Volume 314, number 3, 259–263 FEBS 11839 December 1992

DNA-specific antiidiotypic antibodies in the sera of patients with autoimmune diseases

Igor B. Bronshtein[a], Alexander M. Shuster[a], Gennady V. Gololobov[a], Irina I. Gromova[a], Oksana A. Kvashuk[a], Ksenia M. Belostotskaya[a], Zemfira S. Alekberova[b], Tatiana B. Prokaeva[b] and Alexander G. Gabibov[a]

[a]*Engelhardt Institute of Molecular Biology, Academy of Sciences of Russia, Vavilov str. 32, 117984, Moscow B-334, Russia* and [b]*Institute of Rheumatology, Academy of Medical Science of Russia, Moscow, Russia*

Received 16 October 1992

Blood sera of patients with autoimmune diseases scleroderma (Scl), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) have been shown to yield a specific immune response to topoisomerase I, the product of expression of a cDNA fragment cloned into λgt11 and monoclonal antibodies (MAB) to the enzyme. The 'topoisomerase test' is not absolutely specific for Scl. The stable positive response of autoimmune sera to anti-topoisomerase monoclonal antibodies has a specific character and is associated with the interaction of the Fab fragment of MAB with the IgG fraction of autoimmune serum. The response observed indicates the induction of anti-idiotypic antibodies against topoisomerase. The anti-idiotype, isolated by HPLC and affinity chromatography demonstrated the following functional activities: (i) the immunological reaction against DNA; (ii) high-affinity DNA-binding with topoisomerase-specific consensus; (iii) ability to compete with the native enzyme for binding with DNA and MAB to topoisomerase; (iv) immunological reaction against MAB to topoisomerase.

Autoimmunity; Topoisomerase I; Antiidiotypic antibody; Fusion protein; HPLC of autoimmune sera

1. INTRODUCTION

Production of specific antibodies against various physiologically active compounds, their precursors, transient state analogs and biopolymers has recently gained a new stimulating aspect and entered another phase due to the development of a special field of immunochemistry and enzymology, viz. the catalysis by antibodies or 'abzymes' [1,2] which has wide prospects for use both in fundamental research and for practical purposes.

Marked progress in the new field of abzymes has been so far achieved regarding only a limited number of biologically significant chemical transformations and mostly concerns chemistry of proteolysis [3]. To our knowledge, for example, one of the most interesting objects, DNA, has not been considered yet as an antibody substrate. Many difficulties in this field are connected with the problem of choosing an adequate model for DNA chemical transformations and a design of the corresponding transient states.

Correspondence address: A.G. Gabibov, Institute of Molecular Biology, Academy of Science of Russia, Vavilov str. 32, 117984, Moscow B 334, Russia. Fax: (7) (95) 135-1405.

Abbreviations: Scl, scleroderma; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; MAB, monoclonal antibodies against topoisomerase; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.

A specific feature of our approach is an attempt to discover naturally occurring anti-DNA antibodies possessing an enhanced specificity for certain sequences. Identification of such antibodies and investigation of their properties would provide prerequisites for analyzing the nature of their specificity and possible involvement in catalytic reactions.

2. MATERIALS AND METHODS

2.1. *Antigen–antibody interaction*

Quantitative analysis of antigen-antibody interaction was carried out by the ELISA method [4].

2.2. *Topoisomerase I*

Topoisomerase I was obtained by the HPLC method as described earlier [5].

2.3. *Cloning and expression of the human topoisomerase I gene*

P3 (see Fig. 1) is a fusion protein, expressed from a cDNA fragment cloned in λgt11 vector, which was obtained by immunoscreening 5×10^5 recombinant clones of a human placenta cDNA library using autoimmune Scl sera, biotinylated anti-human antibodies, and avidin-peroxidase conjugate as a non-isotope detection system [6].

2.4. *Isolation of antibodies to topoisomerase I and preparation of FAB-fragments*

Monoclonal antibodies against topoisomerase I were obtained by the method described earlier [7]. Fab-fragments were prepared by proteolysis of IgG molecules with papain [8]. The Fab fraction was purified with HPLC chromatography on a TSK-3000 SW gel-filtration column (21.6 × 600 mm) by elution with buffer A at a flow rate

of 0.2 ml/min. The peak of elution of the Fab-fragment corresponded to a molecular weight of 50 kDa.

Homogeneity was proved with SDS-PAGE according to Laemmli [9], using a 12.5% gel under reducing conditions. MW of FAB-fragment corresponds to 50 kDa.

2.5. *Autoantibodies preparation*

All patients with Scl, SLE and RA were examined in the clinic at the Institute of Rheumatology, Academy of Medical Science. The diagnosis was confirmed and its reliability checked using the criteria developed by the American Rheumatoid Association. The sera of 34 patients with Scl (14), SLE (7) and RA (13) were analysed.

Separation of autoimmune sera involved: (i) precipitation with 40% $(NH_4)_2SO_4$; (ii) HPLC separation on a TSK G 3000 SW gel-filtration column as described above; (iii) IgG fractions, exhibiting a positive response to DNA and MAB to topoisomerase I by the ELISA test were divided into two parts. One was applied to a dsDNA-cellulose column (Sigma, USA) in buffer A (10 mM K-phosphate, 100 mM NaCl, pH 7.2). The chromatography was performed using an increasing gradient of K-phosphate from 10 mM to 1 M, pH 7.3 for 40 min at a flow rate 2 ml/min. The second part was separated on BrCN-Sepharose (Biolar, USSR), coupled with MAB to topoisomerase. Modification was carried out in the mixture: 10 mg antibodies, 0.5 g sepharose per ml of 0.1 M $NaHCO_3$ buffer, pH 9.0, with stirring for 14 h. Remaining groups were blocked with 0.1 M Tris-HCl buffer, pH 9.0, at 4 h. The adsorbent was subsequently washed with 2 M NaCl in Tris-HCl buffer, pH 7.5, and 0.1 M glycine-HCl buffer, pH 3.0. Non-specific adsorption was blocked with 10% fetal serum. Anti-idiotypes were applied to the column in buffer A, washed with the same buffer and eluted with 0.1 M glycine-HCl buffer, pH 2.5, collected fractions being neutralized with 0.5 M Tris-HCl, pH 7.5, followed by dialysis against buffer A. Immunological properties of anti-idiotypes as anti-DNA and anti-MAB antibodies were analysed by ELISA-test. A standard kit was used for anti-DNA antibodies measurements (Biolar, USSR).

2.6. *Assays of competition of topoisomerase and anti-idiotypes for MAB binding*

Topoisomerase was adsorbed onto a plate at a concentration of 0.1 μg/ml. Varying amounts of anti-idiotypes were incubated with biotinylated MAB at a constant concentration of 30 μg/ml, overnight at 4°C. The mixture was re-applied to the plate and the avidin-peroxidase conjugate was added to develop the colour reaction. Using this technique, only the specific interaction of free MAB (anti-idiotype unbound) with topoisomerase is determined.

2.7. *DNA-binding of anti-idiotypes and topoisomerase I*

The fragment of dsDNA from 3'-λ^ globin gene, labeled at the 5'-end, containing different potential sites for topoisomerase binding [10] was incubated with 20 U of topoisomerase I or 1 μg of anti-idiotypic antibodies for 20 min at 37°C (concentration of a fragment was from 0.5 to 50 ng/μl); 20 μg of poly dAT was added to exclude non-specific binding. The incubation mixture contained 20 mM Tris-HCl, pH 7.5, 50 mM KCl, 3 mM $CaCl_2$, 0.1 M sucrose, 1 mM DTT. The reaction mixture was applied to wet nitrocellulose filters and washed three times for 2 min with the same buffer. The radioactivity was measured in a scintillation counter. As a control, a mixture of 5'-labeled DNA with 1–2 mg BSA was used. Using the resulting data, the constant was determined by the method of Lineweaver–Burk.

3. RESULTS AND DISCUSSION

In our investigation of DNA-specific antibodies we examined sera from patients with autoimmune diseases. This pathology is known to be characterized by the accumulation in the patients' blood of substantial amounts of autoantibodies against antigens of the nucleoprotein type [11]. Earlier it was shown that the blood of patients with some forms of Scl had an increased titer of antibodies against one of the key enzymes of DNA metabolism, topoisomerase I [12].

Immunoscreening of the human placenta cDNA library in the expression λgt11 vector revealed clones expressing topoisomerase I in the fusion protein with galactosidase. The DNA structure of the isolated clones was compared with the data of D'Arpa et al. [13] (see Fig. 1). The analysis of 34 patients having different autoimmune pathologies (see Table I) demonstrated that the sera from some patients with Scl, SLE and RA yielded a high stable positive response to the cloned topoisomerase–galactosidase fusion protein (fragment P3, Fig. 1). So, the recombinant protein can successfully substitute the native enzyme in immunological studies as a diagnostic test for autoimmune diseases. However, unlike the results reported by other investigators [14], we have found that apart from 100% positive reactions observed in the case of Scl, the sera from patients with SLE and RA also happen to be immunogenic with respect to topoisomerase. Therefore, the 'topoisomerase test' does not appear specific exclusively for Scl.

Since the recognition system in the case of autoimmunity is characterized by the spontaneous induction of the second antibodies in the series of the suggested anti-idiotypic network [15], it was reasonable to search for specific antibodies against DNA among anti-idiotypic antibodies against topoisomerase. We have analyzed all

Table I

Positive immune response of the blood sera from patients with autoimmune diseases to the expressed topoisomerase–galactosidase fusion protein and anti-topoisomerase monoclonal antibodies (MAB)

Autoimmune disease	Number of patients	Absolute values (%)		
		Fusion protein	Monoclonal antibodies (MAB)	Fusion protein + monoclonal antibodies (MAB)
SLE	14	8 (57.1)	7 (50.0)	4 (28.5)
RA	13	11 (84.6)	9 (69.2)	8 (61.5)
Scl	7	7 (100.0)	6 (85.7)	6 (85.7)

The interaction of MAB with blood sera was analyzed by the ELISA method using MABs to membrane proteins of the potato virus as the negative control. The result exceeding the background by 100% was considered reliable. The serum dilution used was 1:100.

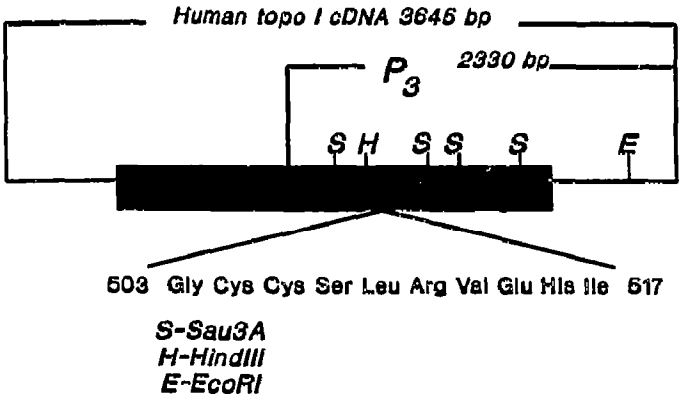


Fig. 1. Human topoisomerase I cDNA map. P3 contains regions coding for the protein fragments responsible for the catalytic function [6] and for the binding of the produced monoclonal antibodies against the calf thymus enzyme. The region coding for the conservative polypeptide common for different DNA binding proteins is indicated by arrows [18].

the available sera for monoclonal antibodies to topoisomerase I which effectively interacted with the expressed fusion protein (dissociation constant, determined by the Scatchard method [16] was below 5.7 nM). Some of the studied sera had a high stable positive response both to topoisomerase and the monoclonal antibodies against it. An especially high level of response was observed from the serum of patients with rheumatoid arthritis. Partially HPLC-purified autoimmune serum (see Fig. 2) was found to possess the same property. We had to check whether the response was specific. As can be seen from Fig. 2 we achieved a good separation of immunoglobulins of autoimmune serum which correlated well with the standard separation [17]. The obtained Fab fragment of monoclonal antibody against topoisomerase I interacted only with the IgG fraction of the autoimmune serum (see Fig. 2). These data indicated that the response of the autoimmune serum to monoclonal antibodies was specific. Indeed, the response was associated with the variable part of the antibody and, moreover, Fab was bound to the IgG fraction. In the case of the rheumatoid factor, the interaction is usually associated with the Fc fragment and the IgM fraction of the serum [11].

All studied sera samples were also tested for the presence of anti-DNA antibodies. The selected group with a high titer against the fusion protein and the monoclonal antibody against topoisomerase responded positively as evidenced by the ELISA test.

The sera exhibiting a positive response to antibodies against topoisomerase and DNA were passed through DNA- and antitopoisomerase-immobilized affinity col-

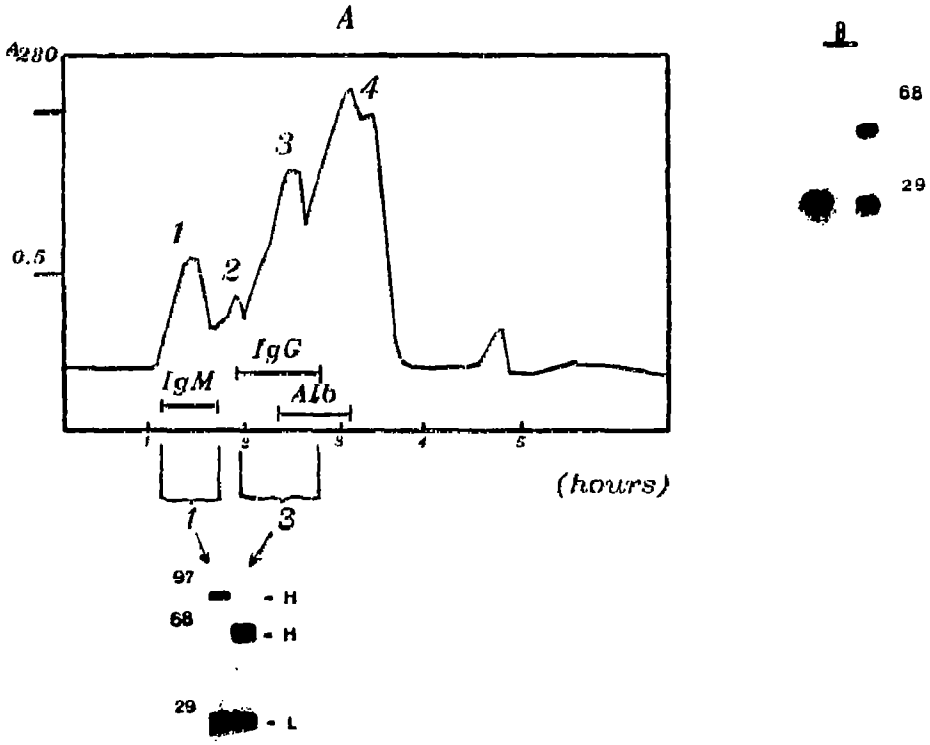
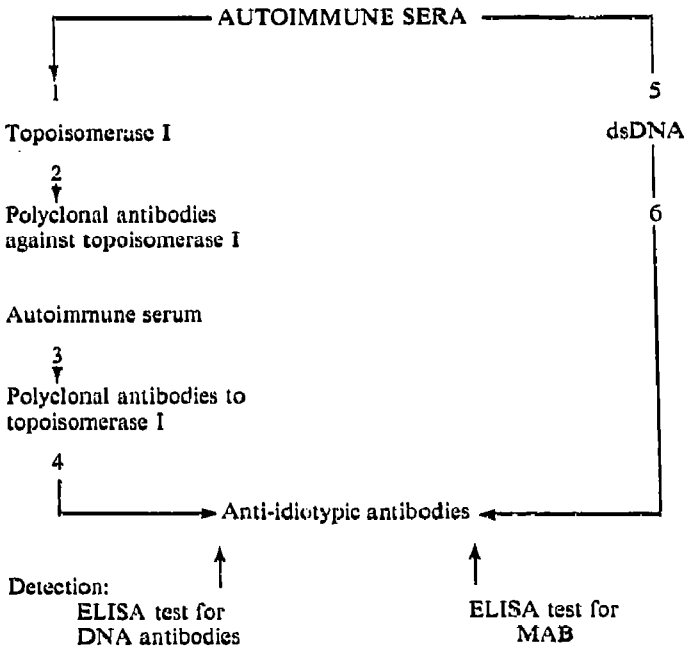


Fig. 2. Separation procedure used for autoimmune sera immunoglobulins and MAB to check the specificity of their interaction. (A) HPLC separation of autoimmune sera. SDS-PAGE of separated immunoglobulins (HPLC fractions 1 and 3). m.wt. of protein standards on the left. 'H' heavy and 'L' light chains indicated on the right. (B) SDS-PAGE of MAB papain digest (on the right) Fab fragment of MAB following protein A Sepharose purification (on the left); m.wt. of protein standards indicated on the right.



Scheme 1. Flow diagram of isolation steps of topoisomerase I anti-idiotypic antibodies.

umns (see Scheme 1). The fractions of antibodies adsorbed specifically onto the affinity supports were eluted and cross-analyzed. The DNA-eluted antibodies were shown to interact with monoclonal anti-topoisomerase antibodies, whereas those eluted from the anti-topoisomerase column gave a stable response to DNA binding. So our experiments have demonstrated that the sera from patients with autoimmune diseases, which respond positively to human topoisomerase contain anti-idiotypic antibodies possessing DNA binding activity.

We studied the properties of isolated anti-idiotypes. The potency of these antibodies to compete with topoisomerase for monoclonal antibody binding has been proven (see Fig. 3). The investigation of interaction be-

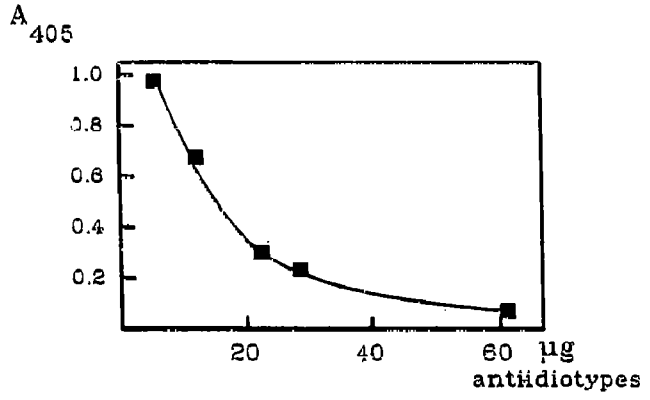


Fig. 3. Competition of anti-idiotypes and topoisomerase for MAB binding.

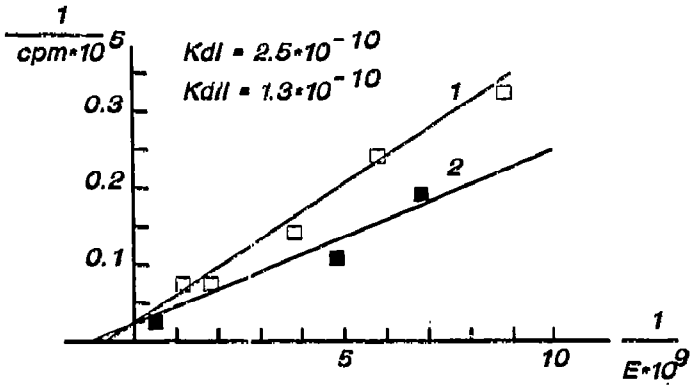


Fig. 4. Determination of dissociation constants of DNA–topoisomerase I complex (line 1) and DNA–anti-idiotype antibody complex (line 2) using the method of Lineweaver–Burk.

tween topoisomerase I and anti-idiotypic antibodies with the fragment of the globin gene, containing consensus sequences specific to topoisomerase I, was carried out. The data are shown in Fig. 4. Their analyses with the method of Lineweaver–Burk allowed to obtain dissociation constants of DNA–enzyme (1) and DNA–antibody (2) complexes. They were 0.13 nM and 0.27 nM respectively. These data show that specific DNA sequences interact both with anti-idiotypes and topoisomerase I, confirming the specific character of the anti-idiotype–DNA interaction.

Studies on the specificity of these anti-idiotypes with respect to various DNA templates are now in progress in this laboratory.

REFERENCES

[1] Jacob, J. and Schultz, P. (1987) J. Am. Chem. Soc. 109, 2174–2176.
[2] Tramontano, A., Jandu, K. and Lerner, R. (1986) Proc. Natl. Acad. Sci. USA 83, 6736–6739.
[3] Lerner, R. (1988) J. Cell Biol. 107, 436a (abstract 2473).
[4] Kendall, C., Jonson-Matiu, J. and Dressma, G.R. (1983) J. Immunol. Methods 56, 329–339.
[5] Bronshtein, I.B., Shuster, A.M., Shevchenko, L.V., Gromova, I.I., Kvashuk, O.A., Geva, O.N. and Gabibov, A.G. (1989) Mol. Biol. 23, 1553–1559 (in Russian).
[6] Shuster, A.M., Kvashuk, O.A., Prassolov, V.S., Chumakov, I.M. and Gabibov, A.G. (1989) Biochemie 71, 599–604.
[7] Bronshtein, I.B., Geva, O.N., Gromova, I.I. and Timofeev, A.V. (1989) Dokl. Akad. Nauk SSSR 305, 460–462 (in Russian).
[8] Harlow, E. and Lane, D. (1988) Antibodies: a laboratory manual, Cold Spring Harbor Laboratory, USA.
[9] Laemmli, U.K. (1976) Nature 227, 680–685.
[10] Vasetsky, E.S., Kalandadze, A.G., Razin, S.V. and Georgiev, G.P. (1989) Dokl. Akad. Nauk SSSR 306, 1493–1496.
[11] Reimer, G., Raska, I., Tan, E.M. and Scheer, U. (1987) Virchows Arch. B54, 131–143.
[12] Maul, G.G., French, B.T., van Venrooij, W.J. and Jiminez, S.A. (1986) Proc. Natl. Acad. Sci. USA 83, 5145–5149.
[13] D'Arpe, P., Machlin, P., Ratrie, H., Rothfield, N.F., Clevelend, D.W. and Earnshaw, W. (1988) Proc. Natl. Acad. Sci. USA 85, 2543–2547.

[14] Maul, G.G., Jimenez, S.A., Riggs, E. and Ziemnicka-Kotula, D. (1989) Proc. Natl. Acad. Sci. USA 86, 8492–8496.
[15] Klinman, D.M. and Steinberg, A.D. (1988) Arthritis and Rheumatism 29, 697–705.
[16] Friguet, B., Chaffotte, A.F., Djavadi-Ohaniance, L. and Goldberg, M. (1985) J. Immunol. Methods 73, 119–126.
[17] High performance liquid chromatography in protein research, LKB Liquid Chromatography Application Note 362, 3–4.
[18] Humphrey, G.M., Newitt, J.A., Kaurmann, S.H., Shaper, N.L. and Shaper, J.H. (1989) 11th Nuclear Workshop, September, USSR, p. 100.