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Mini Review

Catalytic antibodies in healthy humans and patients with autoimmune and viral diseases

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Abstract

Antibodies have been first characterized as proteins produced by the immune system solely for binding other molecules, called antigens, with the goal of eliciting immune response. In this classical conception, antibodies act similarly to enzymes in specific binding to different molecules but cannot catalyze their chemical conversion. However, in 1986 the first monoclonal catalytic antibodies against a chemically stable analog of the transition state of a reaction were obtained and termed abzymes (Abzs). At present, artificial monoclonal Abzs catalyzing more than 100 distinct chemical reactions have been obtained. The discovery of IgG specifically hydrolyzing intestinal vasoactive peptide in the blood serum of asthma patients stimulated studies of natural Abzs. Numerous Abzs discovered afterwards in sera of patients with various autoimmune diseases, viral disorders, or in the milk of healthy mothers, are capable of hydrolyzing proteins, DNA, RNA, polysaccharides, or nucleotides, as well as to phosphorylate proteins and lipids. The phenomenon of catalysis by auto-Abzs is more and more in research focus. In this review we summarize new data on Abzs applications in basic science, medicine and biotechnology.

Keywords: abzymes - autoimmune and viral disorder - medicine - biotechnology

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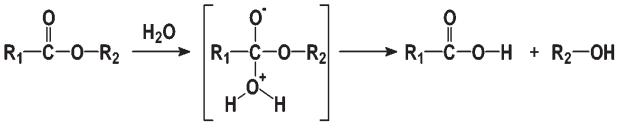
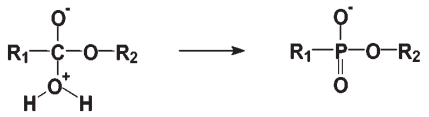
Design and potential of "artificial" abzymes

Conventional antibodies (Abs) were characterized as proteins involved in specific binding of other molecules called antigens. In his 1946 theory of enzyme function, Pauling first hypothesized that the active center of an enzyme closely matches the "strained configuration" of its substrate (in other words, the structure of the transition state) rather than the native conformation of the substrate molecule [1]. This idea led Jencks to propose in 1969 that Abs generated during anti-hapten immune response against chemically stable analogs of the transition-state of a reaction of interest could possibly display enzymatic activity [2]. The first anti-hapten rabbit polyclonal IgG capable of hydrolyzing *p*-nitrophenyl acetate was reported by Slobin in 1966 [3]. Later, several groups confirmed this Jencks' prediction. In 1985 a general method was first described for generating catalytic monoclonal abzymes (Abzs) against transition state analogs, together with a way to use those Abs to accelerate chemical reactions [4]. A year later two groups produced the first monoclonal Abs with catalytic properties, which were generated against hapten analogs of the transition states for *p*-nitrophenylphosphorylcholine [5] or monoaryl phosphonate esters [6]. In order to demonstrate main approaches to design transition state analogs the hydrolysis of esters can be used. In this reaction a carbon atom is bound to three other atoms (I) and is accompanied by the formation (after addition of water molecule) by an unstable transition state (II) characterized by the tetrahedral state of the carbon atom:



I II

To design of the chemically stable transition state analogs a phosphorus atom (III) is usually used for the modeling of tetrahedral carbon atom (II):



II III

Transition state *Analog of transition state*

Such catalytic Abs were termed abzymes (derived from **a**nti**b**ody en**zyme**). At present, artificial monoclonal abzymes (Abzs) catalyzing more than 100 distinct chemical reactions have been obtained.

In 1989, Paul et al. [7] discovered the first natural catalytic IgG specifically hydrolyzing intestinal vasoactive peptide. Numerous natural catalytic Abs were detected afterwards in serum of patients with several autoimmune (AI) and viral disorders, as well as in the milk of healthy women (see below). In this review, for the sake of simplicity and in order to distinguish natural catalytic Abs from those against transition-state analogs, the latter will be termed "artificial abzymes".

The field of artificial Abzs has been amply reviewed recently ([8-11] and refs therein). Artificial Abzs have been described that catalyze hydrolysis of amides and esters, reactions of cyclization, decarboxylation, lactonization, peroxidation, photochemical thymine dimer cleavage, bimolecular amide bond formation, and other reactions not catalyzed by known enzymes. A number of papers describe Abzs with other specific functions towards proteins, including formation of cyclic peptides, peptidyl-prolyl *cis-trans* isomerization during protein folding, and a novel enzymatic activity cleaving the bacterial protein HPr. Some Abzs have been described that require cofactors for activity.

The evolution of the artificial Abzs technology during the last two decades allowed rapid development of direct approaches for the generation of Abs with defined properties, as well as strategies to modify targeting specificity of individual Abzs. A change in antigen binding specificity can be achieved by genetic means, such as site-directed mutagenesis, genetic selection or screening (using phage display or similar approaches). Alternatively, modification can be introduced directly into purified Abs, *via* selective chemical modification of the Ab binding site. Both approaches demonstrated that the substrate specificity (and/or the specific activity) of some artificial Abzs is comparable to or even higher than that of enzymes with the same catalytic activity [10]. The mechanistic basis for the activity of such Abzs is becoming well understood.

Natural catalytic antibodies

As mentioned above, the first example of a natural Abz was an IgG (found in bronchial asthma patients)

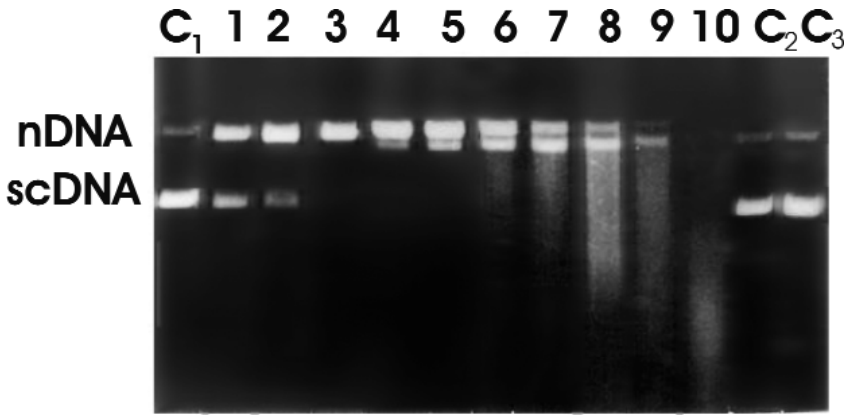


Fig. 1. DNase activities of catalytic IgGs from different AD patients in cleavage of supercoiled (sc) and nicked (n) pBR322 DNA: lanes 1-10 correspond to Abs from the sera of 10 different patients; lane C_1 - DNA incubated alone; lanes C_2 and C_3 - DNA incubated with Ab from the sera of two healthy donors. The estimation of the possible relative DNase activities of Abs from human milk and from the sera of patients with different ADs were carried out as described in [11, 14-16].

that hydrolyzes vasoactive intestinal peptide (VIP) [7]. This was followed by a discovery of IgG with DNase activity in systemic lupus erythematosus (SLE) [12], and an IgG with RNase activity, also in SLE [13]. IgGs and/or IgMs hydrolyzing RNA, DNA [12, 13, 17-19], peptides and proteins [7, 20-23], or polysaccharides [24] have been described in the sera of patients with AI pathologies including SLE, Hashimoto's thyroiditis (HT), polyarthritis, rheumatoid arthritis, multiple sclerosis (MS), lymphoproliferative diseases, polynephritis and malignant tumors, and with two viral diseases, viral hepatitis and AIDS (for a review see [11, 14–16]).

The relative activities of IgGs from the sera varied markedly from patient to patient [13-25]. Fig. 1 illustrates a cleavage of plasmid DNA by Abs from various patients after 2 h of incubation. One can see that in this period some Abs cause only single breaks in one strand of supercoiled DNA (lanes 1–3), whereas others cause multiple breaks and as a result the formation of linear DNA (lanes 4–6). The most active Abs hydrolyze DNA into short and medium length ODNs (lanes 7–10). It should be mentioned that Fig. 1 illustrates a range of possible changes of the relative DNase activities for patients with different ADs and viral disorders analyzed. When passing from one disease to another only the values of a relative percent of patients with low, middle and high DNase activities are usually changed.

We could not detect any nuclease activity in Abs from the sera of 50 healthy subjects or from the patients with influenza, pneumonia, tuberculosis, tonsillitis, duodenal ulcer, several types of cancer and other diseases not accompanied by an immune status upset [17–19]. Some healthy subjects possessed Abzs with low proteolytic and polysaccharide-hydrolyzing activities [16]. We also detected very low nuclease activity in Abs from a small percent of healthy donors, but these activities were usually at the lower limit of sensitivity of measurement [11, 14–16].

Following the initial discovery of anti-VIP Abzs [7], a number of natural catalytic Abs with diverse activities were detected in sera of patients with different AI disorders. Proteolytic IgG directed at thyroglobulin, a precursor of thyroid hormone, were found in the serum of patients with HT and SLE [20, 21]. Auto-Abs to prothrombin are associated with thromboembolism, but the mechanisms by which the Abs modulate the coagulation processes are not understood. A panel of 34 monoclonal Ab light chains isolated from patients with multiple myeloma was screened for prothrombinase activity [22]. Others have reported factor VIII-cleaving allo-Abs in the sera of patients with severe hemophilia [23].

Amidase and peptidase activities were found in IgGs from the sera of patients with rheumatoid arthritis; and DNase, amidolytic and peptidolytic activities, in Bence-Jones proteins from patients with multiple myeloma (reviewed in [11, 14–16]). Abs with amylolitic activity were found in the sera of multiple sclerosis patients [24].

Natural Abzs also occur in the milk of healthy human mothers and include sIgA and/or IgG possessing DNase and RNase [25–27], amylolitic [28] or ATPase activities [29], and protein kinase [30] and lipid kinase activities [31] which are the first examples of natural Abzs with a synthetic activity. Human

milk Abzs show specific activities significantly (5–50-fold) higher than those found in most ADs.

Since Abs can form complexes with other proteins, and Ab-mediated catalysis is sometimes characterized by relatively low reaction rates, it is important to prove that a catalytic activity of Ig fractions is not due to a contamination with conventional enzymes of the same specificity. Application of eight rigid criteria developed by Paul et al [7] in the first papers concerning natural Abzs allowed the authors of the initial study to conclude that the observed VIP-hydrolyzing activity is an intrinsic property of IgGs from the sera of asthma patients. Our studies of catalytic Abs have led us to add further criteria to the Paul's list, and now 15–17 very rigid criteria can be used for assessing Ab catalytic proficiency. Since purification is one of the most complicated aspects of Abz study, in several reviews we have summarized the methods for purifying and characterizing natural Abzs and described the problems of characterization of polyclonal natural Abzs [11, 14–16].

With a hindsight, the first natural Abz was in fact discovered in 1969 when Kulberg [32] found proteolytic activity in highly purified rabbit Abs, followed by catalytic IgGs from human serum and murine IgG-antigen complexes, which demonstrated superoxide dismutase activity [15]. However, in the absence of the rigid criteria, contaminating enzymes as the source of the catalytic activity could not be ruled out. Recently, these first findings of Abs catalyzing conversion of superoxide radical were confirmed using modern approaches [33]. It has been shown that mono- and polyclonal Abs from various sources, as well as their F(ab) fragments, have an intrinsic ability to intercept $^1O_2$ and efficiently reduce it to $O_2^{\bullet-}$, thus offering a mechanism by which oxygen can be reduced and recycled by phagocytes in their cytotoxic activity. Intact Igs and their fragments have been shown to catalyze formation of hydrogen peroxide. Later the same authors have unambiguously proven that hydrogen peroxide formation is a catalytic process and identified the electron source for a quasi-unlimited generation of H_2O_2 [34]. Abs produce up to 500 mole equivalents of H_2O_2 from singlet $^1O_2$*, without decrease in rate; metals or Cl^- were excluded as the electron source. From isotope incorporation experiments and kinetic data, Abs were proposed to use H_2O as an electron source, facilitating its addition to $^1O_2$* to form H_2O_3 as the first intermediate in a reaction cascade that eventually leads to H_2O_2. X-ray crystallographic studies with xenon, point to putative conserved oxygen binding sites within the Ab fold where this chemistry could be initiated. These findings suggest a protective function of Igs against $^1O_2$* and raise the question of whether the need to detoxify $^1O_2$* has played a decisive role in the evolution of the Ig fold.

Recently, it was shown that different $R^\bullet$ or $RO^\bullet$ radicals, including those generated from the saccharide containing Fe-XO/HX system, are efficiently scavenged by selenium-containing Abzs, which may be promising antioxidants [35]. Two types of selenium-containing monoclonal Abzs with glutathione peroxidase activity were developed, and Ab-dependent scavenge of $^\bullet OH$ was examined in the iron-containing xanthine oxidase/hypoxanthine system by a spin trap method. The hydroxyl radical-scavenging proficiency of these two Se-Abzs surpassed the level of native glutathione peroxidase; M1c8 Abz showed the greatest $^\bullet OH$-scavenging effect.

Interaction of human serum IgG (subclasses G1, G2, G4) with different metal ions (Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, and Cr^{3+}) using metal-chelating adsorbent was also investigated, and the IgG-Cu^{2+} complex-dependent hydrolysis of H_2O_2 was demonstrated [36].

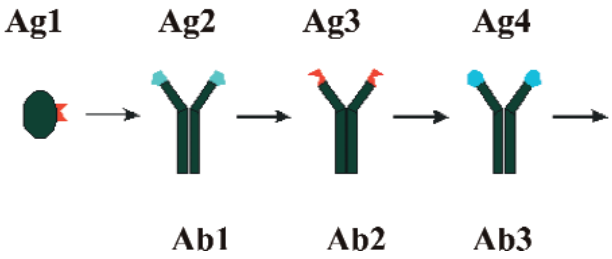
The idea that Abzs are involved in natural immunity arose after the discovery of Abzs, which specifically hydrolyze VIP [7], currently the best-studied natural Abzs whose mechanism and functional roles have been reviewed [16]. Other recently reviewed relevant issues include comparisons of artificial *vs* natural Abzs and *in vitro vs in vivo* Abz generation and analytical approaches [11, 14–16].

The origin of natural autoimmune abzymes

In order to analyze possible reasons for the production of natural Abzs, the following data should be considered. A special feature of autoimmune diseases (ADs) is high concentrations of auto-Abs (Abs to endogenous antigens; for a review see [11, 14–16]). In the case of bronchial asthma, the target of auto-Abs is VIP, which is widely distributed in

the central and peripheral nervous systems. Autoimmune thyroiditis is characterized by increased concentrations of Abs to thyroglobulin, the microsomal fraction of thyrocytes, and to some other antigens. Yet another example is multiple sclerosis, a chronic degenerative demyelinating disease of the central nervous system. Its etiology remains unclear, and the theory of pathogenesis is mainly focused on the degradation of myelin (the protein-lipid sheath of axons) that stems from AI inflammatory processes. Increased levels of Ig and free light chains were observed in the brain tissue and cerebrospinal fluid of MS patients. As in the case of some other ADs, polyspecific DNA-binding Abs interacting with phospholipids have also been recognized in MS patients [37]. Although viral diseases are not related to ADs, some of them, like acquired immune deficiency syndrome and viral hepatitis, greatly affect the immune status of patients. Progressing viral hepatitis and AIDS are accompanied by AI humoral and cellular reactions; similar to ADs, tissue-specific and organ-nonspecific Abs were found in blood of hepatitis patients. Different ADs are characterized by high concentrations of different specific auto-Abs, but increased concentrations of DNA and anti-DNA Abs were found in blood of patients with many ADs: SLE, AI hepatitis, Graves disease, polymyositis, MS, Sjogren's syndrome, HT, as well as with lymphoproliferative and some viral diseases hepatitis and AIDS (review in [11, 15, 16]). Concentrations of DNA and anti-DNA antibodies were especially high in patients with SLE.

Development of different ADs is characterized by spontaneous generation of primary Abs against proteins, nucleic acids and their complexes; then secondary Abs to the primary ones are generated etc. [38, 39]. The origin of natural Abzs is complex. In some cases, they may be directed against transition states analogs of catalytic reactions, or even against substrates *per se*, acting as haptens. For example, natural Abzs hydrolyzing VIP, thyroglobulin, thrombin and factor VIII are Abs against these proteins [7, 20-23]. On the other hand, anti-idiotypic Abs can be induced in ADs by a primary antigen and may show some of its characteristics including catalytic activity. It is believed that Abzs are associated with autoimmunization and that auto-Abs are most probably of anti-idiotypic nature. If the antigen triggering this anti-idiotypic chain is the active site of an enzyme, it is logical to suggest that the secondary anti-idiotypic Abs may resemble the protein's structure, a part of which represents a "mould" of the enzyme's active site and the possibility of Abz induction can be explained by Jerne's anti-idiotypic network [40]:



Consequently, these Abs might possess some properties of this enzyme. The remarkable property of idiotypic mimicry has been exploited to develop anti-idiotypic Abzs; monoclonal Abs were selected by immunizing mice with a monoclonal Ab against the active center of acetylcholinesterase, and a monoclonal anti-idiotypic Ab against carboxypeptidase showed catalytic activity similar to the original antigen [41, 42]. Anti-idiotypic DNase Abs were obtained by immunization of rabbits with DNAse I [43].

Human milk contains IgG, IgM, IgA and sIgA, of which sIgA is the major component (>85–90%) [11]. During pregnancy and immediately after delivery (at the beginning of lactation), women very often experience AI processes similar to those in AD patients. The pregnancy can trigger an appearance of manifestations of different ADs in previously healthy women [44]. A sharp exacerbation of ADs can occur immediately after delivery. Independently of AI status during pregnancy, several AI pathologies may occur postnatally, including SLE, HT, phospholipids syndrome, polymiositis, AI myocarditis etc. Intravenous or peroral administration of antigens (mainly proteinaceous) to animals no more than 1-3 months before giving birth triggers production of the antigen-specific Abs, which then may be detected in high concentration in the milk [11, 14–16, 44]. This means that, in contrast to the rest of the population, pregnant women may be efficiently immunized by viral or bacterial components upon contact with them. Moreover, secretion of Abs in milk 1–3 months after immunization is consistent with the existence of specific "immuno-

memory" in pregnant females. In addition, apoptosis of several types of cells during the last trimester have been demonstrated, as well as the presence of the embryo's cells at low levels in the mother's blood (reviewed in [11, 14–16, 44]). Taken together, these data suggest that during pregnancy women are subject to immunization with many components, including those of anti-idiotypic nature, as in ADs.

As noted above, IgGs and IgMs from the sera of patients with many ADs or viral diseases, as well as Abs from human milk, possess DNase and RNase activities, raising the question of whether the same Ab molecule can have both activities, although RNases usually cannot hydrolyze DNA and *vice versa*. In addition, it is not clear whether Abs against RNA and anti-idiotypic Igs to the RNase active center can hydrolyze DNA, and, *vice versa* whether Abs against DNA and anti-idiotypic Igs to DNase active center can hydrolyze RNA.

When an antigen stimulates B-lymphocytes, Abs are generated, and one antigen may generate up to 10^8 different Ab molecules, a number that may further increase by somatic mutagenesis [9]. Therefore, it seems feasible that different DNA- and RNA-binding non-catalytic Igs, as well as Abs with DNAse and/or RNase activities can be synthesized in the course of immune response, either directed against the substrate or as anti-idiotypic Abs to enzymes hydrolyzing nucleic acids. To address this hypothesis, we have recently immunized rabbits with DNase I, DNase II, pancreatic RNase A, or complexes of DNA or RNA with methylated BSA. Interestingly, in all cases we observed production of Abzs efficiently hydrolyzing DNA, and in each case there were many subfractions of catalytically active Abs with different affinities for DNA (Krasnorutskii, private communication). These data, together with high polyclonality of DNase and RNase Abzs from AI patients, allow us to conclude that a polyclonal Abz pool can contain different types of Abs: Abs to DNA and RNA and anti-idiotypic Igs to DNases and RNases. Additional pathways of Abz generation may exist in AI patients; for example Bronshtein *et al.* [45] suggested that some DNase Abzs of SLE patients are anti-idiotypic Abs to topoisomerase I. Immunization by other enzymes, their complexes with nucleic acids or with other ligands including allosteric enzyme regulators, can likely result in generation of NA-hydrolyzing Abs. Some antigens change conformation upon their association with other proteins, and their structure in such complexes could mimic that of the transition state of a reaction involving the antigen.

Some published data confirm the idea that AI patients may contain an extremely wide repertoire of Abzs of different nature. In our experience, Abs with exclusively DNase or RNase activity could not be efficiently separated by chromatography, except small subfractions of MS IgG that possessed only one activity and were sometimes possible to purify by ion-exchange HPLC [19]. Otherwise, the same electrophoretically homogeneous preparations of catalytic IgGs, IgMs, and sIgAs purified by several chromatographic steps including affinity chromatography on DNA- or RNA-cellulose, always possessed both RNase and DNase activities in the main subfractions [19]. Since monoclonal murine SLE IgGs against specific DNA sequences show both DNase and RNase activities [46], it is likely that in subfractions of polyclonal IgG, IgM, and sIgA both activities can also reside in the same protein. This idea is further supported by our finding that ribo-oligonucleotides inhibit hydrolysis of DNA by Absz and *vice versa* [15, 16]. The RNase activity of IgG and IgM from patients is often 5–400 times higher than their DNase activity, but each activity is different for individual patients or milk donors. DNase and RNase in Abs from patients with different diseases or milk of different donors are correlated [13-19, 26, 27], and recently we obtained a correlation coefficient of 0.75 for IgGs from 90 MS patients (Ershova, Garmashova *et al.*, unpublished data).

As mentioned above, DNase Abs could also be anti-idiotypic Igs to different DNases, but our recent data show that <10% of the total DNase activity of purified IgG from AI patients binds to immobilized DNase I or DNase II (Krasnorutskii, private communication). At the same time, SLE and MS patients demonstrated poor correlation between the relative levels of anti-DNA Abs and DNase Abzs; the major fraction of Abs with high affinity for DNA is usually not catalytically active, whereas the total Abz DNase can be very high in patients with a low concentration of anti-DNA Abs (Ershova, Garmashova *et al.*, unpublished data).

Possible ratio of the Abzs raised directly to specific substrate conformations to the anti-idiotypic Abzs can obviously depend on many factors includ-

ing individual features of the patients' immune systems, type of their AI disorders, and disease stage. Overall, it was shown that patients may bear either a relatively small or an extremely large pool of polyclonal DNA- and RNA-hydrolyzing Abs, which differ in the relative amounts of light chains of α-and λ-types, optimal pH values, net charge, magnesium ion requirements, and substrate specificitiy. Depending on the individual patient and specific disease, the apparent K_m and V_{max} values for RNA substrates, and the substrate specificity of Abs, may either be quite similar or cover a wide range [13–19, 26, 27, 47–51].

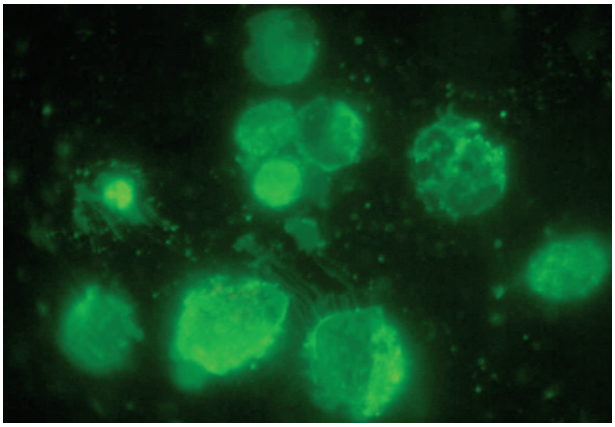


Fig. 2. Apoptosis of HL-60 human cells treated with 1 μM IgG fraction from the sera of MS patient for 16 h revealed by Annexin-V-staining.

Biological role of abzymes

Abzs have been studied primarily in the context of AI diseases where their biological role remains unknown: do they have a function or represent a dysfunction? One cannot exclude that some Abzs may be beneficial, while others may inflict harm (for review see [11, 14-16]). It has been suggested that respiratory tract dysfunction in bronchial asthma may stem from the protease activity of auto-Abzs, resulting in a deficit of VIP, which plays a major role in the pathophysiology. In HT, Abzs hydrolyzing thyroglobulin have been considered a positive factor, since they could minimize AI responses to thyroglobulin and prevent formation of immunoprecipitates [20-21]. On the other hand, anti-VIP Abs are cytotoxic, and mice immunized with the antipeptide IgGs from human sera develop asthma [16]. Abzs hydrolyzing DNA or RNA could at first glance be considered as non-specific side-products of the AI process since they occur in the sera of patients with many AI and viral diseases, but DNase Abzs from SLE [52] and MS patients [16] are as cytotoxic as tumour necrosis factor, which induces cell death by apoptosis. Abs induce internucleosomal DNA fragmentation, a characteristic of apoptotic cell death. Fig. 2 demonstrates morphological changes typical for apoptosis after of HL-60 human cells treated with 1 μM DNase IgGs from the sera of one MS patient.

Some Abzs of healthy donors may also play important role in humans. For example, Abs generating H_2O_2 from singlet oxygen may participate in Ab-mediated cell killing by production of hydrogen peroxide triggered by the immunogenic event [33, 34]. Alternatively, Abs may function in defending the organism against oxygen radicals.

As mentioned above, during last months of pregnancy the "immuno-memory" of the mother "collects information" about inside and outside compounds that can harm infants, and produce Abs to these compounds after beginning of the lactation. We have suggested that DNA-ase and RNA-ase activity Abs from human milk are not only capable of neutralizing viral and bacterial nucleic acids by complexation with them, but also can hydrolyze them; existence of DNA-ase activity in Abs raises the possibility that these Abzs may protect newborns by hydrolysis nucleic acids of infectious agents [26, 27].

While a possible biological role of some Abzs is clear, there are other abzymes whose possible biological roles are difficult to imagine. For example, Abs from human milk phosphorylate more than a dozen different proteins, lipids and polysaccharides [30]. Many proteins change their function after phosphorylation; enzymatic phosphorylation of proteins plays a fundamental role in the regulation of key physiological processes. A dual role of different lipids in cell signalling is now recognized; they can act as intracellular or extracellular signalling molecules. Lipids may regulate heart rate, oxidative bursts, or platelet activation [reviewed in [16]).

We found that a small fraction of sIgA from milk of healthy mothers phosphorylates tightly bound

unusual minor lipids. Similarly to well-known gangliosides GM1, GM3, and GD3, two of these minor lipids contain neuraminic acid moieties. However, in contrast to the gangliosides containing only one or two fatty acid residues, minor lipids have four to five such residues, which are resistant to periodate [31]. Therefore, these minor lipids cannot be identified as any known neuraminic acid-containing lipids. In addition, Abs from human milk phosphorylate polysaccharides of unusual structure. Since human milk provides the breastfed child not only with nutrients but also with passive immunity and the components necessary for protection from aggressive environment, the phosphorylation of proteins and unusual lipids and polysaccharides by human milk Abzs may have biological sense.

Application of natural abzymes

Chemical and enzymatic probes to cleave RNA at specific sequences or structural motifs are crucial tools for studying RNA structure. Although a number of such probes are presently available, the arsenal of these tools has to be expanded to detect structural motifs in RNA molecules in solution ([47], and refs therein). The great variety of RNase Abzs existing in patients with different diseases offers a good source for novel RNase reagents. Nucleases are specific either for sequences (*e.g.*, RNase T1 is specific for guanosines, and RNase A, for Poly-A sequences) or for structural features (*e.g.*, nuclease S1 cleaves exclusively single-stranded domains of RNA). As noted above, Abzs of AI patients show novel RNase activities, including some stimulated by Mg^{2+}, which are not sequence-specific but sensitive to folding changes, as evident from the work with structurally well-characterized tRNA substrates [47–49]. An interesting example of the utility of such RNases is provided by a study in which SLE Abzs were used to cleave two $tRNA^{Lys}$ molecules, one from normal human mitochondria, the other a mutant found in serious neuromuscular disorder, myoclonic epilepsy with ragged-red fibers. This mutation, an A→G transition position, is believed to affect the $tRNA^{Lys}$ structure and cause the functional disorder. RNase A and other RNases used for probing structure showed no difference in their cleavage patterns, but RNase Abzs in the presence of Mg^{2+} revealed different patterns of cleavage sites; the mutant tRNA had significantly different sensitivity in the mutated region, and cleavage also occurred at new positions indicating local structural or conformational changes.

Polyclonal Abzs usually contain a major subfraction characteristic of each disorder and directed against a disease-specific antigen, as seen by their specificity, kinetics and optimal conditions. However, RNase, DNase and amylase Abzs from patients with the same autoimmune disease can demonstrate profound diversity in catalytic parameters [47–51].

Most Mg^{2+}-dependent Abzs from AI and viral disorders display no sequence specificity but are rather sensitive to structural features of tRNAs specific for Phe, Lys, Asp, and Gln [47–49]. Abzs from some patients demonstrate general RNase A-type specificity with minor differences (preference for CpA and UpA sequences), while others contain a major subfraction possessing an RNase T1-type specificity. Mg^{2+}-stimulated IgG-associated RNase possesses in most cases a cobra venom RNase V1-like activity on $tRNA^{Phe}$ with a unique Mg^{2+}-activated specificity for double-stranded regions. Abzs from SLE and other ADs show specificities quite different from those of RNase V1 in spite of some similarities, and the observed specificity differs remarkably from patient to patient. Thus, Abzs can discriminate between sequences and subtle or large structural changes, including stability and folding; they may become tools for investigating RNA structures in solution, but since their specificities are multiple, their further applications will require the development of monoclonal Abzs [15, 16].

We have found a correlation of Abz activities with the progress or remission of human ADs and with biochemical and immunological indices that characterize these diseases [43]. Further, a comparison of the substrate specificity of Abzs, for example those with RNase activity, allows us to distinguish different types of ADs [11, 14–16]. In contrast to auto-Abs, the titers of which are usually very low at the early stages of ADs, catalytic Abzs can be easily detected at initial stages of these diseases. For example, the serum of ~90% of MS patients contains DNase Abzs, while only 17% of these patients are characterized by increased titers of anti-DNA Abs [19].

Abs are believed to play a significant role in pathogenesis of HT. IgG from 65% of patients with

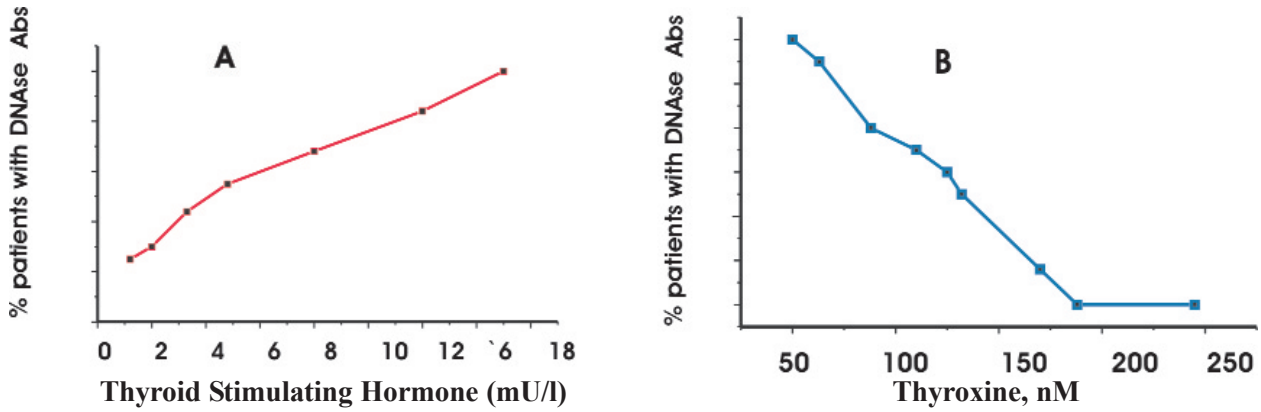


Fig. 3. Dependence of the relative number of HT patients containing DNA-hydrolyzing activity (%) upon the patient's serum concentration of TSH (A), or of thyroxine (B). Most HT patients (83 of 120) can be placed in groups demonstrating comparable concentration of TSH or thyroxine (each group contains 6–16 patients); % value is expressed as the % of patients with Abzs out of all patients in each group demonstrating a comparable concentration of one of the hormones. The details of the analysis are given in [53].

HT possessed DNase and RNase activities, and to correlate these with biochemical and immunological indices we compared them with thyroid hormone status [53]. We found a linear relationships between the proportion of patients having Abzs and an increase of TSH, and between the percent of such patients with a decreased concentration of thyroid hormones (Fig. 3).

All patients with an increase of TSH and/or a very low concentration of thyroxine, which characterize typical hypothyroidism, contained serum Abzs and showed a correlation of Abz activity with thyroid gland damage, while patients at the initial stage or during remission of HT had no or reduced Abz activity [53]. The specific activity of DNase IgGs increased with the relative amount of anti-thyroglobulin Abs and therefore provides a good indicator of progress of AD.

The widely used therapy of HT patients with thyroxine led to an increase of blood hormone concentration but not of Abz levels or AI status, but the immunosuppressive drug plaquenil significantly reduced the DNase activity of Abs correlated with an increase of thyroid hormone concentrations (Fig. 4), elevation of thyroid activity, and improvement of the patient's clinical state without additional hormone therapy [53].

Thus, Abzs may serve as good proxies for alteration of autoimmune processes in different ADs, and could be used in diagnostics of AI diseases as well as for estimation of an efficiency of different drugs [11, 14–16]. The studies of Abzs reveal the ability of the immune system to produce an extremely wide spectrum of Abs possessing different enzymatic activities, which often are not comparable with those of known enzymes. Natural Abs with specified and novel functions have a wide potential for biotechnological and medical applications.

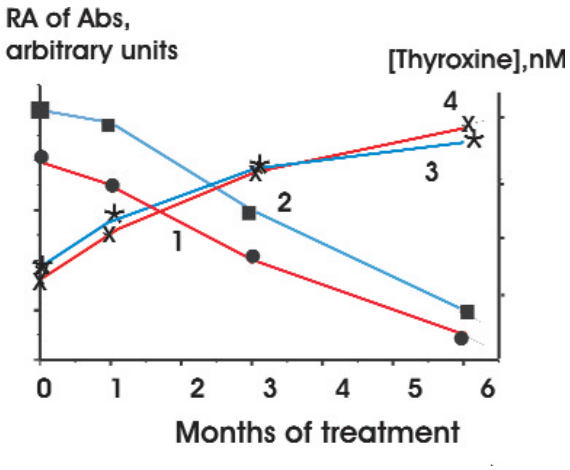


Fig. 4. Time dependencies of the decrease of relative DNase activity of IgG and of increase of thyroxine concentration of two patients (different color of the curves) treated with plaquenil. The relative activity of Abs is expressed in arbitrary units (arbitrary units are equal to the number of the lanes, which are given on Fig. 1).

Conclusion

A growing body of data suggests that catalytic Abzs may be important mediators of immunological defense, regulation, and autoimmune dysfunction. Estimate of the degree to which abzymes contribute to these biological phenomena will require continuing studies of Ab-mediated catalysis following experimental immunization and in AD, as well as mechanistic investigation of catalysis by Abs and their subunits. Highly important from a technological point of view are further studies of artificial and natural Abzs with the goal of understanding their structure-function relationships, to enable production of tailor-made catalysts of potential therapeutic use. Because the catalytic activity of certain Abs is an innate function catalysts specific to virtually any target polypeptide could in principle be developed and then improved by *in vitro* or *in vivo* affinity selection. The increasing number of available X-ray structures of catalytic Abs shows the multiplicity of solutions to the problem of catalysis of an enzyme-like reactions by Abs. These strategies may employ amino acid arrangements analogous to those in enzymes, but also arrangements completely different from those selected in enzymes by natural evolution. Anti-idiotype approaches may enable Abs to mimic many useful enzymes [11, 14–16], enhancing possible functions of Abzs. The phenomenon of abzyme catalysis can potentially be applied to isolate efficient catalysts suitable for passive immunotherapy of major diseases. For example, cocaine-hydrolyzing Abzs have been developed, and may provide a novel approach to the problems of drug addiction [54]. Abzymes that cleave the gp120 protein of HIV may be of use in the treatment of AIDS [55]. Rational design of abzymes with specified and novel catalytic functions allowing the selective cleavage of surface proteins holds a great promise for an unprecedented level of control over *in vivo* protein-protein associations.

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