ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 9, pp. 945-961. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © G. A. Nevinsky, V. N. Buneva, 2009, published in Biokhimiya, 2009, Vol. 74, No. 9, pp. 1165-1183.

REVIEW

Peculiarities of Abzymes from Sera and Milk of Healthy Donors and Patients with Autoimmune and Viral Diseases

G. A. Nevinsky* and V. N. Buneva

Institute of Chemical Biology and Fundamental Medicine, Siberian Division of Russian Academy of Sciences, pr. Lavrentieva 8, 630090 Novosibirsk, Russia; fax: (383) 333-3677; E-mail: nevinsky@niboch.nsc.ru

Received October 22, 2008
Revision received January 22, 2009

Abstract—The detection of catalytic activity of antibodies is the earliest indicator of development of autoimmune diseases (AID). In early stages of AID, the repertoire of abzymes with various properties is relatively small, but it is greatly increased during their development. Catalytic diversity of the abzymes includes DNase, RNase, ATPase, and oxidoreductase activities; there are antibodies phosphorylating proteins, lipids, and polysaccharides. This review summarizes new data on abzyme heterogeneity and possible reasons for this phenomenon. A possible role of abzymes and their exceptional multiplicity in the pathogenesis of different AID is discussed.

DOI: 10.1134/S0006297909090028

Key words: natural abzymes, healthy donors, autoimmune diseases

Antibodies (AB) to stable transition state analogs and natural immunoglobulins (Ig) exhibiting various catalytic activities are known as abzymes and are well described in the literature (see for review [1-9]). IgG hydrolyzing vasoactive intestinal (neuro)peptide (VIP) and isolated from blood of patients with bronchial asthma represent the first example of natural abzymes [10]. Subsequently, IgG with DNase [11] and RNase [12-14] activities were found in blood of patients with systemic lupus erythematosus (SLE). Now IgG and/or IgA and IgM hydrolyzing DNA, RNA [11-21], and polysaccharides [22-27] have been isolated from blood of patients with various autoimmune diseases (AID) including SLE [11-14, 18], autoimmune thyroiditis (AIT) [15], polyarthritis [15], multiple sclerosis (MS) [16], diabetes mellitus [21], lymphoproliferative diseases [18], and also viral hepatitis [17], acquired immunodeficiency syndrome (AIDS) [19, 20], and some diseases related to bacterial infections [22]. Mean values of relative activities of DNA-hydrolyzing abzymes from patients with various diseases (expressed per mg of total polyclonal electrophoretically homogenous AB preparations) increased in the following order: diabetes mellitus < bacterial infections ⩽ viral hepatitis < polyarthritis ⩽ AIT < AIDS ⩽ MS < SLE.

There are abzymes hydrolyzing autoantigenic proteins such as thyroglobulin (AIT and rheumatoid arthritis) [28, 29], prothrombin (multiple myeloma) [30], protein factor VIII (hemophilia) [31], and myelin basic protein (MBP) (multiple sclerosis: antibodies IgG, IgM, and IgA) [32-34] and also casein and serum albumin (human immune deficiency virus (HIV) infection) [35]. In the case of HIV-infected patients, it was demonstrated that viral infections characterized by the development of autoimmune (AI)-reactions are accompanied by abzyme generation against viral proteins. These abzymes specifically hydrolyzed only reverse transcriptase [35] and HIV integrase [36].

According to modern viewpoints, the presence of blood abzymes is considered as a clear indication for the development of AI processes in the human body (see [5-9] and references given there). The existence of natural abzymes in donors without manifestations of any pathology of immune status was considered for a long time as improbable due to the lack of clear immunization. Antibodies from blood of healthy donors and patients with influenza, pneumonia, tuberculosis, tonsillitis, duo-

Abbreviations: AB, antibodies; AI, autoimmune; AID, autoimmune disease; AIDS, acquired immunodeficiency syndrome; AIT, autoimmune thyroiditis; BMSC, bone marrow stem cells; HIV, human immune deficiency virus; HSA, human serum albumin; Ig, immunoglobulins; MBP, myelin basic protein; MS, multiple sclerosis; pIgG, polyclonal IgG; SLE, systemic lupus erythematosus; VIP, vasoactive intestinal (neuro)peptide.

* To whom correspondence should be addressed.

denal ulcer, and some tumor diseases (uterine cancer, breast cancer, intestinal cancer), which were characterized by insignificant changes in immune status, did not exhibit reliably detected DNA- or RNA-hydrolyzing activities [9, 15, 17]. However, it is possible that abzymes with some catalytic activities may be generated in healthy individuals, but their relative activity may be very low, basically below detectable limits of existing methods [9]. Nevertheless, it was demonstrated that blood of healthy donors and animals may contain IgG and IgM antibodies exhibiting reliably tested amylolytic activity, which was, however, several orders of magnitude lower than in AID patients [23-27]. Antibodies with very low VIP- [10] and thyroglobulin-hydrolyzing activities were also found in a small number of healthy donors [29]. It appears that specific clones of lymphocytes synthesizing abzymes in healthy individuals are eliminated via apoptosis; however, in chronic AID they may exist for a long time. In this connection, it should be noted that abzymes can be generated during immunization of animals with various antigens such as DNA. However, the increase in concentration of such abzymes in healthy animals is transient, whereas immunization of experimental animals predisposed to AID results in the development of chronic pathologies [37, 38].

There is a special group of healthy female individuals who are predisposed to the development of AI processes (similar to that of AID patients) during pregnancy and right after delivery [5-9]. During pregnancy, blood of women (as blood of AID patients) contains increased concentrations of DNA [39, 40] and also low concentrations of fetal cells. There is a correlation between increased frequency of fetal cells in maternal peripheral blood and AID morbidity (scleroderma [41]), and detection of fetal DNA is associated with aneuploidy [42]. On one hand, manifestations of such disease as rheumatoid arthritis decrease or even disappear during pregnancy [41], whereas conditions of patients with SLE and antiphospholipid syndrome [43] and also with some other AID markedly deteriorate. A sharp increase in AI reactions (autoimmune shock) can occur right after delivery. Regardless to the development of the puerperal AI-shock, some post-puerperal pathologies can be developed in them [42, 44]. Post-puerperal AID may appear not only right after delivery but also in later periods. Puerperal thyroiditis is one of the most frequent post-puerperal AI disorders. Its frequency varies from 1.9 to 16.7% [45, 46]. Maximal manifestation of this disease is observed within the first 3-6 months, but in some cases AIT can occur later, although during the first year after delivery.

Pregnancy and delivery cause rearrangements in the female immune system, which obviously occur in at least two steps (see [47] and references given there). During pregnancy, compatibility of the maternal organism with a growing fetus is achieved by increased tolerance of the maternal immune system towards the fetus; subsequently the immune system is "prepared" for rearrangement, which occurs simultaneously with the onset of lactation. Trigger of specific immune memory of pregnant women is an important factor of the second step of the immune system rearrangement; this immune memory accumulates information about all internal and external immunogens that have been ingested with food or appeared in the body due to its contact with viruses or bacteria. These factors trigger antibody generation to all these antigens that appeared in the body during the last months of pregnancy. The increase in titer of blood or milk abzymes before and especially after delivery might represent a part of a general process of antibody generation triggered by the immune memory [47].

The first example of abzymes found in clinically healthy women were milk sIgA catalyzing protein phosphorylation [48-50]. Milk is a unique source of abzymes exhibiting both typical and unique catalytic activities. Small subfractions of polyclonal human milk IgG and sIgA catalyzed hydrolysis of DNA, RNA [47, 51-53], ribo- and deoxy-NMP, NDP, and NTP [54, 55] and also cleavage of 5′-terminal DNA and RNA phosphate (phosphatase activity) [51-53] and hydrolysis of polysaccharides [26] and casein [56]. Milk of healthy women also contains sIgA and IgG with unique lipid [57, 58] and polysaccharide kinase [59, 60] activities, which cause phosphorylation of minor lipids and polysaccharides with previously unknown structures.

Recently it has been reported that cow foremilk IgG abzymes hydrolyze DNA with the pH optimum at 3.9-4.0 [61], whereas human milk abzymes exhibit maximal activity at pH 7.0-7.2 [53].

It should be noted that human milk AB are usually characterized by higher catalytic activity than most known abzymes isolated from AID patients [5-9].

The above-described abzymes have been found in patients with various AI reactions. However, there are abzymes exhibiting high catalytic activities that have been found in healthy mammals. It was found that human and animal AB exhibit superoxide dismutase activity and convert singlet oxygen $^1O_2$ into its reduced form $O^{2\cdot}$ [62, 63]. These data suggest that AB might be involved in protection of the mammalian body against reactive oxygen species, and therefore it is possible that special evolution involved Ig as specific blood antioxidants [62, 63]. Recently a mechanism by which oxygen can be reduced by Ig and reutilized in phagocytosis has been described; this suggests the possibility of immune system involvement in microbial regulation. Interestingly, there are abzymes of higher eukaryotes that catalyzed formation of ozone that is utilized by cells during phagocytosis [64].

Recently it was demonstrated that IgG-antibodies from blood of healthy Wister rats exhibit high H_2O_2-dependent peroxidase and H_2O_2-independent oxidoreductase activities, which effectively oxidize aromatic amines and phenols similarly to horseradish peroxidase

[65-68]. Specific activities of a small AB fraction obtained by chelation chromatography of polyclonal rat blood AB on Chelex (a metal chelator) were 2-3 orders of magnitude higher than in other known natural abzymes in the oxidation of these substrates.

In general the biological role of abzymes in AID and other diseases still requires better understanding, and it is possible that some abzymes play a positive role whereas others play a negative role. Anti-VIP abzymes hydrolyze VIP and decrease its blood concentration and hence, they may play a negative role in the pathogenesis of asthma [69]. DNA-hydrolyzing AB from blood of patients with SLE, lymphoproliferative diseases [18], MS [8], and Bence Jones proteins from blood of myeloma patients [70] are cytotoxic; they penetrate into cell nuclei, hydrolyze chromatin DNA, and induce apoptosis of cancer cells.

IgG, IgM, and IgA from MS patients effectively hydrolyze basic myelin protein, a component of the axonal myelin phospholipid membrane; this results in impairments of nerve impulse propagation and development of MS [32-34]. However, AB exhibiting proteolytic activity in sepsis may play a positive role in the regulation of capillary thrombosis, and the increase in their relative activity correlates with improvement of conditions of patients [71]. Maternal milk abzymes increase protective properties of antibodies in passive immunity because they not only bind foreign antigens but also effectively hydrolyze them [5-9]. Abzymes with superoxide dismutase [62, 63] and peroxidase activities can effectively eliminate reactive oxygen species and oxidize various mutagens and carcinogens reaching the circulation [65-68].

POSSIBILITY OF APPLICATION OF ABZYMES FOR DIAGNOSTICS OF AUTOIMMUNE PATHOLOGY

It should be noted that determination of an AB titer against any specific antigen characterizing any AID is a much less sensitive test than analysis of abzyme catalytic activity hydrolyzing this antigen (provided that this activity really exists). For example, using healthy donors as the reference the increased concentrations of AB against native and denatured DNA were found in 17-18 and 53% of MS patients, respectively [72, 73], whereas DNA-hydrolyzing AB with DNase activity were reliably detected in 90-95% of MS patients [16]. In SLE patients increased concentration of AB against native DNA was found in only 38% of patients [72], whereas abzymes with DNase activity were found in 90-95% of patients [14, 74, 75]. This may be associated with the existence of some AB to various autoantigens in healthy people and animals [76, 77]. Frequently, the increase in mean values of AB titers typical for healthy donors and AID patients is detected at late stages or during disease recurrence. In healthy donors abzymes with various catalytic activities are either absent (DNase, RNase, ATPase, MBP-hydrolyzing activities [5-9, 12-17, 32-34, 54, 55]) or are characterized by very low relative activity (hydrolysis of VIP [10], thyroglobulin [28, 29], and polysaccharides [23-26]). The presence of AB activity determined by its intrinsic catalysis, which (in contrast to ELISA) is characterized by generation of a reaction product due to large turnover number of this catalyst and possibility of subsequent increase in the turnover number by extending reaction time provide good opportunity for detection of even a small quantity of abzymes with low activity in polyclonal AB preparations.

Studying AB from blood of patients with various AID, we have demonstrated that statistically significant abzyme appearance is detected at the earliest stages of various pathologies, when changes in antibody titers to antigens specific for various diseases, for example, DNA (SLE, MS, AIT), basic myelin protein (MS), and thyroglobulin (AIT) are within the range of the AB titers determined in healthy donors [5-9]. This was confirmed during analysis of AB titers to DNA and relative abzyme activities hydrolyzing DNA, ATP, and polysaccharides during spontaneous development of SLE in MRL-lpr/lpr autoimmune mice [37, 38] (see below). It was concluded that appearance or a large increase (by tens of times) of the abzyme activity in blood of patients and animals (versus healthy individuals) can serve as the earliest indicators of AID development [9, 38].

Several medical criteria proposed by Poser are used for MS diagnostics [78, 79]; however, the final diagnosis is verified by tomographic detection of plaques in the brains of MS patients, but these plaques appear at later stages of this disease. Anti-DNA antibodies in MS patients were traditionally considered only as additional evidence of systemic imbalance of immune regulation, which did not have independent pathogenic importance. However, in brain plaques and liquor only anti-DNA AB were found; these are the major component of the intracerebral IgG response and bind to the surface of neuronal cells and oligodendrocytes [80]. These results were interpreted as evidence for the leading role of anti-DNA AB in MS pathogenesis [80].

Examining three patients, A. S. Mogelnitskii suggested the possibility of initial stages of MS, but the symptoms did not meet Poser's criteria [81]. However, AB found in blood of these patients exhibited high DNase activity, and this the suggested possibility of an early stage of MS. One and a half years later these patients met Poser's criteria, and after 2-3 consecutive years brain plaques were also found in these patients. According to [32-34], detection of abzymes (IgG, IgA, and IgM) hydrolyzing MBP can serve as a more reliable criterion of the development of MS because such abzymes have not been found in healthy individuals. The IgG-dependent

specific cleavage of an oligopeptide corresponding to one of the specific cleavage sites in MBP has been proposed as a MS marker [82].

It should be noted that the use of detection of abzyme activity for diagnostics of various AID meets some problems. For example, VIP-hydrolyzing abzymes have been found not only in asthmatic patients but also in healthy donors [10], and thyroglobulin-hydrolyzing abzymes were detected in AIT patients and patients with polyarthritis [28, 29]. Polysaccharide-hydrolyzing abzymes were found not only in AID patients but also in healthy individuals [23]. Nevertheless, in AID patients these abzyme activities were 1-3 orders of magnitude higher. Thus, activities of these abzymes can be used in AID diagnostics as additional criteria, and taking into consideration normal ranges of these parameters in healthy donors, as in the case of application of the anti-DNA AB titers, for SLE diagnostics.

Although abzymes hydrolyzing DNA, RNA, and ATP have not been found in healthy donors and they are reliably detected in debut (initial stage) of various diseases (SLE, MS, polyarthritis, Hashimoto's thyroiditis [5-9]), the use of the abzyme activity for diagnostics of these diseases is complicated by some problems. The issue is that such abzymes exhibiting low but reliably detected activity have also been found in some infectious (bacterial and viral) diseases [83]. In contrast to AID, the presence of such abzymes in blood of patients with infectious bacterial pathologies is transient, and they disappear after full recovery. Nevertheless, diagnostics based on abzyme detection can cause diagnostic errors. However, infectious bacterial and viral diseases are recognized using various independent and specific diagnostic methods. Thus, it is clear that AID diagnostics requires the use of known independent methods to exclude other possible diseases. But even in the absence of symptoms typical for other diseases, AID diagnostics should be based on all known criteria, and abzyme detection might help in specification of diagnosis and indicate necessity of more detailed examination of patients. In this connection, it should be noted that the RNA-hydrolyzing abzymes isolated from blood of patients with various AID demonstrate various individual patterns of cleavage of tRNA specific for Phe and other amino acids [84, 85]. This criterion may specify particular type of diagnosed AID (SLE, MS, polyarthritis, AIT, autoimmune viral hepatitis) characterized by generation of the RNA-hydrolyzing abzymes.

Although use of relative catalytic activity of abzymes has limited diagnostic capacity, abzymes might be successfully used for evaluation of severity of AI processes in patients with known diagnosis. Studying 120 AIT patients, it was demonstrated that the increase in relative activity of DNA-hydrolyzing abzymes correlated well with the decrease in thyroid hormone concentration, increase in AB concentration against thyroglobulin and microsomal fraction, impairments in patient conditions, and degree of impairment of thyroid gland function [86, 87]. It was demonstrated that treatment of patients with thyroxin (widely used for therapy of thyroid gland diseases) caused only transient normalization of the concentration of this hormone and general condition of the patients; this treatment did not influence the blood level of DNA-hydrolyzing antibodies and severity of AI processes. At the same time, treatment of AIT patients with Plaquenil, suppressing the immune system, significantly decreased the blood level of abzymes, which occurred in parallel with the decrease in blood thyroglobulin and AB to this protein as well as a decrease in other immunological and biochemical parameters. This was accompanied by increase in thyroid hormone concentrations (basically up to normal values), correlated with improved thyroid gland function and with improvement of general conditions of these patients [86, 87]. Thus, analyses of AB associated DNA-hydrolyzing activity in AIT patients can serve as additional criteria for diagnostics of this disease and can be used for evaluation of significant decrease in thyroid gland function in such patients. It appears that analyses of abzymes with other catalytic activities might be a similar way for diagnostics of various AID.

FEATURES OF DNA- AND RNA-HYDROLYZING ABZYMES

Human abzymes exhibiting nuclease activities are characterized by very high heterogeneity as demonstrated by AB chromatography on DNA-cellulose; use of a NaCl gradient from 0.05 to 3 M resulted in distribution of AB and their catalytic activity over the whole chromatographic profile; a part of the abzymes was eluted from the column only with 2-3 M $MgCl_2$ or acidic buffer [5-9]. Figure 1 shows the distribution of blood abzymes from sick MRL-lpr/lpr mice into fractions characterized by various affinity to DNA and the dependence of their relative activity on Mg^{2+}, Mn^{2+}, and Ca^{2+} [88].

Some studies [84, 85, 89, 90] analyzed a possible spectrum of changes in abzyme specificity using a large set of natural tRNAs specific to various amino acids (and their transcripts) and variations of reaction conditions. It was demonstrated that using a wide range of experimental conditions it is possible to detect not only major but also minor abzyme fractions, and patients with various AID exhibit individual sets of RNase activities (some of them are unique). In contrast to known viral and pro- and eukaryotic RNases, the RNA-hydrolyzing AB are sensitive to slight changes in the spatial structure of the RNA molecule and slight changes in nucleotide sequences including point base substitutions [84, 85, 89, 90]. Summarizing all the data [12, 13, 17, 52, 53, 84-91], we have come to a general conclusion that in AID patients a relatively narrow or rather large repertoire of monoclonal DNA- and RNA-hydrolyzing abzymes can be formed

within polyclonal IgG and IgM. These immunoglobulins are extremely variable, can contain both κ- and λ-type light chains, exhibit maximal activity at various pH values, can have various total charges, and can be characterized by different affinity for DNA and RNA and different dependence of their catalytic activity on the presence of monovalent and bivalent metal ions. Thus, a question concerning the reasons for extreme heterogeneity of polyclonal AB with nuclease activities arises. It should be noted that, theoretically, the human immune system can generate up to 10^6 AB variants against one antigen. According to modern concepts, there are two ways for abzyme generation. Natural abzymes as well as AB against transition state analogs might be AB directly generated against a substrate (DNA, RNA, protein, etc.), which might imitate a chemical reaction transition state [1-4]. For example, the abzymes hydrolyzing VIP [10], thyroglobulin [28, 29], basic myelin protein [32-34], casein [56], and reverse transcriptase and HIV integrase [35, 36] are AB generated directly against these proteins. However, abzymes can be antiidiotypic (i.e. represent AB against active sites of enzymes), and their appearance can be explained by means of the model of Jerne's antiidiotypic network [92]. Monoclonal antiidiotypic abzymes with acetylcholine esterase [93, 94], carboxypeptidase [95, 96], and β-lactamase [97], and urease [98] activities have been described in the literature.

It has been suggested that a part of AB from blood of SLE patients that exhibits DNase activity might be antiidiotypic AB against topoisomerase I [99]. Indeed, immunization of rabbits with DNase I resulted in generation of idiotypic AB1, and subsequent immunization with AB1 raised antiidiotypic AB2 exhibiting DNase activity [100]. Thus, theoretically, DNA-hydrolyzing AB can be both AB against DNA and its complexes with proteins and antiidiotypic AB against active sites of DNA-cleaving enzymes. It is known that DNases and RNases do not hydrolyze RNA and DNA, respectively. However, separation of AB from blood of patients with various AID and human milk on DNA-cellulose yielded AB fractions hydrolyzing both DNA and RNA [5-9]. In addition, it was demonstrated that the rate of RNA hydrolysis (autoimmune mouse) by monoclonal IgG against various DNA sequences was 30-100-fold higher than that of DNA [101]. This raised a question on the nature of monoclonal DNA- and RNA-hydrolyzing AB in blood of patients with AID, ways of their generation, possible immunogens that stimulate their production, and also possibilities for the existence of abzymes exhibiting either DNase or RNase activities as well as chimeric abzymes with both these activities. Therefore, we have tried to clarify this problem by immunizing rabbits with DNA, RNA, DNase I, DNase II, and pancreatic RNase A [102-105] followed by comparative analysis of properties of all resultant pIgG. It was found that the immunization with any of these antigens results in generation of pIgG;

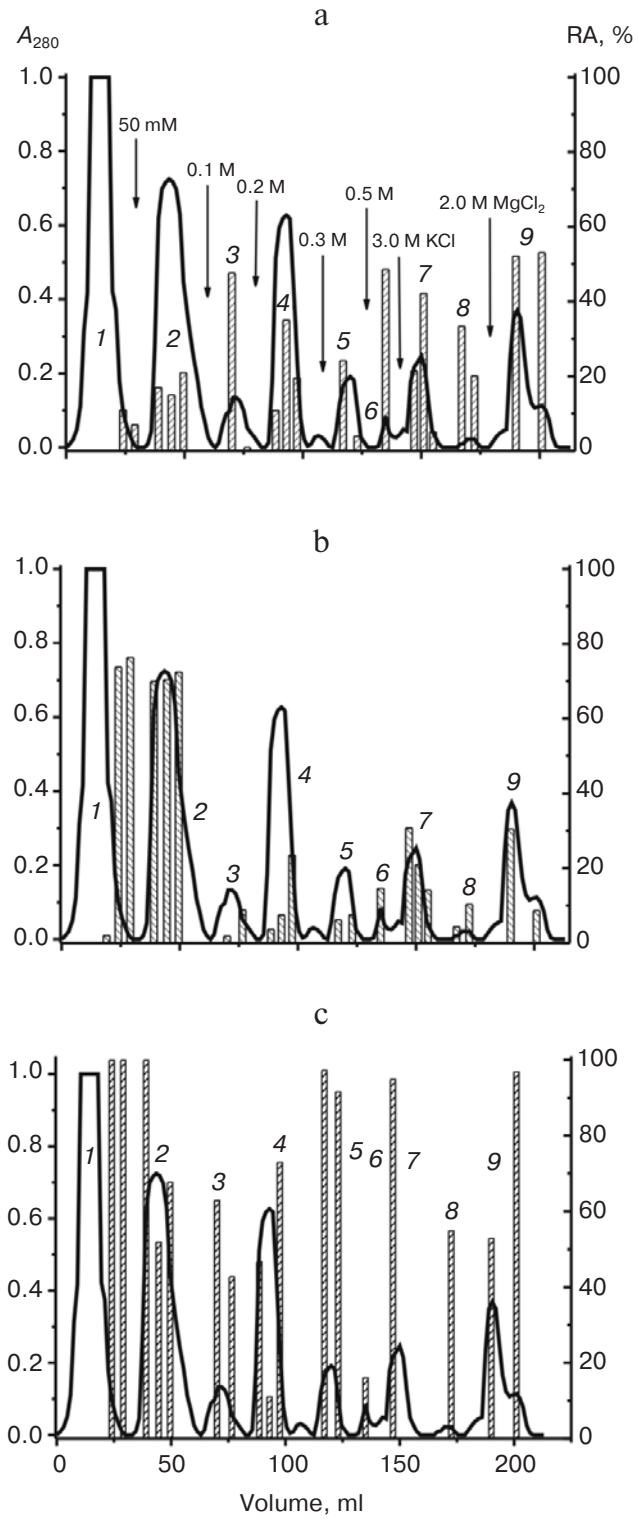

Fig. 1. DNA-cellulose affinity chromatography of pIgG from serum of a sick mouse with high proteinuria. The solid line shows optical absorbance at 280 nm. Columns designate relative DNase activity (RA) of the pIgG fractions eluted from the sorbent by various concentrations of KCl or 2 M $MgCl_2$ in the presence of Ca^{2+} (a), Mg^{2+} (b), and Mn^{2+} (c). Maximal activity of one of the IgG fractions assayed in the presence of Mn^{2+} (c) was defined as 100%.

although the rate of RNA hydrolysis by these pIgG was 10^3-10^4 times higher than that of DNA, the repertoire of monoclonal (within polyclonal) abzymes obtained during immunization by each of these antigens was individual.

Interestingly, blood of healthy mammals contains (in testable amounts) auto-AB against DNA, RNA, and various proteins and enzymes interacting with nucleic acids [76, 77, 106]. In addition, the DNA-sorbents can interact not only with anti-DNA AB, but also with other AB, for example, against phospholipids, polysaccharides, cell surface proteins, and other antigens and thus exhibit cross-reactivity [76, 77, 106]. As in the case of healthy donors [5-9], a small proportion of pIgG (26-31%) from blood of healthy non-immunized rabbits was adsorbed on DNA-cellulose (Fig. 2), but all the fractions were catalytically inactive. Immunization of rabbits with complexes of DNA and RNA with methylated BSA, DNase I, DNase II, and RNase resulted in an increase in AB bound to the DNA-sorbent to 77.5%.

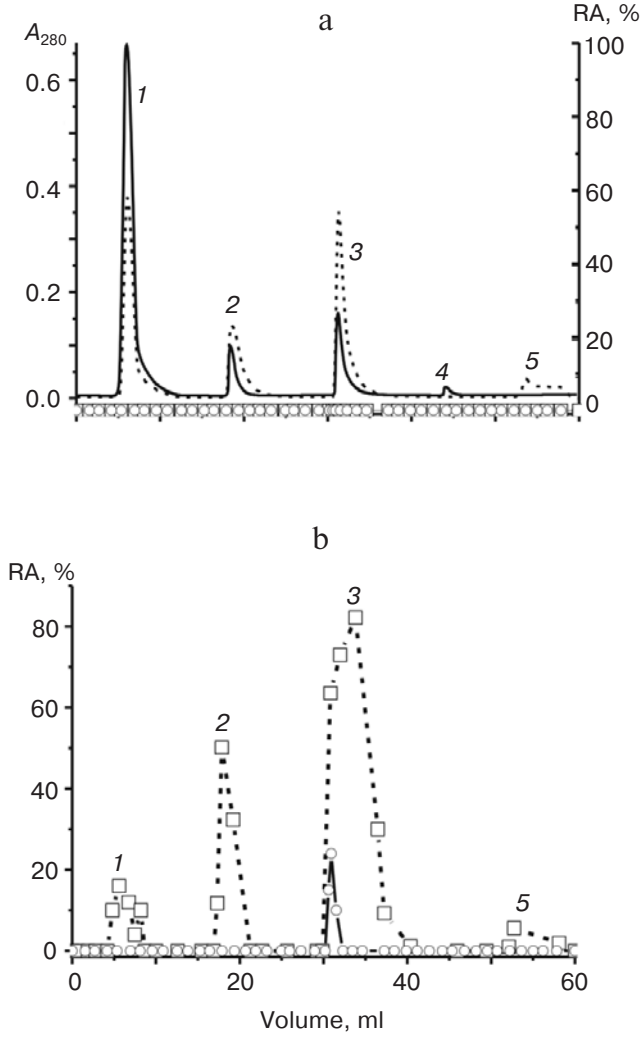

Fig. 2. DNA-cellulose affinity chromatography of pIgG from blood of two rabbits. Solid and dashed lines show optical absorbance at 280 nm for a non-immunized rabbit and the rabbit immunized with DNase I, respectively (a). Relative DNase (□) and RNase (○) activity (RA) of separate fractions of pIgG eluted with NaCl from the sorbent for non-immunized (a) and immunized rabbit (b). Full transition of 4.43 nM scDNA into its hydrolyzed forms during 18 h and total hydrolysis of 140 μM poly(C) after 2.5-h incubation were defined as 100% RA for DNA and RNA hydrolysis, respectively.

Table 1 summarizes data on the relative content and activity of IgG in the peaks eluted from DNA-cellulose during loading (peak *1*), and also elution with 0.1 (peak *2*), 0.5 (peak *3*), and 3 M NaCl (peak *4*) and acidic buffer, pH 2.6 (peak *5*). One can see that immunization with DNA results in generation of abzymes exhibiting the lowest affinity towards DNA; the major proportion of DNase activity (87.4%) was eluted from the sorbent during pIgG loading (peak *1*), and this activity is absent in peaks *3-5*. Fractions with DNase activity were eluted only in peaks *1* and *2*, whereas peak *3* contains only AB hydrolyzing only RNA. Interestingly, immunization of rabbits with RNA resulted in generation of DNA-hydrolyzing AB with higher affinity towards DNA; these AB were eluted in peaks *2-4* (with maximal activity detected in peak *3*). In contrast to anti-DNA AB, peak *3* corresponding to the anti-RNA abzymes also exhibits both DNA- and RNA-hydrolyzing activities, and for these AB it is the only peak with RNase activity. Rabbit immunization with DNase I, DNase II, and a RNase–BSA conjugate results mainly in generation of abzymes that are antiidiotypic AB against active sites of these enzymes. However, some part of the forming abzymes did not bind to sorbents containing immobilized idiotypic AB against these enzymes. This suggests that some abzymes formed during immunization with DNase I, DNase II, and RNase might represent AB against nucleic acid associated with these enzymes. Interestingly, AB exhibiting higher affinity to DNA and eluting from DNA-cellulose by the acid buffer, pH 2.6 (Table 1) appear only after immunization with the enzymes. In the case of DNase II and RNase, AB corresponding to all five peaks exhibit DNase activity, whereas in the case of anti-DNase II AB catalytic activity was not detected only in peak *4* (3 M NaCl). Interestingly, in the case of anti-DNA I AB, RNase activity was detected only in peak *3*, in the case of anti-RNase AB it was detected in peaks *2* and *3*, whereas in the case of anti-DNase II AB this activity was found only in IgG peaks *1-3*. The ratio of relative DNA- and RNA-hydrolyzing activities corresponding to IgG peaks differing by affinity towards DNA was individual for each immunogen (Table 1).

Data of Table 2 indicate that affinity for DNA significantly varies in AB. It is possible that pIgG fractions corresponding to each peak (*1-5*) are also heterogeneous (by affinity for DNA) and they may contain AB subfractions differing by both substrate specificity and k_{cat} values. It should be noted that the k_{cat} values found for each eluted fraction mainly characterize a major subfraction of each analyzed fraction that has maximal k_{cat}.

Table 1. Relative catalytic activity evaluated by hydrolysis of supercoiled DNA and poly(C) and relative content of pIgG in the fractions separated by affinity chromatography on DNA-cellulose [91-94]

Peak*	Elution conditions during chromatography on DNA-cellulose**	RC, %	RA, DNase (RNase), %	RC, %	RA, DNase (RNase), %	RC, %	RA, DNase (RNase), %
		non-immunized		DNA		RNA	
0	before fractionation	100	0 (0)	100	100 (100)	100	100 (100)
1	0.0 M NaCl	68.8	0 (0)	22.5	87.4 (38.3)	56.0	0 (0)
2	0.1 M NaCl	9.3	0 (0)	5.7	12.6 (26.2)	11.0	30.0 (0)
3	0.5 M NaCl	17.1	0 (0)	66.6	0 (35.5)	29.0	68.7 (100)
4	3 M NaCl	4.8	0 (0)	5.2	0 (0)	4.0	1.3 (0)
5	pH 2.6	0	0 (0)	0	0 (0)	0	0 (0)
		DNase I		DNase II		Ribonuclease	
0	before fractionation	100	100 (100)	100	100 (100)	100	100 (100)
1	0.0 M NaCl	37.3	9.3 (0)	55.6	5.5 (19.7)	45.0	6.0 (0)
2	0.1 M NaCl	18.2	29.5 (0)	11.1	25.7 (52.9)	22.6	67.5 (44.7)
3	0.5 M NaCl	34.2	54.6 (100)	28.3	27.8 (27.4)	15.8	6.4 (55.3)
4	3 M NaCl	0	0.0 (0)	2.0	22.3 (0.0)	7.8	11.3 (0)
5	pH 2.6	10.3	6.6 (0)	3.0	18.6 (0.0)	8.8	8.8 (0)

Note: Immunogens, relative content (RC) of IgG in various peaks, and relative total activity (RA) of DNA and RNA hydrolysis are shown. Assay error did not exceed 10-15%.

* Peak numbers are the same for all chromatographic procedures as in Fig. 2.

** Salt concentrations or buffer used for elution of IgG fractions from DNA-cellulose are shown.

Depending on the antigen used, the IgG fractions eluted after affinity column loading were characterized by the K_m values for DNA ranging from 18.3 (anti-RNase) to 68.7 nM (anti-DNase I); AB affinity for DNA (in terms of K_m values) gradually increased with the increase in salt concentration used for abzyme elution (Table 2). For example, in the case of the fractions eluted with the acidic buffer, the K_m values were 9-190 times lower than for the fractions eluted during loading. Anti-DNA IgG demonstrated the lowest affinity to DNA and the highest rate of its hydrolysis ($k_{cat} = 2.5 \cdot 10^{-4}$ min^{-1}). If we define the anti-DNA AB k_{cat} value as 100%, the relative rate of DNA hydrolysis by AB against other antigens decreases in the following order: anti-DNA (100%) > anti-DNase I (~52%) > anti-DNase II (~40%) > anti-RNase (~12%) > anti-RNA (~0.7%). However, the relative activities of separate fractions of AB against each of these antigens may differ by 1.5-26-fold in dependence on the particular antigen (Table 2).

There were fewer IgG fractions with RNase activity than with DNase activity (Table 2), but relative DNA-hydrolyzing activity of rabbit AB (as in the blood AB from AID patients [4-9]) was several orders of magnitude lower (Table 3). In the case of rabbit immunization with RNA or DNase I, only one fraction with RNase activity eluted in peak *3* (0.5 M NaCl) was formed; each of these fractions (from RNA- and DNase I-immunized rabbits) demonstrated comparable relative activity (Table 3). At the same time, immunization with DNA, DNase II, and RNase resulted in formation of two or three abzyme fractions hydrolyzing RNA, and their affinity for poly(C) increase with the increase in NaCl concentration (Table 3). Interestingly, rabbit immunization with DNA caused generation of abzymes exhibiting maximal DNA- and RNA-hydrolyzing activity, although the rate of poly(C) hydrolysis was three orders of magnitude higher. If we define the k_{cat} value for poly(C) hydrolysis by anti-DNA AB as 100%, the relative rates of RNA hydrolysis by non-fractionated (on the DNA sorbent) AB against other antigens decreased in the following order: anti-DNA (100%) > anti-DNase II (~81.8%) > RNase (~54.5%) > anti-DNase I (~12.7%) > anti-RNA (~11.4%) (Table 3). However, the relative activities of individual (separated on DNA-cellulose) fractions of AB against each of these antigens can differ by 2-13-fold.

The abilities of the antigens used to induce generation of abzymes with RNA- and DNA-hydrolyzing activities significantly differed. For example, immunization with DNase I caused generation of abzymes with high DNase but low RNase activity, whereas RNase induced AB with high RNase and low DNase activity. It appears that IgG from each peak eluted from DNA-cellulose at various NaCl concentrations can contain AB hydrolyzing only DNA, only RNA, or AB that can

Table 2. The K_m and k_{cat} values of supercoiled DNA hydrolysis characterizing blood pIgG fractions from rabbits immunized with DNA, RNA, DNase I, DNase II, and RNase

Peak	Elution condition during chromatography on DNA-cellulose	K_m, nM	$k_{cat} \times 10^4$, min^{-1}	K_m, nM	$k_{cat} \times 10^4$, min^{-1}
		DNA		RNA	
0	before fractionation	58.9 ± 13.9	2.5 ± 0.3	20.0 ± 5.0	0.017 ± 0.002
1	0.0 M NaCl	68.1 ± 11.4	6.9 ± 0.7	0	0
2	0.1 M NaCl	21.5 ± 4.0	4.7 ± 0.4	21.0 ± 5.0	0.07 ± 0.02
3	0.5 M NaCl	0.0	0.0	12.0 ± 3.0	0.12 ± 0.2
		DNase I		DNase II	
0	before fractionation	8.8 ± 1.0	1.28 ± 0.02 0.28 ± 0.02	15.8 ± 2.0	1.0 ± 0.1
1	0.0 M NaCl	68.7 ± 8.8	0.1 ± 0.015	60.8 ± 10.5	0.53 ± 0.08
2	0.1 M NaCl	9.5 ± 1.5	0.28 ± 0.035	18.3 ± 3.5	2.2 ± 0.4
3	0.5 M NaCl	7.0 ± 0.8	0.46 ± 0.07	13.5 ± 2.5	8.7 ± 1.7
4	3 M NaCl	0.0	0.0	0.8 ± 0.1	3.9 ± 0.5
5	pH 2.6	0.36 ± 0.05	0.16 ± 0.02	0.5 ± 0.09	1.1 ± 0.2
		RNase			
0	before fractionation	1.0 ± 0.4 15.4 ± 3.7	0.16 ± 0.05 0.61 ± 0.22		
1	0.0 M NaCl	18.3 ± 3.3	0.32 ± 0.04		
2	0.1 M NaCl	15.6 ± 1.6	4.7 ± 1.0		
3	0.5 M NaCl	11.3 ± 3.0	0.18 ± 0.03		
4	3 M NaCl	0.8 ± 0.1	0.3 ± 0.05		
5	pH 2.6	0.5 ± 0.1	0.45 ± 0.06		

Note: Fractions were obtained during separation of total pIgG preparations by affinity chromatography on DNA-cellulose [91-94]; salt concentrations or buffer used for elution of IgG fraction from DNA-cellulose are shown. Assay error did not exceed 15-20%.

hydrolyze both RNA and DNA. However, separation of such three types of IgG fractions by chromatography on DNA-cellulose represents a rather serious problem. Only in the case of anti-DNA IgG was it possible to separate a fraction that actively hydrolyzed RNA but was inactive in DNA hydrolysis (fraction *3*, 0.5 M NaCl) (Tables 2 and 3). This is the first example of pIgG that can hydrolyze only RNA. However, in the case of AB against all the antigens there were fractions hydrolyzing both DNA and RNA, and lack of any fraction exhibiting only DNase activity was only in the case of anti-DNA IgG (Tables 2 and 3). The possibility of the existence of abzymes with a chimeric structure in IgG fractions is supported by the fact that monoclonal (mouse SLE) AB against various DNA sequences were 30-100 times more active in hydrolysis of RNA than DNA [101]. Rabbit immunization with any antigen used (DNA, RNA, DNase I, DNase II, RNase) caused generation of a large number of abzymes exhibiting various affinity to DNA and RNA; these abzymes also differed in relative rates of hydrolysis of these substrates. In addition, each of these antigens is characterized by an individual repertoire of monoclonal abzymes exhibiting only DNA- or only RNA-hydrolyzing activity and also abzymes possessing both these activities.

These data suggest that DNA, RNA, their various complexes with histones and other proteins, and enzymes of cell nuclei, cytoplasm, and blood, and also DNases, RNases, as well as other various cell and blood enzymes cleaving DNA and RNA can represent antigens in AID. Consequently, one of reasons underlying extreme heterogeneity of DNA- and RNA-hydrolyzing abzymes in patients with some autoimmune and viral diseases consists of extremely large number of antigens and autoimmunization with these antigens causes generation of a large number of monoclonal abzymes with nuclease activities.

In this connection, it should be mentioned again that, theoretically, the human immune system is able to generate up to 10^6 variants of AB against one antigen. Development of AI reactions is accompanied by active apoptosis of cells, and various nuclear and cytoplasmic components including degraded DNA and RNA and also their complexes with various proteins and enzymes enter

Table 3. The K_m and k_{cat} values of poly(C) hydrolysis characterizing blood pIgG fractions from rabbits immunized with DNA, RNA, DNase I, DNase II, and RNase

Peak	Elution conditions during chromatography on DNA-cellulose	K_m, μM	k_{cat}, min^{-1}	K_m, μM	k_{cat}, min^{-1}
		DNA		RNA	
0	before fractionation	33.9 ± 7.8	0.22 ± 0.02	13.0 ± 2.0	0.025 ± 0.005
1	0.0 M NaCl	73.2 ± 14.8	0.32 ± 0.03	0	0
2	0.1 M NaCl	28.5 ± 3.0	3.5 ± 0.4	0	0
3	0.5 M NaCl	25.2 ± 5.6	1.1 ± 0.1	14.0 ± 2.0	0.1 ± 0.02
		DNase I		DNase II	
0	before fractionation	13.4 ± 2.0	0.028 ± 0.003	32.9 ± 3.7	0.18 ± 0.02
1	0.0 M NaCl	0	0	83.7 ± 9.8	0.07 ± 0.01
2	0.1 M NaCl	0	0	34.2 ± 4.9	0.88 ± 0.09
3	0.5 M NaCl	12.2 ± 1.8	0.11 ± 0.015	15.4 ± 2.0	0.17 ± 0.025
		RNase			
0	before fractionation	18.4 ± 2.7	0.12 ± 0.2		
1	0.0 M NaCl	0.0	0.0		
2	0.1 M NaCl	18.4 ± 2.2	0.39 ± 0.04		
3	0.5 M NaCl	18.7 ± 2.6	0.69 ± 0.08		

Note: Fractions were obtained during separation of total pIgG preparations by affinity chromatography on DNA-cellulose [91-94]; salt concentrations or buffer used for elution of IgG fractions from DNA-cellulose are shown. Assay error did not exceed 15-20%.

the mammalian circulation. The immune system can raise AB against relatively short DNA and RNA fragments of any sequences, especially if they are in complexes with proteins [107-110]. According to [104, 105], for rabbit immunization with DNase I, DNase II, and RNase the major proportion of DNA- and RNA-hydrolyzing AB represent antiidiotypic AB against active sites of these enzymes, but a small part does not bind to IgG against these enzymes and might thus represent AB against nucleic acid existing in complexes with these enzymes. Besides five RNases and a few DNases of human blood, various enzymes from human cells, for example, topoisomerase I [99] capable of cleaving DNA, can act as antigens inducing generation of antiidiotypic AB.

Thus, a large number of antigens and many AB variants formed in response to each antigen are the most probable reasons underlying extreme diversity of DNA- and RNA-hydrolyzing abzymes. Using immunization of healthy rabbits with various antigens, it is possible to model only formation of DNA- and RNA-hydrolyzing abzymes in healthy mammals. Since immunization of AI mice results in much higher increase in the relative content of abzymes and their catalytic activity than in ordinary mice [111, 112] it is possible that additional mechanisms extending the repertoire of monoclonal AB variants interacting with DNA and RNA and hydrolyzing these substrates also exist in patients with AID.

ASSOCIATION OF ABZYME GENERATION WITH CHANGES IN DIFFERENTIATION AND PROLIFERATION OF BONE MARROW STEM CELLS

MRL-lpr/lpr mice are characterized by spontaneous development of SLE. This disease starts at approximately 4 month of age, and at the age of 7 months the major proportion of these mice has clear symptoms and also biochemical (proteinuria ⩾3 mg/ml) and immunological (high concentration of anti-DNA AB) parameters typical for this disease. At the age of 2-3 months all parameters of healthy AI-MRL-lpr/lpr mice did not differ from control healthy non-autoimmune mice, and blood AB from these mice lacked catalytic activity (Table 4) [38]. Some MRL-lpr/lpr mice did not have statistically significant changes of proteinuria and titers of anti-DNA AB even at the age of 7 months; however, AB of these mice already exhibited reliably detected DNase and ATPase activities. The condition of these mice may be considered as a pre-disease, and 1-1.5 months after identification of reliably detected catalytic activity in mice of any age (2-7 months) they usually demonstrate all symptoms and biochemical and immunological parameters of severe SLE pathology; these parameters are typical for sick males and females at the age of 7 months (Table 4). We have demonstrated that as in the case of humans [4-9] at the earliest stages of SLE, in MRL-lpr/lpr mice the only statistically signifi-

Table 4. Biochemical and immunological characteristics of autoimmune MRL-lpr/lpr and control non-autoimmune mice [38]

Group type	Group number	Proteinuria, mg/ml	AB to native DNA, A_{450}	AB to denatured DNA, A_{450}	DNase activity, %	ATPase activity, %	Amylase activity, %
Control mice, males and females							
(CBA × C57BL) F1 (3-7 months)	1	0.12 ± 0.07	0.04 ± 0.01	0.02 ± 0.01	0**	0	1.0 ± 0.5
BALB/c (3-7 months)	2	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 months)*	3	0.38 ± 0.1	0.032 ± 0.01	0.09 ± 0.07	0	0	1.9 ± 1.2
Healthy, pre-disease (7 months)*	5	0.8 ± 0.3	0.1 ± 0.05	0.16 ± 0.05	**3.0 ± 1.0**	**0.4 ± 0.25**	n.d.
Sick (7 months)	7	**8.0 ± 3.1****	**0.2 ± 0.05**	**0.23 ± 0.11**	**22.0 ± 24.0**	**68.3 ± 98.0**	3.7 ± 1.0
Immunized, healthy (2-3 months)	9	**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 months)*	4	0.31 ± 0.03	0.08 ± 0.03	0.12 ± 0.06	0	0	1.8 ± 1.1
Healthy, pre-disease (7 months)*	6	**0.9 ± 0.2**	0.18 ± 0.1	0.08 ± 0.04	**6.1 ± 2.8**	**2.4 ± 1.7**	n.d.
Sick (7 months)	8	**5.0 ± 3.8****	**0.23 ± 0.1**	0.21 ± 0.12	**20.0 ± 21.0**	**65.0 ± 93.0**	**9.2 ± 5.4**
Pregnant (2-3 months)	10	0.31 ± 0.2	**0.24 ± 0.05**	0.25 ± 0.07	**7.3 ± 6.0**	**39.3 ± 42.8**	**3.9 ± 3.6**
Lactating (3 months), 4 days after delivery	11	0.32 ± 0.1	**0.54 ± 0.3**	0.35 ± 0.21	**44.4 ± 40.6**	**367 ± 548**	**31.7 ± 27.3**
Lactating (3 months), 14 days after delivery	12	0.70 ± 0.3	**0.57 ± 0.28**	0.39 ± 0.18	**19.0 ± 24.0**	**191 ± 173**	**13.7 ± 11.0**

Note: Proteinuria corresponded to ≥3 mg protein per ml urine. n.d., not determined. Parameters significantly different ($p \leq 0.05$) from those in healthy MRL-lpr/lpr male and female mice are shown in bold.

* MRL-lpr/lpr mice without symptoms of SLE; the mice with all parameters basically the same as in healthy control or autoimmune mice are conditionally defined as healthy mice; the other group of these mice with the same parameters as in healthy mice, but demonstrating detectable abzyme activity, is defined as pre-diseased mice.

** 100% of relative activity corresponded to total conversion of substrate to product in the presence of 0.1 mg/ml IgG.

cant parameter indicating the onset of this disease is detectable abzyme activity [37, 38]. It should be noted that immunization of MRL-lpr/lpr mice with the complex of DNA with methylated BSA resulted in appearance of all symptoms of SLE, and their blood contains high concentrations of anti-DNA AB characterized by high catalytic activities (Table 4).

Pregnancy is another special condition of both mice and humans [5-9], when AB with high catalytic activities are detected. Lactation is characterized by 6-10-fold increase in abzyme activity (Table 4). Generation of abzymes in pregnant and lactating mice is not associated with proteinuria; however, there is statistically significant increase in AB titers to native but not denatured DNA.

According to literature data, numerous AI impairments can be associated with changes in proliferation and differentiation of bone marrow stem cells (BMSC) [113]. Taking into consideration these data, we have performed correlation analysis between biochemical and immunological parameters and also correlation analysis between relative activities of abzymes and relative content of three types of blood cell progenitors — erythroid burst-forming

unit (BFU-E), granulocytic-macrophagic colony-forming unit (CFU-GM), granulocytic-erythroid-megakaryocytic-macrophagic colony-forming unit (CFU-GEMM) — in bone marrow of 12 groups of mice [38]. In healthy control and MRL-lpr/lpr mice (2-7 month old) there were no reliably detected abzyme activities and the levels of proliferation and differentiation of BMSC were also comparable (groups 1, 3, and 4; Table 5). Appearance of reliably detected abzyme activity in the pre-diseased mice was accompanied by a sharp and statistically significant change in the differentiation profile and increase in the proliferation level of BMSC; there was a significant increase in the size of colonies (groups 5 and 6). Transition from the pre-disease (groups 5 and 6) into the true pathological condition (groups 7 and 8) was accompanied by a decrease in colony size to normal and also by a sharp change in the differentiation profile, the level of BMSC proliferation, and a sharp increase in the catalytic activity of abzymes (Tables 4 and 5).

In contrast to the situation with spontaneous SLE, immunization of mice with DNA caused the highest increase in abzyme activity and manifestation of immunization-induced symptoms of this disease (Table 4). However, this was not associated with alteration of the bone marrow immune system: the differentiation profile and the level of differentiation of BMSC of these mice (group 9) did not differ from those seen in healthy individuals (groups 1, 3, and 4). This suggests that immunization of healthy mice with various antigens would have no influence on BMSC. We have demonstrated [38] that immunization of MRL-lpr/lpr mice with DNA caused a sharp increase in lymphocyte proliferation, first of all in lymph nodes, and to a lesser extent in spleen and thymus; this was accompanied by a sharp inhibition of lymphocyte apoptosis in these organs. During spontaneous development of SLE the increase in lymphocyte proliferation in these organs was markedly lower, and inhibition of apoptosis was less pronounced than in immunized mice. In other words, the development of SLE induced by immunization of MRL-lpr/lpr mice occurs at the level of predifferentiation and due to the increase in proliferation and simultaneous suppression of lymphocyte apoptosis in internal organs; spontaneous development of SLE is characterized by altered differentiation profile and the level of BMSC proliferation (the lymphocyte system of internal organs is less affected). These data explain why abzyme repertoire and relative level of abzyme activity is significantly lower during immunization of mice that are not prone to AID as compared with AI mice [111, 112].

Special attention ought to be paid to analysis of data obtained using pregnant and lactating MRL-lpr/lpr mice. Appearance of reliably detected abzyme activities in preg-

Table 5. Formation of various blood cell progenitors in bone marrow of MRL-lpr/lpr and control non-autoimmune mice [38]

Type of the group	Visual symptoms	Group number	Number of colonies		
			BFU-E**	CFU-GM	CFU-GEMM
CBA (3-7 months)	no	1	3.0 ± 0.5	7.3 ± 1.0	0.25 ± 0.05
			MRL-lpr/lpr, males		
Healthy (2-3 months)*	no	3	6.5 ± 1.5	7.0 ± 1.0	0.5 ± 0.1
Healthy, pre-disease (7 months)*	no	5	**12.7 ± 1.4**	**30.0 ± 1.3**	**9.2 ± 1.9**
Sick (7 months)	yes	7	**25.3 ± 9.8**	7.4 ± 0.4	**3.9 ± 2.0**
Immunized, healthy (2-3 months)	yes, weak	9	7.0 ± 2.1	6.0 ± 2.6	0.9 ± 0.7
			MRL-lpr/lpr, females		
Healthy (2-3 months)*	no	4	5.5 ± 0.5	11 ± 2.5	0.5 ± 0.2
Healthy, pre-disease (7 months)*	no	6	**11.5 ± 2.0**	**23.0 ± 3.0**	**8.2 ± 3.0**
Sick (7 months)	yes	8	**22.1 ± 8.0**	9.0 ± 3.9	2.4 ± 1.8
Pregnant (2-3 months)	no	10	6.8 ± 2.0	**7.8 ± 1.5**	**0.1 ± 0.08**
Lactating (3 months), 4 days after delivery	no	11	8.8 ± 2.0	**19.1 ± 1.8**	0.25 ± 0.2
Lactating (3 months), 14 days after delivery	no	12	**21.0 ± 8.0**	9.7 ± 0.5	**2.1 ± 0.7**

Note: Parameters significantly different ($p \le 0.05$) from those in healthy MRL-lpr/lpr male and female mice are shown in bold.

* MRL-lpr/lpr mice without symptoms of SLE; the mice with all parameters basically the same as in healthy control or autoimmune mice are conditionally defined as healthy mice; the other group of these mice with the same parameters as in healthy mice but demonstrating detectable abzyme activity is defined as pre-diseased mice.

** BFU-E, erythroid burst-forming unit; CFU-GM, granulocytic-macrophagic colony-forming unit; CFU-GEMM, granulocytic-erythroid-megakaryocytic-macrophagic colony-forming unit.

nant mice was associated with a sharp decrease in the differentiation profile and suppression of BMSC proliferation (Tables 4 and 5) and also with simultaneous and very potent decrease in lymphocyte apoptosis in lymph nodes, thymus, spleen, and bone marrow. It should be accepted that under normal conditions abzyme-synthesizing lymphocytes are eliminated via apoptosis. In this case, suppression of apoptosis in pregnant mice might be the main cause for abzyme generation in reliably detected quantities.

As it has already been mentioned, the immune memory of pregnant women accumulates information about all hazardous environmental factors that appeared during the third trimester of pregnancy. A switch of the immune memory occurs right after deliver with onset of lactation; foremilk, early milk, and blood contain AB to all the hazardous factors penetrating into the female body orally or due to some viral or bacterial infections. Four days after delivery there was a sharp increase in the relative activity of abzymes in mice (Table 4); this was associated with the first step of alteration of the differentiation profile and the increase in the proliferation level of BMSC of lactating mice (Table 5); 14 days after birth there was a marked decrease in relative activity of abzymes, and this activity gradually decreases up to total disappearance by the end of lactation and the differentiation profile and the proliferation level of BMSC returns to the norm. Women were characterized by basically the same pattern of altered relative activity of abzymes during pregnancy and onset and termination of lactation [47, 55]. Transition from pregnancy to lactation is characterized by a 6-10-fold increase in abzymes from blood of women [47, 55] and female mice [38], but the abzyme activity in milk of parturient women can be 10-1600-fold higher than in blood. Interestingly, relative activity of DNA-hydrolyzing abzymes in blood of pregnant women with pregnancy-induced AIT was 6-10 time higher than in women without clear symptoms of AID [47].

There is some similarity in abzyme production and changes in differentiation profiles and the levels of BMSC in MRL-lpr/lpr mice with SLE, on one hand, and in pregnant and lactating individuals, on the other hand. At the same time, differentiation profiles and relative proliferation levels of BMSC had marked differences in these groups of mice (Table 5). In addition, autoimmune-like proteins observed in parturient women are transient, and in most cases their immune system returns to the normal level after termination of lactation. However, typical AID are chronic diseases with transient recurrence and remissions, and they are basically incurable. This suggests existence of significant differences in changes in BMSC in lactating woman and typical AID patients.

Based on the above-considered data, we should conclude that the appearance of new clones of abzyme-synthesizing lymphocytes attributed to changes in differentiation profiles and the level of BMSC proliferation represent another reason underlying extreme heterogeneity of DNA- and RNA-hydrolyzing abzymes in AID-patients as compared with healthy non-immunized and immunized individuals. This might account for the sharp increase of monoclonal (within polyclonal) abzymes in AID patients.

HETEROGENEITY OF ABZYMES WITH PROTEOLYTIC ACTIVITY

Theoretically, catalytic heterogeneity of proteolytic abzymes specifically hydrolyzing just one oligopeptide or protein should be significantly lower than in the case of DNA- and RNA-hydrolyzing abzymes because in this case the number of antigens stimulating generation of such abzymes is significantly lower (frequently, one antigen). Originally, such clear catalytic heterogeneity was found in IgG abzymes from blood of MS patients; these abzymes specifically hydrolyzed MBP [32-34]. It was demonstrated that pIgG contained not only subfractions of abzymes with proteolytic activity similar to that of serine proteases, but also subfractions exhibiting metalloprotease-like activity [32-34]. Chromatography on a metal ion-chelating sorbent resulted in separation of AB with metalloprotease activity into many subfractions exhibiting different affinity to metals and effectiveness of MBP hydrolysis in the presence of various metal ions [34]. Later it was demonstrated [114] that IgG abzymes from MS patients hydrolyze MBP by four sequences corresponding to four main antigenic determinants of MBP. Using the MBP-hydrolyzing abzymes from blood of MS patients, we have demonstrated for the first time that the proteolytic activity can be associated with IgG λ- and κ-light chains and also with IgG antibodies, subclasses I-IV.

The IgG antibodies with specific β-casein hydrolyzing activity isolated from blood of HIV-infected patients are a second example of catalytic heterogeneity of proteolytic abzymes [35]. During IgG chromatography on Sepharose with immobilized β-casein, they were separated into many catalytically active subfractions exhibiting different affinity to casein. Being isolated from various patients, pIgG demonstrated various profiles of their relative activity on pH; this suggests the existence of various repertoires of monoclonal AB with acidic, neutral, and alkaline proteolytic activities, which obviously depend on individual features of patients and also on severity of AI processes.

CATALYTIC HETEROGENEITY OF NUCLEOTIDE-DEPENDENT ABZYMES

Originally, catalytic heterogeneity of nucleotide-dependent abzymes was found in milk sIgA from parturient women; these abzymes phosphorylated serine

residues in approximately 15 various milk proteins [49, 50]. Later this was found in milk AB hydrolyzing ATP, ADP, and AMP [55]. Subsequently, high catalytic heterogeneity was also demonstrated for human milk sIgA and IgG catalyzing phosphorylation of minor lipids [57, 58] and polysaccharides [59, 60]. During chromatography on ATP-Sepharose, abzymes with all these activities were separated into many subfractions different in affinity for ATP. Analysis of K_m and V_{max} values for reactions catalyzed by these AB revealed from two to four values of each of these parameters (in dependence on preparation used). In some cases the increase in ATP concentrations caused a wave-like increase in the reaction rate during a wide-range variation of substrate concentration (up to 3-4 orders of magnitude); this suggested the presence of an extremely high number of monoclonal abzymes with different affinity to ATP in the total AB pool.

CATALYTIC HETEROGENEITY OF ABZYMES WITH OXIDOREDUCTASE ACTIVITIES

In contrast to abzymes with DNase, RNase, nucleotide-dependent, and proteolytic activities isolated from animal and human blood, IgG antibodies isolated from blood of healthy Wistar rats [64-67] were characterized by single K_m and V_{max} values for oxidized substrates (aromatic amines and phenols) in the reactions of H_2O_2-dependent peroxidation and H_2O_2-independent oxidoreductase oxidation. At the same time, pIgG contained subfractions strongly different in affinity for hydrogen peroxide, and pH optima varied from very acidic (3-4) to alkaline pH values (9-10) [67]. During chromatography on the Chelex sorbent chelating metal ions, there was adsorption up to 40% of total AB pool and distribution of peroxidase and oxidoreductase activities over the whole chromatographic profile (during elution with NaCl gradient from 10 mM to 1 M) [65]. Some very small AB fractions exhibited higher activity in the presence of Cu^{2+} than Fe^{2+}, whereas others demonstrated the opposite preference.

All known plant, bacterial, and eukaryotic oxidases, peroxidases, and dismutases utilizing reactive oxygen species as substrates are enzymes exhibiting dependence of their catalytic activity on metal ions [115]. Selenium-dependent glutathione peroxidase is the only enzyme that does not depend on metal ion. Two known eukaryotic superoxide dismutases are the Mn- and Cu,Zn-dependent enzymes, whereas in some bacteria Ni- and Fe-dependent dismutases have been recognized. Human Fe^{2+}-dependent catalase is mainly localized in hepatocytes and peroxisomes. There are some plant peroxidases that share enzymatic properties with horseradish peroxidase, one of the most active enzymes, which also exhibits wide substrate specificity. All known plant peroxidases are porphyrin-dependent enzymes.

In contrast to horseradish (and other plant) peroxidase and also oxidoreductases, abzymes exhibiting oxidoreductase activities can be activated by various transition metal ions (Fe^{2+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}) [66]. After separation on Chelex, individual pIgG fractions exhibited different effectiveness of their activation by various metal ions. Moreover, these abzymes exhibited the highest activity in the presence of two types of metal ions: Cu^{2+} + Mn^{2+}, Cu^{2+} + Zn^{2+}, Fe^{2+} + Mn^{2+}, Fe^{2+} + Zn^{2+} [116]. All these data suggest very high catalytic heterogeneity of rat abzymes with oxidoreductase activities.

Thus, there is evidence that the immune system of higher organisms can generate many various AB variants to each foreign and in the case of AID own (auto)antigen. It appears that among all AB variants against one antigen, the major proportion of AB lacks catalytic activity because even generation of AB against chemically stable transition state analogs (as haptens) results in AB generation, but only a part of them exhibits catalytic activity [4]. However, in contrast to canonic proteases cleaving any proteins, small AB fractions generated in various AID selectively hydrolyze just one protein: VIP in asthma [10], thyroglobulin in AIT [28, 29], MBP in MS [32-34], and HIV reverse transcriptase and integrase and also casein and human serum albumin (HSA) in AIDS [35]. Taking into consideration these facts, abzymes should be considered as a source of enzymes with new catalytic activities otherwise absent in nature.

It is known that auto-AB are generated not only in patients with AID, but also in healthy donors; in the latter case the auto-AB titer could be rather high. However, abzymes with some reliably detected activities (e.g. DNase and RNase) that can be harmful for the body are not detected in blood of healthy donors. It appears that clones of lymphocytes synthesizing such abzymes are constantly eliminated by apoptosis in healthy individuals. However, blood of healthy people may contain AB hydrolyzing polysaccharides, VIP, and thyroglobulin [10, 23, 29]. Although this suggests abzyme generation in healthy people, it is possible that such abzymes are less harmful for them.

Blood AB with DNase and ATPase activities are detected in both pregnant women and female mice even in the cases of lack of such activities in pIgG before pregnancy. First of all this can be attributed to suppression of lymphocyte apoptosis in all organs during pregnancy; this can result in accumulation of lymphocyte clones generating abzymes. Pregnancy is characterized by rearrangements of the mouse bone marrow immune system, and when the immune memory begins to function (with onset of lactation) it synthesizes large quantities of various AB and abzymes. It is possible that small clones of abzyme-synthesizing lymphocytes might be formed even during pregnancy, and appearance of these clones is associated with an altered differentiation profile of BMSC.

It should be emphasized that immunization of healthy mice mainly involves lymphocytes of peripheral

organs (lymph nodes, spleen, and thymus) but not BMSC [38]. This suggests that the huge diversity of DNA-hydrolyzing abzymes that appeared during immunization of healthy rabbits with DNA, RNA, DNase I, DNase II, RNase [102-105] is determined by processes of alternative lymphocyte differentiation in the internal organ. Rabbit immunization with DNA and RNA resulted in generation of abzymes with low affinity to DNA only in the case of generation of antiidiotypic AB against active sites of DNase I, DNase II, and RNase [102-105]. Although separation of the pIgG obtained from various AID patients on DNA-cellulose revealed AB distributed over the whole chromatographic profile (0-3 M NaCl), small abzyme fractions were eluted by means of 3 M $MgCl_2$ or acidic buffer (pH 2.6) [4-9]. Thus, it is clear that DNA- and RNA-hydrolyzing abzymes detected in AID represent a "cocktail" of AB against DNA and RNA and antiidiotypes against various DNA- and RNA-hydrolyzing enzymes.

Since mice with spontaneous SLE and healthy mice subjected to immunization with DNA are characterized by increased lymphocyte proliferation in internal organs, it appears that abzyme generation in sick mice might be partially determined by alternative lymphocytes similar to that observed in healthy mice. However, in mice with SLE specific change in the differentiation profile and the increase in BMSC proliferation level might significantly influence the lymphocyte repertoire diversity in internal organs. Obviously, the change in the BMSC differentiation profile results in appearance of lymphocytes synthesizing abzymes of higher activity and possibly with other biological functions. In this connection, it should be noted that DNA-hydrolyzing IgG from blood of healthy rabbits immunized with DNA, RNA, DNase I, DNase II, and RNase are not cytotoxic to cancer cells. Only DNA-hydrolyzing abzymes from about 30% of MS patients exhibited marked cytotoxicity [8]. At the same time, DNA-hydrolyzing abzymes from blood of all SLE-patients exhibit reliably detected cytotoxicity, but there was no strict correlation with relative activity of these abzymes (AB with lower DNA activity could exhibit higher cytotoxicity and vice versa). This suggests that not all DNA-hydrolyzing abzymes are cytotoxic and that formation of specific lymphocytes generating abzymes with the highest cytotoxicity might occur mainly in AID. In addition, RNA-hydrolyzing abzymes from blood of patients with various AID differ in patterns of cleavage of various tRNAs [84, 85]. Consequently, structure of active sites of abzymes may also depend on type of diseases.

Thus, it appears that a part of lymphocytes, which synthesize various abzymes in patients with AI reactions, can be eliminated by apoptosis. However, our data indicate that many clones remain, and our pilot estimations indicate that their number may exceed tens and in some cases even hundreds, and this underlies the extreme heterogeneity of abzymes exhibiting various activities. In the case of DNA- and RNA-hydrolyzing AB, the number of abzymes also increases due to the presence of many antigens in the human body. These antigens can induce generation of such abzymes.

This work was supported by the Programs of Basic Research of Presidium of Russian Academy of Sciences "Molecular and Cell Biology" and "Basic Sciences for Medicine", Russian Foundation for Basic Research (grant No. 07-04-00387), and a joint grant from the Russian Foundation for Basic Research and the Byelorussian Foundation for Basic Research (grant No. 08-04-90014).

REFERENCES

1. Tramontano, A., Gololobov, G., and Paul, S. (2000) *Chem. Immunol.*, **77**, 1-17.
2. Gololobov, G., Tramontano, A., and Paul, S. (2000) *Appl. Biochem. Biotechnol.*, **83**, 221-231.
3. Nevinsky, G. A., Semenov, D. V., and Buneva, V. N. (2000) *Biochemistry (Moscow)*, **65**, 1233-1244.
4. Keinan, E. (ed.) (2005) *Catalytic Antibodies*, Wiley-VCH Verlag GmbH, and Co. KgaA, Morlenbach, Germany, pp. 1-586.
5. Nevinsky, G. A., Kanyshkova, T. G., and Buneva, V. N. (2000) *Biochemistry (Moscow)*, **65**, 1245-1255.
6. Nevinsky, G. A., and Buneva, V. N. (2002) *J. Immunol. Meth.*, **269**, 235-249.
7. Nevinsky, G. A., Favorova, O. O., and Buneva, V. N. (2002) in *Protein–Protein Interactions. A Molecular Cloning Manual* (Golemis, E., ed.) Cold Spring Harbor Laboratory Press, N. Y., pp. 523-534.
8. Nevinsky, G. A., and Buneva, V. N. (2003) *J. Cell Mol. Med.*, **7**, 265-276.
9. Nevinsky, G. A., and Buneva, V. N. (2005) in *Catalytic Antibodies* (Keinan, E., ed.) VCH-Wiley Press, Weinheim, pp. 503-567.
10. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989) *Science*, **244**, 1158-1162.
11. Shuster, A. M., Gololobov, G. V., Kvashuk, O. A., Bogomolova, A. E., Smirnov, I. V., and Gabibov, A. G. (1992) *Science*, **256**, 665-667.
12. Andrievskaya, O. A., Buneva, V. N., Baranovskii, A. G., Gal'vita, A. V., Benzo, E. S., Naumov, V. A., and Nevinsky, G. A. (2002) *Immunol. Lett.*, **81**, 191-198.
13. Andrievskaya, O. A., Buneva, V. N., Naumov, V. A., and Nevinsky, G. A. (2000) *Med. Sci. Monit.*, **6**, 460-470.
14. Buneva, V. N., Andrievskaya, O. A., Romannikova, I. V., Gololobov, G. V., Yadav, R. P., Yamkovoi, B. I., and Nevinsky, G. A. (1994) *Mol. Biol. (Moscow)*, **28**, 738-743.
15. Vlasov, A. V., Baranovskii, A. G., Kanyshkova, T. G., Prints, A. V., Zabara, V. G., Naumov, V. A., Breusov, A. A., Buneva, V. N., and Nevinsky, G. A. (1998) *Mol. Biol. (Moscow)*, **32**, 559-569.
16. Baranovskii, A. G., Kanyshkova, T. G., Mogilnitskii, A. S., Naumov, V. A., Buneva, V. N., Boiko, A. N., Favorova, O. O., and Nevinsky, G. A. (1998) *Biochemistry (Moscow)*, **63**, 1239-1248.

17. Baranovskii, A. G., Matyushin, V. G., Vlasov, A. V., Zabara, V. G., Naumov, V. A., Buneva, V. N., and Nevinsky, G. A. (1997) *Biochemistry (Moscow)*, **62**, 1358-1366.
18. Kozyr, A. V., Kolesnikov, A. V., Aleksandrova, E. S., Sashchenko, L. P., Gnuchev, N. V., Favorov, P. V., Kotelnikov, M. A., Iakhnina, E. I., Astsaturov, I. A., Prokaeva, T. B., Alekberova, Z. S., Suchkov, S. V., and Gabibov, A. G. (1998) *Appl. Biochem. Biotechnol.*, **75**, 45-61.
19. Kharitonova, M. A., Sizyakina, L. P., and Nevinsky, G. A. (2003) *Immunologiya*, **24**, 68-69.
20. Odintsova, E. S., Kharitonova, M. A., Baranovskii, A. G., Sizyakina, L. P., Buneva, V. N., and Nevinsky, G. A. (2006) *Mol. Biol. (Moscow)*, **40**, 857-864.
21. Gal'vita, A. V., Baranovsky, A. G., Kuznetsov, I. A., Vinshu, N. V., Galenok, V. A., Buneva, V. N., and Nevinsky, G. A. (2007) *Rus. Immunol. Zh.*, **1**, 116-131.
22. Odintsova, E. S., Parkhomenko, T. A., Buneva, V. N., Generalov, I. I., and Nevinsky, G. A. (2006) *Immunopatol. Allergol. Infektol.*, **2**, 23-31.
23. Savel'ev, A. N., Eneyskaya, E. V., Shabalin, K. A., Michael, V., Filatov, M. V., and Neustroev, K. N. (1999) *Protein Peptide Lett.*, **6**, 179-184.
24. Neustoev, K. N., Ivanen, D. R., Kulminskaya, A. A., Brumer, I. H., Saveliev, A. N., and Nevinsky, G. A. (2003) *Human Antibodies*, **12**, 31-34.
25. Saveliev, A. N., Ivanen, D. R., Kulminskaya, A. A., Ershova, N. A., Kanyshkova, T. G., Buneva, V. N., Mogelnitskii, A. S., Doronin, B. M., Favorova, O. O., Nevinsky, G. A., and Neustroev, K. (2003) *Immunol. Lett.*, **86**, 291-297.
26. Savel'ev, A. N., Kanyshkova, T. G., Kulminskaya, A. A., Buneva, V. N., Eneyskaya, E. V., Filatov, M. V., Nevinsky, G. A., and Neustroev, K. N. (2001) *Clin. Chim. Acta*, **314**, 141-152.
27. Ivanen, D. R., Kulminskaya, A. A., Shabalin, K. A., Isaeva-Ivanova, L. V., Ershova, N. A., Saveliev, A. N., Nevinsky, G. A., and Neustroev, K. N. (2004) *Med. Sci. Monit.*, **10**, BR273-BR280.
28. Li, L., Kaveri, S., Tyutyulkova, S., Kazatchkine, M. D., and Paul, S. (1995) *J. Immunol.*, **154**, 3328-3332.
29. Kalaga, R., Li, L., O'Dell, J. R., and Paul, S. (1995) *J. Immunol.*, **155**, 2695-2702.
30. Thiagarajan, P., Dannenbring, R., Matsuura, K., Tramontano, A., Gololobov, G., and Paul, S. (2000) *Biochemistry*, **39**, 6459-6465.
31. Lacroix-Desmazes, S., Moreau, A., Sooryanarayana, S., Bonnemain, C., Stieltjes, N., Pashov, A., Sultan, Y., Hoebeke, J., Kazatchkine, M. D., and Kaveri, S. V. (1999) *Nat. Med.*, **5**, 1044-1047.
32. Polosukhina, D. I., Kanyshkova, T. G., Doronin, B. M., Tyshkevich, O. B., Buneva, V. N., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2004) *J. Cell Mol. Med.*, **8**, 359-368.
33. Polosukhina, D. I., Buneva, V. N., Doronin, B. M., Tyshkevich, O. B., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2005) *Med. Sci. Monit.*, **11**, BR266-BR272.
34. Polosukhina, D. I., Kanyshkova, T. G., Doronin, B. M., Tyshkevich, O. B., Buneva, V. N., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2006) *Immunol. Lett.*, **103**, 75-81.
35. Odintsova, E. S., Kharitonova, M. A., Baranovskii, A. G., Sizyakina, L. P., Buneva, V. N., and Nevinsky, G. A. (2006) *Biochemistry (Moscow)*, **71**, 251-261.
36. Baranova, S. V., Buneva, V. N., and Nevinsky, G. A. (2007) *Vestnik NGU. Biol. Ser.: Biol. Klin. Med.*, **5**, 74-81.
37. Andryushkova, A. S., Kuznetsova, I. A., Orlovskaya, I. A., Buneva, V. N., and Nevinsky, G. A. (2006) *FEBS Lett.*, **580**, 5089-5095.
38. Andryushkova, A. S., Kuznetsova, I. A., Buneva, V. N., Toporkova, L. B., Sakhno, L. V., Tichonova, M. A., Chernykh, E. R., Orlovskaya, I. A., and Nevinsky, G. A. (2007) *J. Cell Mol. Med.*, **11**, 531-551.
39. Mohan, C., Adams, S., Stanic, V., and Datta, S. K. (1993) *J. Exp. Med.*, **177**, 1367-1381.
40. Kazakov, V. I., Bozhkov, V. M., Linder, V. A., Repina, M. A., and Mikhailov, V. M. (1995) *Tsitologiya*, **37**, 232-235.
41. Amino, N., Tada, H., and Hitaka, Y. (1999) *Thyroid*, **9**, 705-713.
42. Bauer, M., Hutterer, G., Eder, M., Majer, S., Leshane, E., Johnson, K. L., Peter, I., Bianchi, D. W., and Pertl, B. (2006) *Prenat. Diagn.*, **26**, 831-836.
43. Hazes, J. M. (1997) *Ann. Rheum.*, **50**, 71-72.
44. Dayan, C. M., and Daniels, G. H. (1996) *New Engl. J. Med.*, **335**, 99-107.
45. Tanaka, A., Lindor, K., Ansari, A., and Gershwin, M. E. (2000) *Liver Transpl.*, **6**, 138-143.
46. Freeman, R., Rosen, H., and Thysen, B. (1986) *Arch. Int. Med.*, **146**, 1361-1364.
47. Buneva, V. N., Kudryavtseva, A. N., Gal'vita, A. V., Dubrovskaya, V. V., Khokhlova, O. V., Kalinina, I. A., Galenok, V. A., and Nevinsky, G. A. (2003) *Biochemistry (Moscow)*, **68**, 890-900.
48. Kit, Y. Y., Kim, A. A., and Sidorov, V. N. (1991) *Biomed. Sci.*, **2**, 201-204.
49. Nevinsky, G. A., Kit, Y. Ya., Semenov, D. V., Khlimankov, D. Yu., and Buneva, V. N. (1998) *Appl. Biochem. Biotechnol.*, **75**, 77-91.
50. Kit, Y., Semenov, D. V., and Nevinsky, G. A. (1996) *Biochem. Mol. Biol. Int.*, **39**, 521-527.
51. Kanyshkova, T. G., Semenov, D. V., Khlimankov, D. Yu., Buneva, V. N., and Nevinsky, G. A. (1997) *FEBS Lett.*, **416**, 23-26.
52. Nevinsky, G. A., Kanyshkova, T. G., Semenov, D. V., Vlassov, A. V., Gal'vita, A. V., and Buneva, V. N. (2000) *Appl. Biochem. Biotechnol.*, **83**, 115-129.
53. Buneva, V. N., Kanyshkova, T. G., Vlassov, A. V., Semenov, D. V., Khlimankov, D. Yu., Breusova, L. R., and Nevinsky, G. A. (1998) *Appl. Biochem. Biotechnol.*, **75**, 63-76.
54. Semenov, D. V., Kanyshkova, T. G., Kit, Yu. A., Khlimankov, D. Yu., Akimzhanov, A. M., Gorbunov, D. A., Buneva, V. N., and Nevinsky, G. A. (1998) *Biochemistry (Moscow)*, **63**, 935-943.
55. Semenov, D. V., Kanyshkova, T. G., Karotaeva, N. A., Krasnorutskii, M. A., Kuznetsova, I. A., Buneva, V. N., and Nevinsky, G. A. (2004) *Med. Sci. Monit.*, **10**, BR23-BR33.
56. Odintsova, E. S., Buneva, V. N., and Nevinsky, G. A. (2005) *J. Mol. Recognit.*, **18**, 413-421.
57. Gorbunov, D. V., Semenov, D. V., Shipitsin, M. V., Kit, Yu. Yu., Kanyshkova, T. G., Buneva, V. N., and Nevinsky, G. A. (2000) *Rus. J. Immunol.*, **5**, 267-278.
58. Gorbunov, D. V., Karataeva, N. A., Buneva, V. N., and Nevinsky, G. A. (2005) *Biochim. Biophys. Acta*, **1735**, 153-166.

59. Karataeva, N. A., Gorbunov, D. V., Prokudin, I. V., Buneva, V. N., Kulminskaya, A. A., Neustroev, K. N., and Nevinsky, G. A. (2006) *Immunol. Lett.*, **103**, 58-67.
60. Karataeva, N. A., Buneva, V. N., and Nevinsky, G. A. (2006) *Biochemistry (Moscow)*, **71**, 1207-1221.
61. Cao, J., Guo, C., Wang, X., He, C., and Zhang, P. (2008) *Food Agric. Immunol.*, **19**, 107-116.
62. Wentworth, A. D., Jones, L. H., Wentworth, P., Jr., Janda, K. D., and Lerner, R. A. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 10930-10935.
63. Wentworth, P., Jr., Jones, L. H., Wentworth, A. D., Zhu, X., Larsen, N. A., Wilson, I. A., Xu, X., Goddard, W. A., III, Janda, K. D., Eschenmoser, A., and Lerner, R. A. (2001) *Science*, **293**, 1806-1811.
64. Wentworth, P., Jr., McDunn, J. E., Wentworth, A. D., Takeuchi, C., Nieva, J., Jones, T., Bautista, C., Ruedi, J. M., Gutierrez, A., Janda, K. D., Babior, B. M., Eschenmoser, A., and Lerner, R. A. (2002) *Science*, **298**, 2143-2144.
65. Ikhmyangan, E. N., Vasilenko, N. L., Buneva, V. N., and Nevinsky, G. A. (2005) *FEBS Lett.*, **579**, 3960-3964.
66. Ikhmyangan, E. N., Vasilenko, N. L., Buneva, V. N., and Nevinsky, G. A. (2006) *J. Mol. Recognit.*, **19**, 91-105.
67. Ikhmyangan, E. N., Vasilenko, N. L., Sinitsina, O. I., Buneva, V. N., and Nevinsky, G. A. (2006) *J. Mol. Recognit.*, **19**, 432-440.
68. Ikhmyangan, E. N., Vasilenko, N. L., Sinitsina, O. I., Buneva, V. N., and Nevinsky, G. A. (2006) *Immunopatol. Allergol. Infektol.*, **2**, 32-48.
69. Paul, S. (1998) *Appl. Biochem. Biotechnol.*, **75**, 13-23.
70. Sinohara, H., and Matsuura, K. (2000) *Appl. Biochem. Biotechnol.*, **83**, 85-94.
71. Lacroix-Desmazes, S., Bayry, J., Kaveri, S. V., Hayon-Sonsino, D., Thorenoor, N., Charpentier, J., Luyt, C. E., Mira, J. P., Nagaraja, V., Kazatchkine, M. D., Dhainaut, J. F., and Mallet, V. O. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 4109-4113.
72. Shoenfeld, Y., Ben-Yehuda, O., Messinger, Y., Bentwitch, Z., Rauch, J., Isenberg, D. I., and Gadoth, N. (1988) *Immunol. Lett.*, **17**, 285-291.
73. Ershova, N. A., Garmashova, N. V., Mogelnitskii, A. S., Tyshkevich, O. B., Doronin, B. M., Konenkova, L. P., Buneva, V. N., and Nevinsky, G. A. (2007) *Rus. Immunol. Zh.*, **1**, 229-245.
74. Andrievskaya, O. A., Buneva, V. N., Zabara, V. G., Naumov, V. A., Yamkovoi, V. I., and Nevinsky, G. A. (2000) *Mol. Biol. (Moscow)*, **32**, 908-915.
75. Nevinsky, G. A., Kanyshkova, T. G., Semenov, D. B., and Buneva, V. N. (2001) *Vestnik RAMN*, **2**, 38-45.
76. Berneman, A., Cuilbert, B., Enschrich, S., and Avrames, S. (1993) *Mol. Immunol.*, **30**, 1499-1510.
77. Coutinho, A., Kazatchkine, M. D., and Avrameas, S. (1995) *Curr. Opin. Immunol.*, **7**, 812-818.
78. Poser, C. M., Paty, D. W., and Scheinberg, L. (1983) *Ann. Neurol.*, **13**, 227-231.
79. Kurtzke, J. F. (1983) *Neurology*, **33**, 1444-1452.
80. Williamson, R. A., Burgoon, M. P., Owens, G. P., Ghausi, O., Leclerc, E., Firme, L., Carlson, S., Corboy, J., Parren, P. W., Sanna, P. P., Gilden, D. H., and Burton, D. R. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 1793-1798.
81. Mogelnitskii, A. S. (2001) *The Role of Catalytically Active Antibodies in Pathogenesis and Diagnostics of Multiple Sclerosis (A Clinico-Biological Study)*: Candidate's dissertation [in Russian], State Medical Academy, Novosibirsk.
82. Belogurov, A. A., Kurkova, I. N., Friboulet, A., Thomas, D., Misikov, V. K., Zakharova, M. Y., Suchkov, S. V., Kotov, S. V., Alehin, A. I., Avalle, B., Suslova, E. A., Morse, H. C., 3rd, Gabibov, A. G., and Ponomarenko, N. A. (2008) *J. Immunol.*, **180**, 1258-1267.
83. Parkhomenko, T. A., Odintsova, E. S., Buneva, V. N., Kunder, E. V., Zhyltsov, I. V., Senkovich, S. A., Generalov, I. I., and Nevinsky, G. A. (2008) *J. Cell Mol. Med.*, doi:10.1111/j.1582-4934.2008.00441.x.
84. Vlassov, A., Florentz, C., Helm, M., Naumov, V, Buneva, V., Nevinsky, G., and Giege, R. (1998) *Nucleic Acids Res.*, **26**, 5243-5250.
85. Vlassov, A. V., Helm, M., Florentz, C., Naumov, V. A., Breusov, A. A., Buneva, V. N., Giege, R., and Nevinsky, G. A. (1999) *Rus. J. Immunol.*, **4**, 25-32.
86. Nevinsky, G. A., Breusov, A. A., Baranovskii, A. G., Prints, A. V., Kanyshkova, T. G., Galvita, A. V., Naumov, V. A., and Buneva, V. N. (2001) *Med. Sci. Monit.*, **7**, 201-211.
87. Breusov, A. A., Gal'vita, A. V., Benzo, E. S., Baranovskii, A. G., Prints, A. V., Naumov, V. A., Buneva, V. N., and Nevinsky, G. A. (2001) *Rus. J. Immunol.*, **6**, 17-28.
88. Kuznetsova, I. A., Orlovskaya, I. A., Buneva, V. N., and Nevinsky, G. A. (2007) *Biochim. Biophys. Acta*, **1774**, 884-896.
89. Vlassov, A. V., Andrievskaya, O. A., Kanyshkova, T. G., Baranovskii, A. G., Naumov, V. A., Breusov, A. A., Giege, R., Buneva, V. N., and Nevinsky, G. A. (1997) *Biochemistry (Moscow)*, **62**, 474-479.
90. Vlassov, A. V., Helm, M., Naumov, V. A., Breusov, A. A., Buneva, V. N., Florentz, C., Giege, R., and Nevinsky, G. A. (1999) *Mol. Biol. (Moscow)*, **33**, 866-872.
91. Baranovskii, A. G., Ershova, N. A., Buneva, V. N., Kanyshkova, T. G., Mogelnitskii, A. S., Doronin, B. M., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2001) *Immunol. Lett.*, **76**, 163-167.
92. Jerne, N. K. (1974) *Ann. Immunol.*, **125**, 373-398.
93. Izadyar, L., Friboulet, A., Remy, M. H., Roseto, A., and Thomas, D. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 8876-8880.
94. Kolesnikov, A. V., Kozyr, A. V., Alexandrova, E. S., Koralewski, F., Demin, A. V., Titov, M. I., Avalle, B., Tramontano, A., Paul, S., Thomas, D., Gabibov, A. G., and Friboulet, A. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 13526-13531.
95. Hu, R., Xie, G. Y., Zhang, X., Guo, Z. Q., and Jin, S. (1998) *J. Biotechnol.*, **61**, 109-115.
96. Friboulet, A., Izadyar, L., Avalle, B., Roseto, A., and Thomas, D. (1994) *Appl. Biochem. Biotechnol.*, **47**, 229-237.
97. Debat, H., Avalle, B., Chose, O., Sarde, C.-O., Friboulet, A., and Thomas, D. (2001) *FASEB J.*, **15**, 815-822.
98. Hifumi, E., Morihara, F., Hatiuchi, K., Okuda, T., Nishizono, A., and Uda, T. (2008) *J. Biol. Chem.*, **283**, 899-907.
99. Bronshtein, I. B., Shuster, A. M., Gololobov, G. V., Gromova, I. I., Kvashuk, O. A., Belostotskaya, K. M., Alekberova, Z. S., Prokaeva, T. B., and Gabibov, A. G. (1992) *FEBS Lett.*, **314**, 259-263.
100. Crespeau, H., Laouar, A., and Rochu, D. (1994) *CR Acad. Sci.* III, **317**, 819-823.

101. Andrievskaya, O. A., Kanyshkova, T. G., Yamkovoi, V. I., Buneva, V. N., and Nevinsky, G. A. (1997) *Dokl. Ros. Akad. Nauk*, **355**, 401-403.
102. Krasnorutskii, M. A., Buneva, V. N., and Nevinsky, G. A. (2008) *Biochemistry (Moscow)*, **73**, 1242-1253.
103. Krasnorutskii, M. A., Buneva, V. N., and Nevinsky, G. A. (2008) *J. Mol. Recognit.*, **21**, 338-347.
104. Krasnorutskii, M. A., Buneva, V. N., and Nevinsky, G. A. (2008) *J. Mol. Recognit.*, **21**, 233-242.
105. Krasnorutskii, M. A., Buneva, V. N., and Nevinsky, G. A. (2008) *Int. Immunol.*, **20**, 1031-1040.
106. Raz, E., Ben-Bassat, H., Davidi, T., Shlomoz, Z., and Eilat, D. (1993) *Eur. J. Immunol.*, **23**, 383-390.
107. Kubota, T. (1977) *J. Med. Dent. Sci.*, **44**, 37-44.
108. Marion, T. N., Krishan, M. R., Desai, D. D., Jou, N. T., and Tillman, D. M. (1997) *Methods*, **11**, 3-113.
109. Mitsuhashi, S., Saito, R., Kurashige, S., and Yamashugi, N. (1978) *Moll. Cell. Biochem.*, **20**, 131-147.
110. Gottieb, A. A., and Shwartz, R. H. (1972) *Cell. Immunol.*, **5**, 341-362.
111. Nishi, Y. (2002) *J. Immunol. Meth.*, **269**, 213-233.
112. Tawfik, D. S., Chap, R., Green, B. S., Sela, M., and Eshhar, Z. (2002) *Proc. Natl. Acad. Sci. USA*, **92**, 2145-2149.
113. Ikehara, S., Kawamura, M., and Takao, F. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 8341-8344.
114. Ponomarenko, N. A., Durova, O. M., Vorobiev, I. I., Belogurov, A. A., Kurkova, I. N., Petrenko, A. G., Telegin, G. B., Suchkov, S. V., Kiselev, S. L., Lagarkova, M. A, Govorun, V. M., Serebryakova, M. V., Avalle, B., Tornatore, P., Karavanov, A., Morse, H. C., 3rd, Thomas, D., Friboulet, A., and Gabibov, A. G. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 281-286.
115. Zenkov, N. K., Lankin, V. Z., and Men'shchikova, E. B. (1993) *Oxidative Stress* [in Russian], MAIK Nauka/ Interperiodika, Moscow.
116. Tolmacheva, A. S., Zaksas, N. P., Buneva, V. N., Vasilenko, N. L., and Nevinsky, G. A. (2009) *J. Mol. Recognit.*, **22**, 26-37.