

Novel Functional Activities of Anti-DNA Autoantibodies from Sera of Patients with Lymphoproliferative and Autoimmune Diseases

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ABSTRACT

DNA-hydrolyzing activity of IgG autoantibodies from sera of patients with various types of lymphoproliferative diseases was investigated. The association of DNA-hydrolyzing activity with the antibody (Ab) fraction has been proved by newly developed affinity-capture assay. Study of abzyme incidence in blood tumors and systemic lupus erythematosus (SLE) revealed linkage of anti-DNA Ab catalysts to mature B-cell tumors, and increased probability of DNA-abzymes formation on the background of autoimmune manifestations. These data suggest possible similarity between mechanisms of abzyme formation in SLE and B-cell

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lymphomas. A new mechanism of formation of DNA-specific catalytic Abs has been proposed based on the increased crossreactivity of polyclonal DNA-abzymes to DNA-depleted nuclear matrix proteins. The possibility of the abzyme production as Ab to the energetically destabilized ground state of the antigen has been discussed. Preliminary results were obtained that indicate the complement-independent cytotoxicity of anti-DNA autoantibodies isolated from blood of patients with SLE and chronic lymphocytic leukemia.

Index Entries: DNA-hydrolyzing antibodies; lymphoproliferative diseases; chronic lymphocytic leukemia; affinity-capture assay; autoantibody cytotoxicity; DNA-protein complexes; nuclear matrix.

INTRODUCTION

Two types of natural catalytic antibodies (Abs) were found to be associated with autoimmune pathologies: peptidase-like activity of immunoglobulin G (IgG) from patients with asthma (1) and Hashimoto's thyroiditis (2), and DNase-like activity of IgG from the systemic lupus erythematosus (SLE) and acquired immune deficiency syndrome (AIDS) (3,4). Anti-DNA autoantibodies existing in the blood of patients with SLE are heterogeneous and display crossreactivity with various proteins (5). The participation of anti-DNA autoantibodies in pathological manifestations is well documented (*see ref. 6 for recent review*), yet the complete spectrum of Ab-mediated tissue injury remains to be understood.

A number of lymphoproliferative diseases display autoimmune manifestations (hemolytic anemia, immune thrombocytopenia, glomerulonephritis, dermatitis, and so on). B-cell chronic lymphocytic leukemia (CLL), characterized by a high level of autoantibody production and a significant proportion of anti-DNA Abs (7,8), could serve as a new model for investigation of DNA-abzyme activity. Moreover, recent data on peptide-hydrolyzing activity of Abs from lymphoproliferative diseases (9) and autoimmune disease (2) strongly support the assumption that the mechanisms of spontaneous abzyme formation are similar in both types of the diseases. An earlier article (10) mentioned the phenomenon of Ab-associated DNA-hydrolyzing activity in sera of patients with lymphoproliferative diseases. Here is presented the data on distribution of the DNA-abzymes in various types of lymphoproliferative diseases, compared with their frequency in SLE. Also introduced is a rapid and efficient assay for Ab DNA-hydrolyzing activity that employs the use of streptavidin-coated paramagnetic particles, and that is convenient for large-scale screening of serum samples.

The mechanism of induction of DNA-specific catalytic Abs was previously hypothesized (11), but precise events leading to formation of these catalysts require further investigation. It cannot be excluded that multiple types of the antigen (Ag)–Ab interfaces are favorable for induction of the Ab catalysts. It was assumed that natural DNA-abzymes can rise as autoantibodies to DNA–protein complexes with stabilized high-energy state of DNA molecule (12), such as DNA associated with nuclear matrix proteins. This article presents some preliminary data in favor of this hypothesis.

The pathological role of DNA-abzymes is even more difficult to estimate, considering the fact that DNA-cleaving activity of Ab is likely to be harmful only upon direct interaction of the abzyme with nuclear DNA. Recent progress in the investigation of the mechanism of penetration of anti-DNA Abs into living cells (13) allows one to speculate that cleavage of chromosomal DNA by DNA-abzymes might contribute to the pathological processes in autoimmunity. Furthermore, frequent crossreactivity of anti-DNA autoantibodies with protein Ags, especially with membrane receptors (14,15) and nuclear proteins (16), has prompted analysis of whether direct interaction of DNA-specific Abs with cells can cause any cell damage without the involvement of complement or cytotoxic T-lymphocytes. This article presents an analysis of autoantibody-mediated cytotoxicity toward cultured tumor cells.

MATERIALS AND METHODS

Materials

Blood samples of patients suffering from lymphoproliferative and autoimmune diseases, and those of healthy donors, were provided by the physicians of the Russian Hematological Center, the Institute of Rheumatology, and the Moscow Regional Research Institute of Clinical Investigations. Salts and chemicals were from Sigma (St. Louis, MO) and Merck (Rahway, NJ). Chromatography equipment and sorbents were from Pharmacia (Uppsala, Sweden). Cell culture materials were from Life Technologies (Gaithersburg, MD).

Purification of IgG and Determination of DNA-Hydrolyzing Activity

Abs were precipitated from serum with ammonium sulfate (40%), and further steps of purification were conducted as described by Kozyr (10). The homogeneity of the final preparation was checked by silver-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with mouse antihuman IgG Abs (Sigma)

(data not shown). DNA-hydrolyzing activity was assayed by monitoring conversion of supercoiled plasmid DNA into circular or linear form, as previously described (3,10). Complete conversion of supercoiled plasmid DNA into circular form after 10 h of incubation with Abs in buffer, containing 20 mM Tris-HCl, 50 mM NaCl, and 10 mM MgCl₂, was considered as 1 unit of DNA-hydrolyzing activity.

Affinity-Capture Assay

One unit of DNA-hydrolyzing Abs isolated by the authors' standard technique (10), was brought to pH 2.5 with acetic acid (Merck, Germany), to exclude incubated for 30 min, then the pH of the Ab solution was brought to neutral by addition of a premeasured amount of Tris base, the Ab solution was diluted to the concentration of approx 20 ng/ μ L in binding buffer (20 mM Tris-HCl, pH 7.5; 50 mM NaCl), and was immediately mixed with 10-fold molar excess of biotinylated mouse antihuman IgG Abs in sterile binding buffer (calculated considering total amount of polyclonal Abs in the sample). Prior to the experiments, the biotinylated Abs were purified on the human IgG-Sepharose column in order to remove any traces of nuclease activity (3). The final volume was adjusted to 50 μ L and the reaction mixture was incubated at 4°C for 5 h with gentle shaking. Streptavidin-coated paramagnetic beads (Dynal, Oslo, Norway) were equilibrated with binding buffer following the protocol recommended by the supplier, and added to the reaction mixture, so that the immobilized streptavidin was roughly in twofold molar excess over the biotinylated Ab. Incubation was continued for 1 h in the same conditions. The beads with bound IgG-biotinylated antihuman IgG Ab complexes were removed from the solution using a magnetic separation stand, and washed thrice with sterile binding buffer. The final wash was assayed for the presence of DNA-hydrolyzing activity. No activity was observed.

The Ab complexes attached to the beads were further treated with 100 μ L glycine-HCl, pH 2.6, in order to disrupt Ab-Ab interaction and to detach DNA-abzymes. After treatment, the beads were removed by magnetic separation, the pH of the solution containing detached Abs was immediately brought to 7.5 with 1 M Tris base, and the final preparation was checked for DNA-hydrolyzing activity. Similar purifications were performed using protein A-coated Sepharose 6MB beads (Pharmacia), instead of paramagnetic particles.

Cytotoxicity Assay

L929 tumor cell line (mouse fibroblasts) obtained from American Type Culture Collection was cultured in RPMI 1640 media (Life Technologies) containing 0.002 M L-glutamine, 10 mg/mL gentamicin, and 10% heat-inactivated fetal bovine serum (Life Technologies). Antibodies were iso-

lated using the authors standard procedure (10), and the final Ab preparations were dialyzed twice against 2000 vol of phosphate buffered saline.

All experiments were conducted in RPMI 1640 medium with all aforementioned supplements, but without serum. The samples contained 3×10^4 target cells in 100 μ L RPMI 1640 and Abs at concentrations ranging from 10^{-6} to 10^{-12} M, taken at the order of magnitude increments. Trypan blue exclusion was used to calculate the percent of dead cells after incubation as, described in Sashchenko et al. (17). Each dilution was tested using eight replicate wells. Each experiment was repeated 5 \times and the average deviation in the percent of dead cells was determined between separate wells and further between the repeats of the experiment.

Mock reactions were performed in 100 μ L RPMI 1640 medium in the absence of Abs. Incubation time varied from 3 to 48 h, depending on the purpose of the experiment.

Preparation of Affinity Support Harboring Immobilized Nuclear Matrix Proteins

Nuclear matrix was isolated by the method described by Belgrader et al. (18). Resulting preparation was treated with 3% SDS to dissociate nuclear matrix proteins from DNA. The dissociated preparation was dialyzed against coupling buffer containing 0.5 M Na_2CO_3 adjusted to pH 8.2 by CH_3COOH , 1 M NaCl, and 3% SDS, to remove any traces of Tris and primary amines. Dialyzed preparation was mixed with activated CH-Sepharose 4B (Pharmacia), so that the reactive groups of the CH-Sepharose were in approximately three-fold excess to the amino groups of the protein. After 1 h incubation in coupling buffer, the reaction was terminated by addition of 50 mM ethanolamine, and the affinity support was packed into the column. DNA remains uncoupled because *N*-hydroxysuccinimide esters are not strong enough acylating agents to react with aromatic amines of cytidine and adenine in the provided conditions, and can be easily removed from the packed support with excess of coupling buffer.

At the next step, SDS was removed from the column by washing with gradually decreasing concentrations of the detergent and finally, by washing with 200 column volumes of SDS-free coupling buffer. The column was then equilibrated with the buffer containing 20 mM Tris-HCl, pH 7.6, and 50 mM NaCl. The degree of immobilization of nuclear matrix proteins was assayed by SDS-PAGE, followed by Coomassie brilliant blue staining. Initial nuclear matrix preparation was compared with the column flowthrough. More than 90% of protein appeared to be immobilized. The same samples were electrophoresed in Tris-Borate-EDTA (TBE) buffer and the gel was stained with ethidium bromide. The amounts of DNA in the initial nuclear matrix preparation and the unconjugated fraction were

equivalent. To analyze whether any detectable amount of DNA remained attached to the solid phase as DNA-protein complexes, 50 μ L affinity support was radiolabeled by Klenow fragment (Promega, Madison, WI), according to the standard procedure (19). The mock reaction contained the same components, except the enzyme. Both reactions were washed free from unincorporated label and counted using a scintillation counter (Interteknique, France). No differences were observed between the two samples, thus indicating that virtually no DNA remained attached to the coupled protein.

Purified anti-DNA Abs from autoimmune sera (10) were applied to the nuclear matrix protein-Sepharose (NMP-Sepharose) HR 5/2 FPLC column in buffer containing 20 mM Tris-HCl, pH 7.6, and 50 mM NaCl. Matrix-bound fraction was eluted by 100 mM glycine-HCl, pH 2.6, with following neutralization by 1 M Tris-HCl, pH 9.0. All solutions were autoclaved in order to eliminate any residual nuclease activity. DNA-hydrolyzing activity of bound and unbound fractions, as well as of original purified Abs was determined as described in Schuster et al. (3) and Kozyr (10).

RESULTS AND DISCUSSION

Affinity-Capture Assay and Distribution of DNA-Hydrolyzing Antibodies in Sera of Patients with Lymphoproliferative Diseases and Systemic Lupus Erythematosus

The activity of spontaneously occurring abzymes is relatively low. Similarly low is the proportion of catalytic Abs among total immunoglobulins isolated from sera. Usually, the association of enzymatic activity with Abs is assessed by several different techniques in order to exclude the possibility of enzymatic contamination. Most widely applied include gel filtration in acid shock conditions (1 M acetic acid) (3), guanidine chloride denaturation with further refolding (9), and comparison of DNA-abzyme patterns of DNA hydrolysis with those of nucleases (20). The use of the aforementioned techniques has been applied as the standard in the large-scale isolation of DNA-abzymes. Ab structure is generally resistant to acidic pH, but treatment of abzymes under more harsh conditions, such as strong denaturants, may cause disruption of the active center. Patterns of DNA hydrolysis would probably differ in different preparations because of the diversity of polyclonal abzyme species. Multistep purification and assay procedures employed for isolation of abzymes are too cumbersome to be viable for analysis of multiple serum samples necessary to study the clinical relevance of catalytic Abs and for analysis of the correlation between abzyme incidence and disease type, stage, and manifestations. In addition to SLE, in which anti-DNA autoantibodies appear to be a hallmark of the

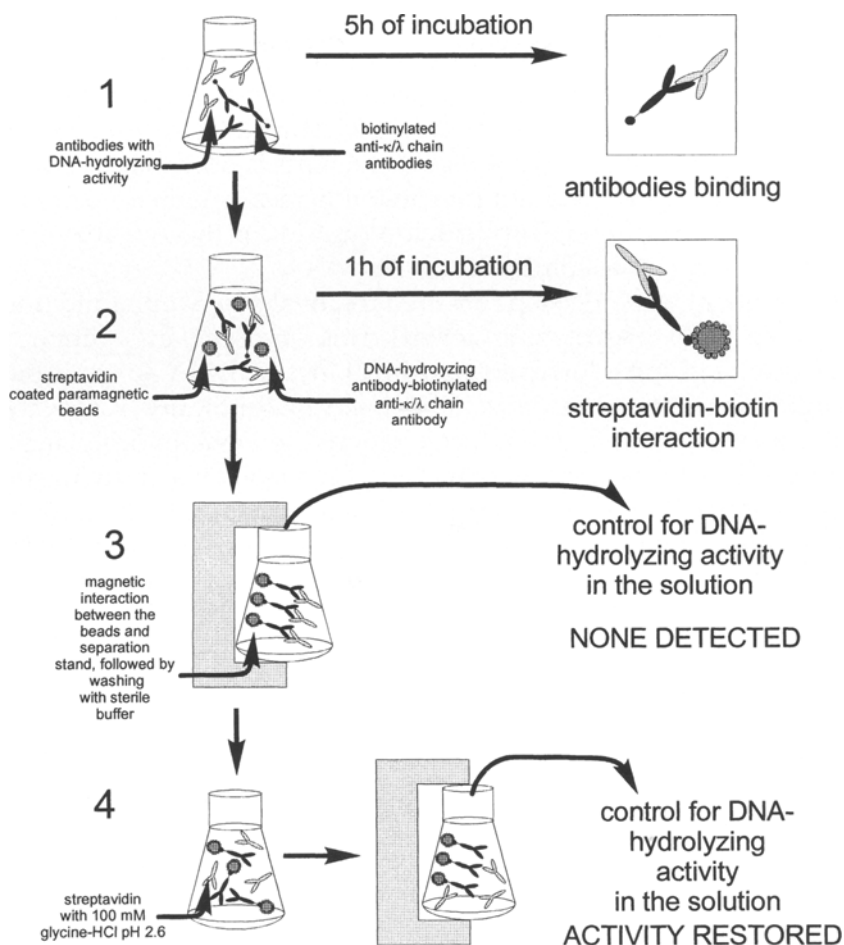


Fig. 1. Scheme of the affinity-capture assay (details provided in Materials and Methods section).

disease, the authors demonstrated the presence of DNA-abzymes in blood of patients with (CLL) (4,10). Autoimmune disorders are frequently manifested at the late stages of lymphoproliferative diseases (21). It is therefore important to understand which types of leukemic disease manifest DNA-specific abzymes. This would be helpful both for determination of the pathological role of these catalysts and for understanding of the disease background under which catalytic autoantibodies can occur.

A method was sought that provides fast and accurate attribution of catalytic activity detected in multiple Ab preparations. This new approach for assaying serum samples for the presence of DNA-specific Ab catalysts is readily applicable for rapid analysis of large number of samples. The authors developed a scheme (Fig. 1), according to which Abs first bind to

biotinylated Abs raised against their Fab fragments, and further are specifically removed from the solution using streptavidin-coated paramagnetic particles. Acid shock helps to dissociate any possible nuclease contaminants, and subsequent dilution of the preparation and addition of light (L) chain-specific Abs are likely to severely hinder potential reassociation events. The streptavidin–biotin interaction is ideally suited for this assay, because it is strong enough to provide capture of the Ab–abzyme complexes from very dilute solutions.

Subsequent washing steps ensured removal of any unbound nuclease activity. No DNA-hydrolyzing activity was observed in solution at the final step of washing. However, after breaking the DNA-abzyme–biotinylated antihuman L chain Ab complexes in acidic conditions, removal of the beads from the solution, and neutralization, the DNA-hydrolyzing activity appeared to be restored. Restoration of nucleolytic activity in solution coincided with the reappearance of Abs, as judged by SDS-PAGE with subsequent immunoblotting. Thus, the authors proved that DNA hydrolysis is the property of the captured Ab fraction. The results of the experiment are presented in Fig. 2. Since both anti- κ and anti- λ Abs captured the catalytic activity, one can conclude that there is more than one abzyme clone in the preparation. Abs with kappa chains make up nearly 70% of Abs in normal human serum (22). However, the ratio of kappa- and lambda-chain containing Abs in lymphoproliferation diseases can be disturbed. The possibility also cannot be excluded of nonspecific binding of lambda-chain IgG Abs to anti-chain Abs and vice versa, because of the disease-mediated changes in their sequence and high level of autoantibody crossreactivity.

Using the above-described technique, the authors have tested the Abs isolated from 142 serum samples of patients with various types of lymphoproliferative diseases, 42 serum samples from patients with SLE, and 12 serum samples from healthy donors for presence of DNA-hydrolyzing Abs. In 10 randomly selected cases that included Abs positive and negative for the DNA-hydrolyzing activity, the results of the affinity capture were compared to those obtained by the standard assays (3). No difference was observed between the data obtained using these two different methods.

No DNA-hydrolyzing activity of Abs was detected by the affinity-capture assay in sera of healthy individuals. No activity was found in eight cases of T-lymphocyte proliferation, including T-cell non-Hodgkin's lymphomas, Sezary syndrome, and acute T-lymphoblastic leukemia, five cases of acute B-cell leukemia, and 12 cases of Hodgkin's lymphoma. The other 117 studied cases included marginal zone lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, and multiple myeloma with Bence-Jones syndrome.

The DNA-hydrolyzing activity of IgGs was observed in 56% of cases with various types of B-cell proliferation, including acute B-cell leukemia,



Fig. 2. Analysis of the DNA-hydrolyzing activity of IgG Ab preparation from serum of patient with CLL by affinity-capture assay. The assay was performed as described in Materials and Methods. One microgram of pUC19 plasmid DNA was incubated overnight in the assay buffer (20 mM Tris-HCl, 50 mM NaCl, and 10 mM MgCl₂), unless otherwise indicated, and subjected to gel electrophoresis in 1% agarose. The amount of Abs brought into reaction was 10 µg, unless otherwise stated. Lane 1, control for the absence of nuclease activity in the assay buffer; lane 2, control for absence of DNA-hydrolyzing activity in glycine-HCl solution, pH 2.6 (the plasmid was incubated with the solution, neutralized with Tris-HCl, pH 9.0, and supplemented with 10 mM MgCl₂); lane 3, the plasmid incubated with initial DNA-abzyme preparation; lane 4, 3 µg pUC19 marker plasmid in three forms (supercoiled, linear, and circular). Lanes 5 and 6, controls for absence of the DNA-hydrolyzing activity in the final washing step from anti-κ and anti-λ particles, respectively, prior to elution of captured Abs; lane 7, restoration of DNA-hydrolyzing activity of Abs after elution from anti-κ L chain mouse antihuman Abs attached to the particles; lane 8, same as lane 7, except the DNA-abzymes were eluted from anti-λ L chain Abs.

CLL, mantle cell lymphoma, marginal zone lymphoma, follicular lymphoma, and multiple myeloma. Among 122 cases of the aforementioned diseases, DNA-hydrolyzing activity caused by Abs was detected in 2 of 5 cases of follicular lymphoma, 2 of 10 cases of mantle cell lymphoma, 23 of 41 cases of CLL, 9 of 27 cases of marginal zone lymphoma, and 26 of 34 cases of multiple myeloma. These data indicate the increased probability for the formation of cell clones producing the DNA-hydrolyzing Abs in tumors derived from relatively mature B-cells, compared to other types of malignant lymphoproliferation. Whether or not the abzymes are produced by the tumor cells remains to be investigated.

Remarkably, DNA-abzymes were found to be preferentially present in blood of patients with lymphoproliferation disease complicated by var-

ious autoimmune disorders. DNA-hydrolyzing Abs were detected in 66% of patients with autoimmune manifestations associated with lymphoproliferation (autoimmune hemolytic anemia, immune thrombocytopenia, dermatitis, and glomerulonephritis: 33 cases out of 50 studied). This observation supports the hypothesis of an autoimmune origin of the DNA-abzymes, and allows us to assume that the pathways of catalytic Ab production in SLE and blood tumors are similar.

Among patients with SLE, the catalytic activity of IgG fraction assayed by the affinity-capture technique was found in 18 of 42 cases studied (42.8%).

Cytotoxic Activity of CLL and SLE Anti-DNA Autoantibody Preparations

Spontaneous abzyme occurrence is strictly linked to autoimmunity. However, the clinical relevance of catalytic Abs remains to be determined. The mechanism of pathogenicity of DNA-binding autoantibodies is also far from being completely elucidated. Although proteolytic abzymes can exert harmful effects regardless of their localization, theoretically DNA-specific Ab catalysts should enter the cell nucleus in order to cause any direct DNA damage. In SLE, DNA-binding Abs represent the major part of autoantibody pool, and frequently crossreact with various proteins (14–16), thus providing the basis for the possible interaction of antinucleic acid immunoglobulins with membrane receptors and other proteinaceous targets.

The question whether the Ab can penetrate into the cell and enter the nucleus has been highly debatable for the past 20 yr (13). Recent investigations have provided direct evidence for the ability of DNA-binding autoantibody to enter the cell via interaction with the myosin I, and subsequently localize to the nucleus (15). Loss of the ability of another nuclear-localizing anti-DNA Ab to cross the cellular membrane after mutagenesis (23) strongly supports the receptor-mediated pathway of the Ab penetration. On the other hand, crossreactivity of anti-DNA Abs with membrane proteins raises the possibility of existence of autoantibodies that may cause cell death if acting through the appropriate receptors. Discovery of anti-tumor necrosis factor (TNF) receptor antibodies mimicking TNF actions (24), along with the phenomenon of triggering of apoptosis by monoclonal anti-Fas Abs led to the hypothesis that DNA-abzymes may be cytotoxic to the cells in a complement-independent manner, acting either directly by entering the nuclei and cleaving DNA, or indirectly by binding to the membrane receptors. Therefore, the authors determined the cytotoxicity of DNA-binding fraction containing DNA-abzymes, and compared the results to those obtained with the fraction of Abs that are not specific to DNA.

Table 1
Cytotoxicity of Total IgG, DNA-Binding, and Non-DNA-Binding IgG Fractions Isolated from Blood of Healthy Donor and Patients with Lymphoproliferative and Autoimmune Diseases

Preparation	Total IgG	DNA-binding IgG	IgG fraction not binding to DNA
Source			
Healthy donor	No cytotoxicity detected	No cytotoxicity detected	No cytotoxicity detected
Patient with systemic lupus erythematosus	Cytotoxic	Cytotoxic	No cytotoxicity detected
Patient with chronic lymphocytic leukemia (B-cell proliferation)	Cytotoxic	Cytotoxic	No cytotoxicity detected
Patient with B-cell lymphosarcoma	Cytotoxic	Cytotoxic	No cytotoxicity detected
Patient with T-cell lymphoma	No cytotoxicity detected	No cytotoxicity detected	No cytotoxicity detected

The authors studied the influence of total IgG preparations, DNA-binding IgG, and non-DNA-binding IgG from sera of SLE patients, lymphoproliferative disease patients (CLL, B-cell lymphosarcoma, T-cell lymphoma), and healthy donors on tumor cell line L929. There were no noticeable changes in cell viability after treating the cells with total IgG preparations from healthy individuals, T-cell lymphoma patients, and B-cell lymphosarcoma patients. However, the increased level of cell mortality was evident after 3 h of incubation with 10^{-8} M Abs derived from SLE and CLL patients (13–18% and 10–15% of dead cells, respectively) (Table 1; Fig. 3).

The DNA-binding Ab fraction was isolated from the total Ab preparation using FPLC chromatography on DNA-Sepharose (10). DNA-binding Abs isolated by this technique constituted on average one-tenth of the total IgG fraction. Cells were incubated with identical concentrations of DNA-binding and nonbinding Abs (10^{-8} M). TNF- α at the same concentration was used as a positive control for the cytotoxic activity (25). DNA-binding Abs from SLE and CLL sera were cytotoxic at concentrations of up to 10^{-10} M. Thus, a 100-fold enrichment of cytotoxic Abs is observed in DNA-specific fraction, compared to the total IgG. The IgG fraction from which anti-DNA Abs were depleted appeared not to be cytotoxic at all (Table 1). The authors determined the time-dependence of cytotoxicity exerted by DNA-binding Abs. The results (Fig. 3) outline three different

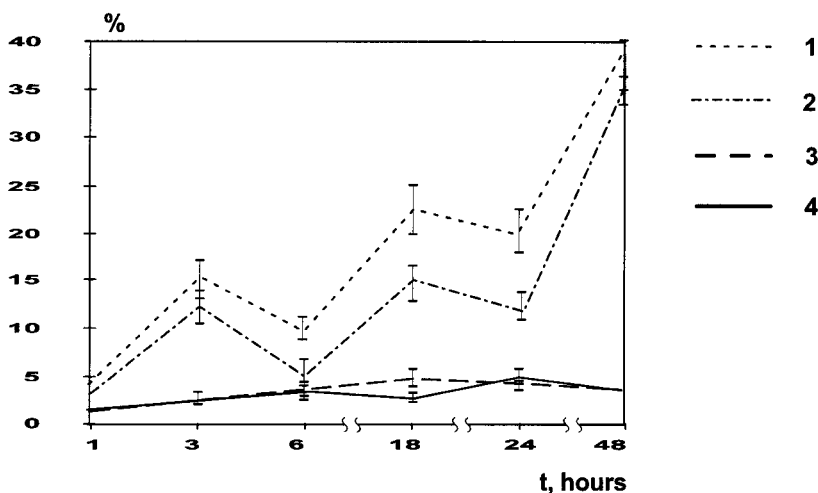


Fig. 3. Time-dependent pattern of cytotoxicity revealed by DNA-binding Abs from serum of CLL patient toward the tumor cell line L929. The percent of dead cells is shown on the vertical axis; time-course of incubation is shown at the horizontal axis. All experiments were conducted in the serum-free media as described in Materials and Methods section. The cells were incubated with 10^{-8} M of TNF- α (1), 10^{-8} M of DNA-binding fraction of IgG (2), 10^{-8} M of non-DNA-binding fraction (3); with medium alone (4).

peaks of cell mortality during the course of incubation with 10^{-8} M of CLL-derived DNA-binding Abs, at 3 h (10–15%), 18 h (13–17% of dead cells), and at 48 h (32–37% of dead cells). Decreases in the percent of the dead cells were observed after 6 h (4–6%) and after 24 h of incubation (10–12%). A similar time dependence of the cytotoxic effect of TNF- α (10^{-8} M) was evident (Fig. 3).

In some cases, the cytotoxicity of DNA-binding Abs was unexpectedly very high (Fig. 4). One case of SLE yielded anti-DNA Abs that caused cell death at concentrations as low as 10^{-10} M, thus rivalling the toxicity of TNF- α (Fig. 4; 25).

Various cytotoxic substances induce a plethora of distinct cytolytic processes in tumor cells that can be characterized by differences in the time-course and resulting proportion of killed cells (25). It is not known whether anti-DNA Abs induce single cytotoxic pathway, or whether the observed pattern of cell death is the result of induction of multiple overlapping mechanisms.

The results obtained outline the phenomenon of complement-independent autoantibody-mediated cytotoxicity. It is not known whether DNA hydrolysis or DNA binding by the Abs is responsible for the described phenomenon, nor it is clear whether the DNA-abzymes can localize to the nuclei and cleave DNA in living cells. The data presented indicate a new mechanism of pathogenicity of anti-DNA autoantibodies

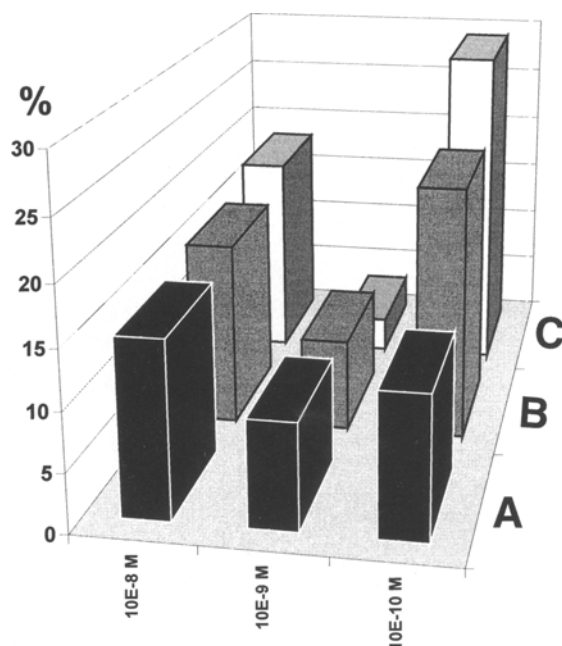


Fig. 4. Comparison of cytotoxicity of different concentrations of the DNA-binding Abs from serum of a SLE patient toward the tumor cell line L929. The vertical axis shows the percent of dead cells, the horizontal axis depicts the Ab concentration. A, 3 h incubation; B, 48 h incubation; C, control substance (TNF- α), 48 h incubation.

isolated from various sources, and provide the basis for detailed examination of the role of DNA-abzymes in complement-independent cytotoxicity.

Interaction of DNA-Abzymes with Nuclear Matrix Proteins

The origin of catalytic activity of anti-DNA autoantibodies remains elusive. An earlier article proposed that such Abs can arise as anti-idiotypes to the enzymes involved in DNA metabolism (11). Later work described crossreactivity of the DNA-abzyme fraction with nuclear matrix proteins, and hypothesized that tightly associated DNA-protein complexes composing nuclear matrix can be natural autoantigens for DNA-abzymes (4), as well as for some other DNA-specific autoantibodies (26). DNA complexed with certain nuclear matrix proteins was shown to be in a severely bent and energetically unstable conformation (12). The distorted, energetically unstable state of DNA in DNA-protein complexes may render the latter favorable autoantigens for formation of corresponding abzymes. Transition from binding to such DNA-protein interfaces to hydrolysis of naked DNA may recruit the induced-fit mechanism (27). The DNA-binding constant of polyclonal DNA-hydrolyzing Abs toward heterogeneous double-stranded oligonucleotide, calculated using Scatchard

plots, is $(1.8 \pm 0.2) \times 10^{-10} M$ (28). The high value of the binding constant of the DNA-abzyme fraction provides indirect evidence for the complex mechanism of the interaction between abzyme and DNA. More important, recent study of catalytic BV04-01 monoclonal Ab strongly supports the hypothesis that the interaction between abzyme and its substrate occurs via an induced fit (29).

Abs to the distorted ground state most closely mimic classic abzymes raised artificially to the transition-state analog of the Ag. The half-life of the transition state is too short to be considered sufficient for induction of catalytic Ab response. Alternatively, a molecule in distorted conformation can exist long enough to serve as the Ag provoking the formation of the autoantibody catalyst.

According to this hypothesis, significant proportions of DNA-specific abzymes would be crossreactive with the protein component of nuclear matrix. To test this, the authors prepared immobilized nuclear matrix proteins devoid of DNA (*see Materials and Methods for details*), and analyzed the affinity of polyclonal abzyme fractions to the immobilized proteins.

Anti-DNA Abs purified from sera of patients with CLL and SLE were applied to the NMP-Sepharose column, prepared as described in Materials and Methods. The DNA-hydrolyzing activity of Abs in the original IgG preparation, and the NMP-bound and unbound fractions, was assayed with supercoiled plasmid substrate, as described (3,10). The concentration of Abs and the reaction conditions were equivalent for all assayed samples. Of four CLL and five SLE sera analyzed, all revealed selective retention of DNA-hydrolyzing activity on the NMP-Sepharose column. Typical results from such experiments are presented in Fig. 5. The DNA-hydrolyzing activity is nearly twofold higher in the column-bound fraction. Decreasing the amount of Abs loaded on the column did not significantly affect the distribution of catalytic activity between bound and flowthrough fractions. The presence of DNA-abzymes in the unbound fraction may reflect occurrence of catalysts formed via mechanisms other than proposed above, e.g., the unbound fraction may comprise Abs occurring as anti-idiotypes to the enzyme paratope. In addition, certain nuclear matrix-specific abzymes can be specific to complex conformational epitopes that can be disrupted by treatment with SDS. Such Abs would not be bound by the NMP-Sepharose.

The data obtained support the hypothesis that a significant proportion of natural DNA-specific abzymes are formed as autoantibodies to complex and relatively stable molecular interfaces, such as the nuclear matrix, which contains the target Ag in an energetically destabilized state. Both CLL and SLE sera contain DNA-abzymes of this type, thus suggesting a similarity in the mechanisms of catalytic Ab formation in these diseases. Isolation of individual protein components of the nuclear matrix

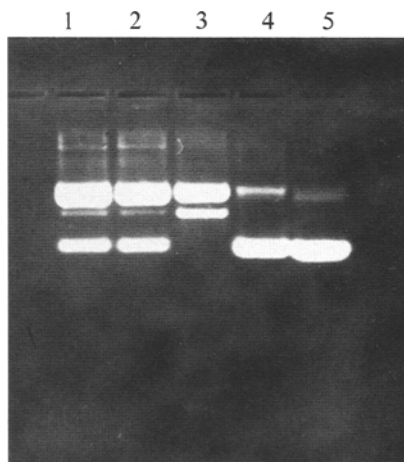


Fig. 5. Crossreactivity of DNA-abzymes with nuclear matrix proteins. Anti-DNA IgG isolated from serum of a CLL patient were resolved on NMP-Sepharose, as described in Materials and Methods, and subjected to the standard plasmid cleavage assay (see Fig. 2), followed by gel electrophoresis. Mock elution was performed by passing glycine-HCl, pH 2.6, through the column in the absence of loaded Abs, the eluate was neutralized with Tris-HCl, pH 9.0, and $MgCl_2$ was added to a final concentration of 10 mM. Lane 1, total anti-DNA IgG incubated with the substrate prior to the NMP-Sepharose chromatography step; lane 2, the plasmid incubated with the column flowthrough; lane 3, plasmid incubated with column-bound Abs; lanes 4 and 5, control for absence of DNA-cleaving activity in the mock eluate from NMP-Sepharose.

reactive with DNA-hydrolyzing Abs and dissection of the mechanism of Ab-mediated DNA hydrolysis would provide the final proof on the newly proposed mechanism of DNA-abzymes formation in autoimmunity.

CONCLUSIONS

The following conclusions summarize the data of this investigation.

1. Abs from sera of patients with lymphoproliferative diseases reveal DNA-hydrolyzing activity. This activity belongs to the Ab fraction as judged by the affinity-capture assay.
2. According to data from analysis of serum samples obtained from patients with various lymphoproliferative diseases, DNA-abzymes occur preferentially in low-grade B-cell proliferation. The percentage of DNA-abzyme incidence in B-cell tumors (56%) exceeds the frequency of DNA-abzyme occurrence in SLE (42%). Autoimmune disorders associated with lymphoproliferation substantially increase the possibility of formation of DNA-abzymes (66% of patients with B-cell proliferation and autoimmune manifestations were positive for presence of DNA-abzymes).

3. Anti-DNA autoantibodies from sera of patients with SLE and CLL reveal cytotoxicity toward the tumor cell line L929. The cytotoxic effect was observed at Ab concentrations of 10^{-8} – 10^{-10} M, which is comparable to cytotoxicity of TNF- α .
4. DNA-hydrolyzing Abs from blood of patients with both SLE and CLL display crossreactivity with nuclear matrix proteins, as assayed by affinity chromatography of polyclonal DNA-abzymes on the column with immobilized nuclear matrix proteins from which the bound DNA was purportedly depleted.

Abs from patients with autoimmune and lymphoproliferative diseases reveal extraordinary properties compared to the Abs of normal individuals. The catalytic and cytotoxic activity of these Abs deserve detailed investigation, because they may not only play an important role in the development of the disease, but they could alter the traditional view at Ab function.

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