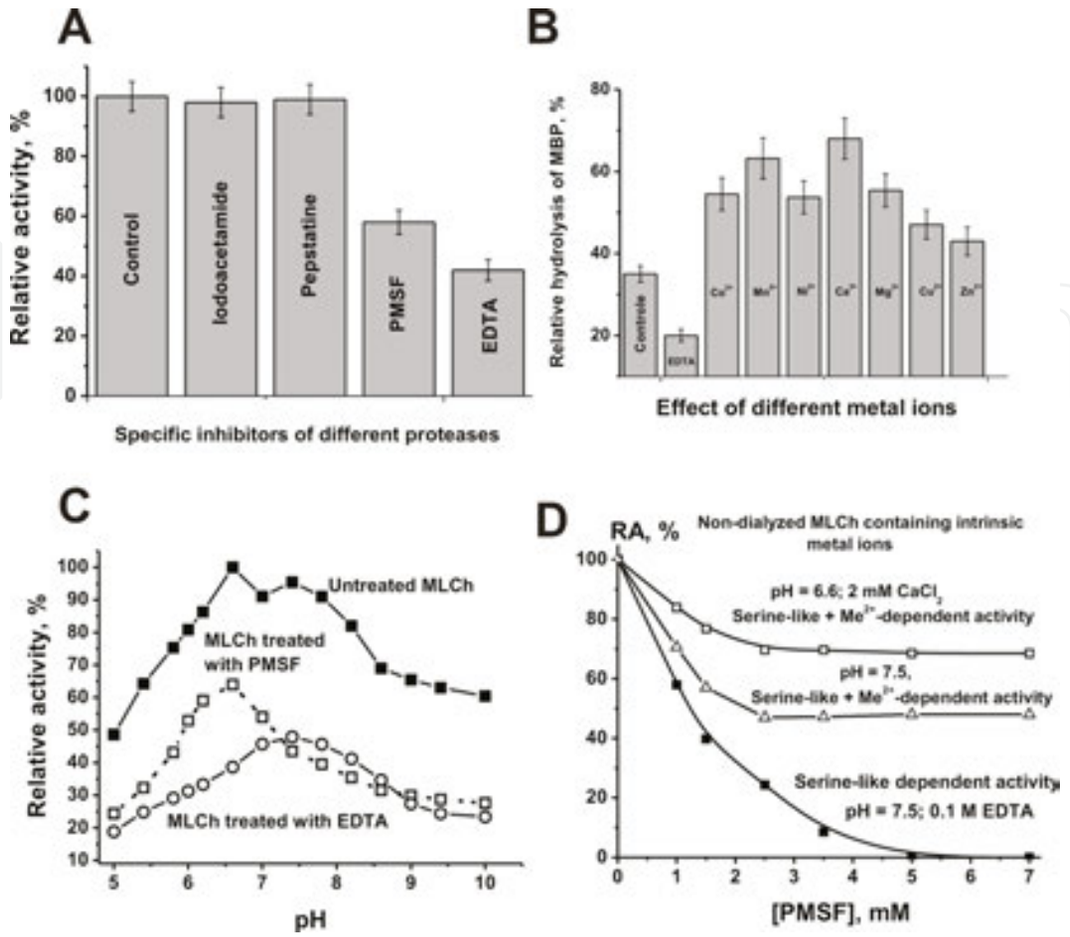

Figure 23. The RAs of the activity of NGTA2-Me-pro-Tr in the hydrolysis of MBP after its preincubation with specific inhibitors of different type proteases (A) [119]. MLCh (0.3 mg/ml) was preincubated alone (control), or the presence of iodoacetamide, PMSF, pepstatin, or EDTA; 1.0 μl of these mixture was added to 29 μl of MBP-containing standard reaction mixtures (A). The relative activity of NGTA2-Me-pro-Tr after preincubation with without different inhibitors (control) was taken for 100%. Effects of 10 mM EDTA and various Me^{2+} ions (2 mM) on the RAs of MLCh are shown (B). Dependences of the relative proteolytic activity of NGTA2-Me-pro-Tr before and after its treatment with PMSF and EDTA upon pH of reaction mixtures are shown (C). Dependence of the MBP-hydrolyzing activity on concentration of CaCl2 at pHs 6.0 and 8.5 (D).

Intact polyclonal Abs interact with various metal ions and they do not lose completely intrinsically bound ions during the standard purification procedures [121]. Addition of EDTA to NGTA2-Me-pro-Tr containing only intrinsically bound Me^{2+}-ions results in a decrease in its activity for 58 ± 5% (**Figure 23A**) [119]. Average serine-like activity of NGTA2-Me-pro-Tr containing only intrinsically bound Me^{2+} ions was ~1.4-fold lower than its Me^{2+}-dependent protease activity. Seven various external metal ions activate this MLCh in the following order: $Ca^{2+} \geq Mn^{2+} \geq Mg^{2+} > Co^{2+} > Ni^{2+} \geq Cu^{2+} \geq Zn^{2+}$ (**Figure 23B**). After NGTA2-Me-pro-Tr treatment with PMSF, its metalloprotease activity demonstrated pH optimum at 6.5–6.6 (**Figure 23C**). After dialysis of this MLCh against EDTA or in the presence of EDTA, serine-like protease activity showed pH optimum at 7.4–7.5. **Figure 23(D)** demonstrates that the increase in PMSF concentration results in a complete suppression of the activity at pH 7.5 in the presence of 50 mM EDTA, conditions corresponding to serine-like activity. NGTA2-Me-pro-Tr containing

no intrinsic metal ions demonstrated in the absence of external metal ions at pH 7.5 K_m and k_{cat} (9.0 ± 1.0 μM, 8 ± 0.6 min^{-1}) different as in the presence of $CaCl_2$ at pH 6.5 (24.0 ± 2.0 μM, 15.2 ± 1.1 min^{-1}) [119]. Thus, NGTA2-Me-pro-Tr is the first example of recombinant MLCh having two combined serine-like and metalloprotease activities.

It should be emphasized that all recombinant MLChs were obtained using affinity chromatography of phage particles on MBP-Sepharose and all electrophoretically homogeneous preparations of MLChs have affinity for MBP-Sepharose; similar to phage particles homogeneous MLChs were eluded from the sorbet by 0.5 M NaCl. Taking this into account, a very unexpected result was obtained from the analysis of enzymatic activities of NGTA3-pro-DNase [Timofeeva and Nevinsky, personal communication].

The homogeneity of ~26–27-kDa NGTA3-pro-DNase was confirmed using SDS-PAGE with following silver staining (**Figure 24B**, lane 1). NGTA3-pro-DNase demonstrated positive answer with horseradish peroxidase conjugated with mouse IgGs against human Abs light chains at Western blotting and positive ELISA answer using plates with immobilized MBP and DNA.

After SDS-PAGE, MBP-hydrolyzing activity was revealed only in the band corresponding to the light chains in the presence of $CaCl_2$ (o) and in the absence of external metal ions (□); the positions of proteolytic (o, □) and DNase (x) activities of MLCh are coincided (**Figure 24A**). NGTA3-pro-DNase hydrolyzed specifically only MBP and not hydrolyzed foreign control proteins (**Figure 24C**).

Only one (NGTA3-pro-DNase) of 25 recombinant MLChs analyzed by us efficiently hydrolyzed not only MBP, but also DNA (for example, **Figure 24D**). DNase activity of NGTA3-pro-DNase was determined *in situ* after separation of proteins using SDS-PAGE gels copolymerized with calf thymus DNA (**Figure 24E**). Ethidium bromide staining of the gels after the electrophoresis of the NGTA3-pro-DNase revealed sharp dark bands against a fluorescent background of DNA in the gel zone corresponding only to the MLCh and there were no other peaks of proteins or DNase activity (**Figure 24E**).

NGTA3-pro-DNase containing intrinsic metal ions was not sensitive to treatment with iodoacetamide and pepstatin, while its preincubation with PMSF led to decrease in the activity for 67 ± 5% (**Figure 25A**).

The dialysis of NGTA3-pro-DNase containing only intrinsically bound Me^{2+} ions against EDTA or addition of EDTA to reaction mixture led to a decrease in its activity for 33 ± 3% (**Figure 25A**). And average Me^{2+}-dependent protease activity of MLCh containing only intrinsically bound Me^{2+} ions was approximately 2.0-fold lower (**Figure 25A**), but after addition of external Ca^{2+} ions became to be 2.2-fold higher than its serine-like activity (**Figure 25B**). Seven various external metal ions activate NGTA3-pro-DNase in the following order: $Ca^{2+} \geq Ni^{2+} > Co^{2+} \sim Mn^{2+} \geq Cu^{2+} \sim Zn^{2+} \geq Mg^{2+}$ (**Figure 25B**). An optimal concentration of $CaCl_2$, which is the best activator of this MLCh, was 3 mM. NGTA3-pro-DNase demonstrates two different optimal pHs (**Figure 25C**). After treatment of MLCh with PMSF, its metalloprotease activity was maximal at pH 8.6, while in the presence of EDTA, serine-like protease activity demonstrated pH optimum at 7.0 (**Figure 25B**). NGTA3-pro-DNase treated with PMSF in the presence of 3 mM $CaCl_2$ (pH 7.0) demonstrated K_m for intact MBP (15 ± 1.1 μM) and k_{cat} value 0.4 ± 0.03 min^{-1}, while in the presence of EDTA at pH 8.6, K_m and k_{cat} values were different (45 ± 1.1 μM and 0.2 ± 0.04 min^{-1}).

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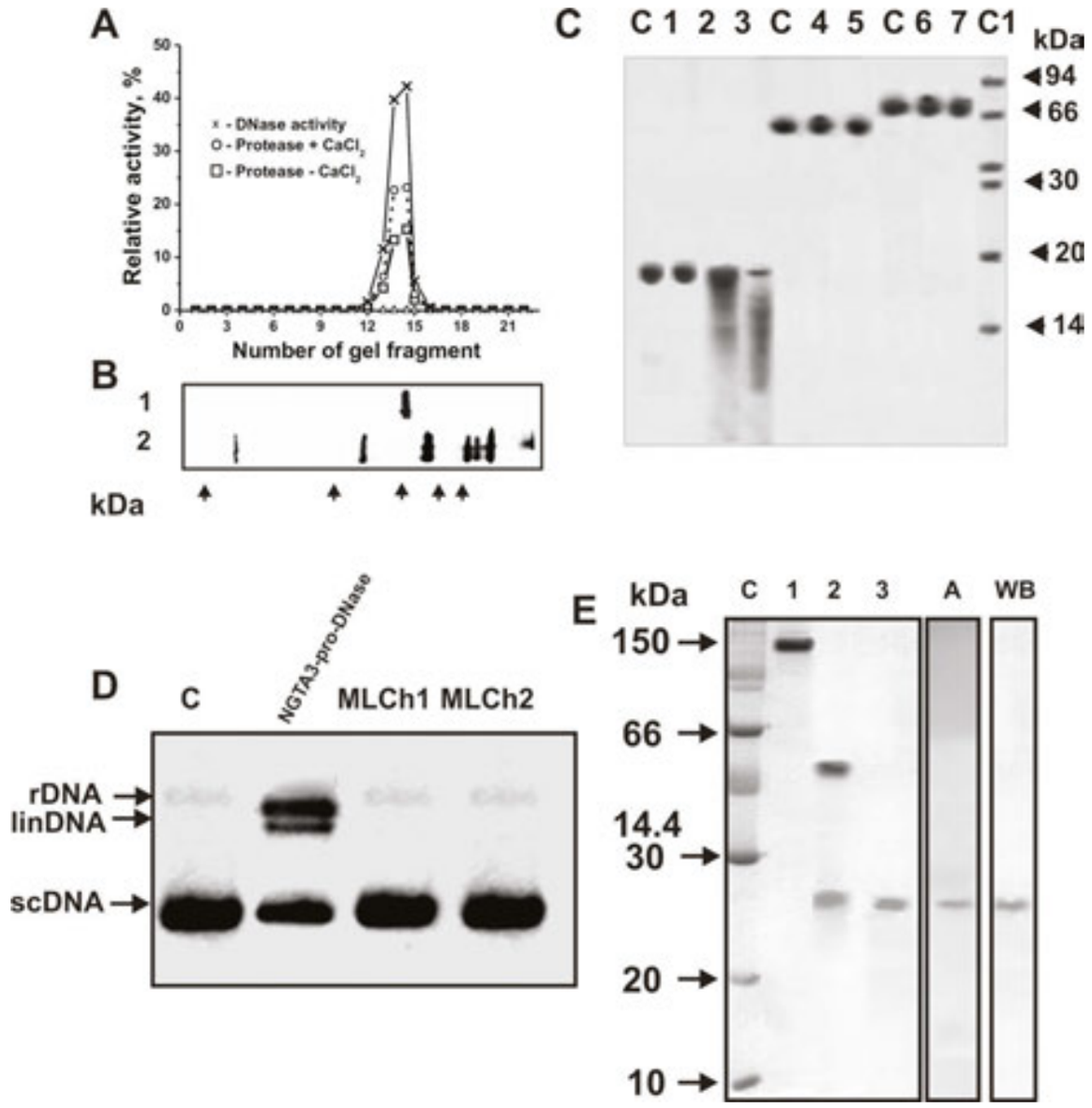

Figure 24. SDS-PAGE analysis of MBP- and DNA-hydrolyzing activities (A) and homogeneity of NGTA3-pro-DNase (7 μg) using a reducing 5–16% gradient gel followed by silver staining (B, lane 1); the arrows (B, lane 2) indicate the positions of protein markers. After SDS-PAGE the gel was incubated using conditions for renaturation of NGTA3-pro-DNase. The relative MBP- and DNA-hydrolyzing activity (%) was revealed using the extracts of 2- to 3-mm gel fragments (A). The activity of NGTA3-pro-DNase corresponding to a complete hydrolysis of 0.5 mg/ml MBP (or 18 nM scDNA) after 24 h of incubation of 25 μl reaction mixture containing 10 μl of the gel extracts was taken for 100%. SDS-PAGE analysis of hydrolysis of MBP by inact-MLChmix (lane 1) or NGTA3-pro-DNase (lanes 2 and 3, different time of incubation) (C). Hydrolysis of control proteins (0.5 mg/ml) by inact-MLChmix and NGTA3-pro-DNase was analyzed: human albumin (lanes 4 and 5) and lactoferrin from human milk (lanes 6 and 7) (C). The mixtures were incubated for 6 h with inact-MLChmix (lanes 4 and 6), or NGTA3-pro-DNase (lanes 5 and 7). All lanes C correspond to different proteins incubated alone without MLChs, while lane C1- to standard protein markers. DNase activity of NGTA3-pro-DNase and two control MLCh1 and MLCh2 (10 nM) was analyzed in the presence of 5 mM MgCl2 (D); lane C corresponds to scDNA incubated alone. *In situ* assay of DNase activity of the NGTA3-pro-DNase (8 μg) after treatment with DTT (lane A) (E). DNase activity was revealed by ethidium bromide staining as a dark band on the fluorescent background. A part of the gel was stained with Coomassie R250 to show the position of the SLE IgGs before (lane 1) and after incubation with DTT (lane 2), as well as NGTA3-pro-DNase (lane 3) (E). MLCh was analyzed by Western blotting to a nitrocellulose membrane using mouse IgGs against light chains of human Abs conjugated with horsedish peroxidase (lane WB) (E).

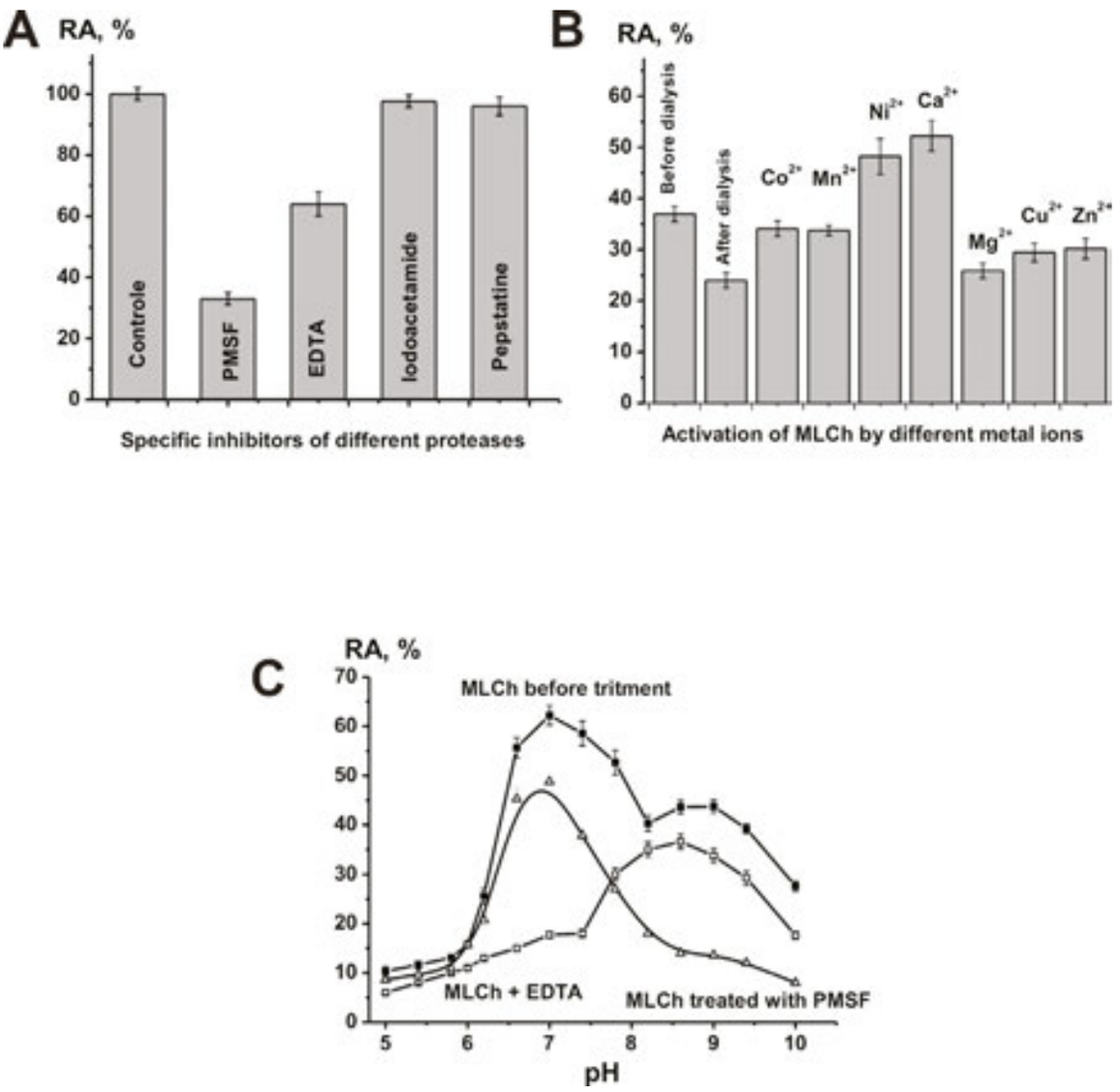

Figure 25. The RAs of MBP-hydrolyzing activity of NGTA3-pro-DNase after its preincubation with specific inhibitors of different types proteases (A). MLCh (0.1 mg/ml) was preincubated alone (control), in the presence of iodoacetamide, PMSF, pepstatin, or EDTA, and then 1.5 μl added to 29 μl of standard reaction mixture (A). The RA of NGTA1-Me-pro before its preincubation with various inhibitors was taken as 100%. Effect of EDTA and different metal ions (2 mM) on the RA of MLCh is shown (B). Dependence of RA of MBP-hydrolyzing activity of NGTA1-Me-pro on pH of reaction mixture before and after its treatment with EDTA and PMSF is given (C).

It is known that Mg^{2+} (10 mM) is optimal cofactor of DNase I, while other Me^{2+} ions very weakly activate DNase I [109, 110]. Optimal concentration for Mn^{2+}, Mg^{2+}, and Ni^{2+} in activation NGTA3-pro-DNase was ~4–5 mM, for Ca^{2+} and Zn^{2+} 2 mM, while Co^{2+} and Cu^{2+} activate MLCh up to 10 mM concentration. DNase activity increased in the presence of metal ions in the following order: $Mn^{2+} \approx Co^{2+} \geq Mg^{2+} > Cu^{2+} \approx Ni^{2+} \geq Ca^{2+} > Zn^{2+}$), which is completely different in comparing with that for DNases I and other recombinant MLChs analyzed.

DNase activity for NGTA3-pro-DNase in the presence of Mg^{2+} or Mn^{2+} at fixed concentration (5 mM) was increased at optimal concentrations of NaCl or KCl (30–40 mM) for only 27–28%.

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In optimal conditions, NGTA3-pro-DNase demonstrated well expressed optima at pH 6.5–6.6. The K_m (2 ± 0.2 nM) and k_{cat} (1.1 ± 0.1) × 10^{-3} min^{-1} values for scDNA were estimated. The affinity of NGTA3-pro-DNase for supercoiled DNA is about 3.5 orders of magnitude higher than affinity of scDNA for DNase I (K_m = 46–58 μM [122].

8. Conclusion

In several articles, it was demonstrated that polyclonal RNA-, DNA-, MBP- integrase-, and oligosaccharide-hydrolyzing antibodies of different classes and subclasses from patients with SLE, MS, AIDS, and other diseases are very catalytically heterogeneous. These abzymes can contain lambda- and kappa- types of light chains, may be of different subclasses (IgG1–IgG4), can demonstrate different affinity for specific sorbents and free antigens-substrates, very different pH optima, and may be independent or dependent on metal ions. Different abzymes can catalyze the hydrolysis of MBP, HIV integrase, and other proteins as serine-, thiol-, and acidic-like or metalloproteases. Various IgGs of four subclasses (IgG1–IgG4) and/or IgAs and IgMs from the sera of patients with autoimmune and viral diseases are catalytically active in the hydrolysis of RNA, DNA, oligosaccharides, and various proteins with their different contribution to the total activity of the Abs in the hydrolysis of these substrates in the case of every individual patient.

At the same time, the analysis of polyclonal antibodies does not allow to obtain detail characteristics of monoclonal abzymes entering to small pools of polyclonal antibodies separated by affinity chromatography on sorbents with different immobilized antigens-substrates. As it was shown on the example of polyclonal IgGs with DNase and MBP-hydrolyzing activities from sera of SLE and MS patients, elution of Abs by a NaCl concentration gradient leads to their distribution all over the chromatography profiles. In this case, each eluted Ab fraction contains abzymes with comparable affinity for immobilized ligand, but demonstrating a significant diversity of various enzymatic properties described above. These data are strong evidence of exceptional diversity abzymes in the blood of some patients with SLE, MS, and other diseases. In this regard, it should be mentioned that theoretically immune system of human can produce up to 106 different Abs against one antigen. It is evident that all theoretically possible variants of antibodies are in reality not realized and much less than one million. However, in the case of some patients, a possible number of abzymes can be very large. In our studies, we used a cDNA library only kappa light chains of Abs from three patients with SLE [111, 112, 118–120]. We have analyzed only 45 of 451 single of colonies corresponding one peak eluted from DNA-cellulose with 0.5 M NaCl and 33 of 687 colonies of peak eluded with acidic buffer. In the first case 15 of 45 (~33%) [111] and in the second 19 of 33 MLChs (58%) demonstrated DNase activity [112]. For analysis of MBP-hydrolyzing activity, we have used 72 of 440 individual colonies corresponding to phage particles eluted from MBP-Sepharose with 0.5 M NaCl; 25 of 72 MLChs (~35%) effectively hydrolyzed MBP [118–120]. Since we analyzed abzymes corresponding only one or two of ≥10 phage particles, it is obvious that the number of MLChs with DNase and MBP-hydrolyzing activity with very different enzymatic properties may be at least ≥ 500–1000.

The question is why many abzyme with nuclease and protease activities exist in SLE and other AI patients. First, immunization of autoimmune mice leads to a dramatically higher incidence of Abzs with a higher activity comparing to conventionally used normal mouse strains [51, 52]. The immune response to RNA and DNA and their complexes with histones and other proteins only partially depends on the length and sequence of nucleic acid [123, 124]. In addition, antiidiotypic Abs against the active centres of different DNA- and RNA-dependent enzymes can also possess catalytic activity. We have shown that polyclonal nuclease abzymes of autoimmune patients are usually different cocktails of Abzs against DNA and RNA and their complexes with proteins as well as antiidiotypic Abzs to active centers of various DNA- and RNA-hydrolyzing enzymes [13–22].

It is possible to explain to some extent in a similar way the exceptional diversity of abzymes hydrolyzing MBP and other proteins. At the same time, possible ways of production of monoclonal abzymes having two or even three different catalytic centers have a special interest. It should be noted that the known antigenic determinants of different proteins are usually relatively long and the active centers of some abzymes with two activities can correspond at once to variable parts of the antibodies to different contiguous parts of these determinants. One cannot exclude that metal-dependent active centers may be against specific part of protein antigenic determinants bound with one or several metal ions.

The second question is why NGTA3-pro-DNase against MBP can hydrolyze DNA. It is believed that MBP and anti-MBP Abs cannot interact with DNA or RNA. However, it was recently shown that anti-MBP IgGs can efficiently interact with nucleic acids [125]. Using quenching of MBP tryptophan fluorescence emission, we have shown that MBP bind oligonucleotides showing two K_d values: 65 ± 5 and 250 ± 20 μM [Timofeeva and Nevinsky, personal communication]. Therefore, it is possible to suggest that 24 of 25 MLChs interacting only with MBP correspond to Abzs directly against this protein, while NGTA3-pro-DNase may be against the complex of MBP with DNA. In the latter case, it is impossible to exclude possibility of a formation of the chimeric MLChs possessing affinity for MBP and for DNA and also hydrolyzing these absolutely different substrates.

As mentioned above, DNA-hydrolyzing Bence-Jones proteins [60] and DNase abzymes of patients with SLE [59] and MS [16] are dangerous since they are cytotoxic, can penetrate to cell nuclear, and hydrolyze nuclear DNA resulting in cell apoptosis. Abzymes against vasoactive peptide are harmful since they decrease in the concentration of the peptide and have an important negative role in pathogenesis of patients with asthma [126]. RAs of DNase abzymes of patients with Hashimoto thyroiditis well correlate with different immunological and biochemical indices of this disease including a concentration of thyroid hormones, while decrease in their activity is related to decrease in thyroid gland damage and improvement of the clinical status [105]. Protease IgGs of patients with sepsis participate in the control of disseminated microvascular thrombosis and play important role in recovery from the disease [127]. Thus, various abzymes can play both a negative and positive role in the pathogenesis of SLE and other autoimmune or viral diseases. Meanwhile, it should be mentioned that in the later stages of SLE, MS, and other diseases, the blood of these patients contains abzymes not only with DNase and MBP-hydrolyzing activities, but also hydrolyzing other proteins, oligosaccharides, and lipids [13–22].

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As it was shown in the example of Hashimoto thyroiditis production of harmful abzymes can be suppressed by using therapy with suppressing immune system drug plaquenil [102]. In MS and SLE, anti-MBP abzymes with proteolytic activity can attack MBP of the myelin-proteolipid sheath of axons. The established MS drug Copaxone was shown to be a specific inhibitor of abzymes with MBP-hydrolyzing activity [128]. One cannot exclude that the same drugs can be used for the treatment of SLE and other autoimmune diseases, which characterized by high level of abzymes with nuclease and MBP-hydrolyzing activities.

The presence of anti-DNA Abs is known as the main important diagnostic index for SLE. High-affinity anti-DNA Abs was recently shown to be major component of the intrathecal IgG in cerebrospinal fluid and brain of MS patients [48]. Moreover, DNase abzymes from SLE and MS patients are cytotoxic and induce cell death by apoptosis [16, 59]. The sera of patients with SLE and MS patients contain different free light chains [61, 62]. Therefore, we propose that exceptional diverse of intact antibodies and their free light chains hydrolyzing DNA, MBP, nucleotides, and polysaccharides may cooperatively all together promote important neuropathologic pathogenic mechanisms in SLE and MS.

Our data on the study of abzymes production in SLE patients associated with the change in profile differentiation of brain stem cells seem to be very important for understanding possible mechanisms of various autoimmune diseases development.

Acknowledgements

This research was made possible by grant from the Russian Science Foundation (no 16-15-10103 to G.A. Nevinsky).

Abbreviations

Abs	Antibodies
Abzs	Abzymes, or catalytically active antibodies
AI	Autoimmune
AD	Autoimmune disease
AIDS	Acquired immunodeficiency syndrome
a/u	Arbitrary units
BSA	Bovine serum albumin
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
OP	Oligopeptide
SLE	Systemic lupus erythematosus
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
RA	Relative activity

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