breaks in DNA molecules is increased, and second, by degradation of a certain proportion of hepatocytes as a result of a disturbance in denervated liver cells to activation of DNA-tropic factors.

The experimental results suggest that on the 30th day after vagotomy (Fig. 1c) there is a tendency for the chromatin to return to its original state. This view is supported by the closeness of the character of dependence of the relative viscosity of the chromatin on concentration of the intercalating agent (this is clearly visible when curves I and IV are compared, see Fig. 1c).

Thus for the first time a difference has been found in the state of isolated chromatin from rat hepatocytes of the control group and in different stages after the operation of bilateral subdiaphragmatic vagotomy. A characteristic feature of these changes is absence of domains with a high degree of supercoiling, present in chromatin isolated from the liver of intact rats. Restoration of the state of rat hepatocyte chromatin on the 30th day after vagotomy suggests that the nervous system can influence activity of topoisomerases, which determine the topologic and, consequently, the transcriptional state of the genome.

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INTERACTION OF BLOOD SERA FROM PATIENTS WITH AUTOIMMUNE DISEASES WITH EXPRESSED cDNA FRAGMENT OF TOPOISOMERASE I AND WITH MONOCLONAL ANTIBODIES TO THE ENZYME

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Until recently the molecular theory of the origin and development of the autoimmune process was far from being expressed in its final form. For this reason many authorities paid increased attention to the study of idiotype — anti-idiotype interaction in autoimmune processes [4, 6]. One approach to the study of the molecular bases of autoimmune pathology is to undertake the discovery, cloning, and immunochemical investigation of the individual characteristics of genes and of proteins that are products of their expression [8]. In particular, the appearance of positive reactions in autoimmune sera to antigens of nucleoprotein nature has been observed in many investigations [5]. We know that in systemic scleroderma (SSD) a high titer of antibodies to one of the main enzymes of DNA metabolism, namely type I topoisomerase, is found in the patients' sera [3]. Previously, we described the cloning of this enzyme in the expressed vector λgt 11, and also the obtaining of monoclonal

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antibodies to it [1, 2]. In this communication we describe the study of interaction of the product of expression of a cloned cDNA fragment and of monoclonal antibodies to topoisomerase, with sera from patients with various types of autoimmune pathology, with the aim of discovering possible prognostic criteria of these specific reactions.

EXPERIMENTAL METHOD

Monoclonal antibodies to type I topoisomerase were obtained from calf thymus [2]. They were eluted in one peak by high-performance liquid chromatography (HPLC) on a DEAE-5PW column, with NaCl in a concentration of 0.35 M, and were placed in class IgG 1 on the basis of data of affinity chromatography on protein A-sepharose [7]. Topoisomerase I was isolated from human placenta by the method we described in [2]. The stages of chromatography on hydroxyapatite were carried out with the use of HPLC. In the first stage we used HPLC, a TSK 3000 SWG gel-filtration column, followed by Phenyl 5 PW for hydrophobic chromatography. Fractionation was carried out in a diminishing ammonium sulfate gradient from 1.5 M to 10 mM, and a rising gradient of glycerol from 10 to 30% in 50 mM Tris-HCl, pH 7.2. The duration of the gradient was 40 min. The flow rate was 0.5 ml/min. Cloning and isolation of the hybrid topoisomerase — β-galactosidase protein were carried out exactly as in the method described in [1]. Chromatography of the immunoglobulins of the autoimmune sera was carried out by HPLC, gel-filtration on a TSK 4000 SWG preparative column in 50 mM K-phosphate buffer, pH 7.2, containing 200 mM NaCl. The flow rate was 2 ml/min. The Fab-fragment of monoclonal antibodies to topoisomerase was obtained [1] by proteolytic digestion of the IgG molecule with papain. Anti-idiotypic antibodies were purified from autoimmune sera by affinity chromatography on a column with covalently bound monoclonal antibodies to topoisomerase I and to double-helical DNA. The specifically adsorbed antibodies were eluted with 0.2 M Tris-glycerine buffer, pH 2.5, followed by rapid neutralization of the eluent with 1 M Tris-HCl buffer, pH 8.0. Quantitative immunochemical analysis of antigen—antibody interaction was carried out by ELISA. All conjugates of secondary antibodies with peroxidase were commercial preparations from "Sigma" (USA). The antibodies were exhausted against *E. coli* proteins. The negative control for the study of interaction of monoclonal antibodies with autoimmune sera consisted of monoclonal antibodies to potato virus membrane proteins. The result was taken to be significant if, on spectrophotometric detection of E405 of the immunologic planchet on a multichannel spectrophotometer the optical density of the experimental sample exceeded that of the control by 100%. The working dilution of the sera was 1:100. Interaction of DNA with topoisomerase and with the isolated anti-idiotypic antibodies was studied in 20 mM Tris-HCl buffer, pH 7.5, containing 5 mM $CaCl_2$, 1 mM mercaptoethanol, 1 mM EDTA, and 5% glycerol. The reaction mixture contained 100 μg of topoisomerase or 0.5-1 μg of anti-idiotypic antibodies, 4-100 mg DNA, and 1 μg poly-d(AT). The mixture was incubated for 20 min at 37°C and then applied to nitrocellulose filters. The filter was washed 4 times for 5 min each time in incubation buffer and counted on an S1-3000 scintillation counter. Binding was assessed in double reciprocal coordinates.

Sera of 34 patients with various autoimmune diseases were used. After the standard procedure for their preparation the sera were frozen at —135°C. The patients' diagnoses were established in accordance with the criteria of the American Rheumatoid Association.

EXPERIMENTAL RESULTS

We analyzed blood sera from 34 patients with various autoimmune diseases (Table 1). As the parameters for investigation of the specific response of the autoimmune sera we chose the test, classical for these investigations, for antibodies to DNA and to topoisomerase I, for the product of expression of its cDNA fragment in the composition of the fusion-protein with β-galactosidase, and for monoclonal antibodies to topoisomerase. It follows from Table 1 that all cases of systemic lupus erythematosus (SLE) gave a positive response for the presence of antibodies to DNA. In all patients with SSD a positive reaction was obtained for topoisomerase, in agreement with observations by other workers [3]. The values of optical density obtained correlated fully with the results of experiments in which native topoisomerase was replaced by the expressed product. It could accordingly be concluded that replacement of this enzyme, which is difficult to obtain, by its genetic engineering analog is adequate. As Table 1 shows, antibodies to topoisomerase were found in blood sera of patients with SLE, and also with rheumatoid arthritis (RA). Thus the topoisomerase test is not absolutely characteristic for SSD, as has been stated in the literature [3]. During analysis of the clinical manifestations in 11 patients with RA, positive for the presence of antibodies to topoisomerase, revealed that five of them had systemic manifestations and a positive result for rheumatoid factor. Among patients with SLE a positive reaction was observed (8 and 14 patients tested) for the presence of antibodies to topoisomerase, but by contrast with cases of RA, the activity of the disease under these circumstances was moderate or low.

TABLE 1. Positive Immunologic Reaction of Blood Sera of Patients with Autoimmune Diseases for Expressed Hybrid Topoisomerase — β-Galactosidase Protein, Monoclonal Antibodies to Topoisomerase, and Antibodies to DNA

Disease	Number of patient	Hybrid protein		Monoclonal antibodies		Antibodies to DNA	
		abs.	%	abs.	%	abs.	%
SLE	14	8	57,1	7	50	14	100
RA	13	11	84,6	9	69,2	13	100
SSD	7	7	100	6	85,7	7	100

One trend in the study of the autoimmune process is to examine the idiotypic grid [4]. The pathological state may perhaps be determined by idiotype—anti-idiotype in the case of antibodies to DNA can be used as a prognostic criterion of the autoimmune process [8]. Since the topoisomerase test has been found to be characteristic of many autoimmune diseases, we postulated the presence of anti-idiotypic antibodies to topoisomerase in the autoimmune process. It follows from Table 1 we recorded a positive response of several autoimmune sera to monoclonal antibodies to topoisomerase. The positive response often corresponded to cases giving a positive topoisomerase test, but correlation between the absolute values could not be established. As a rule, a high signal was found by the ELISA method in cases with RA, characterized by a particularly rapid course. It was necessary to demonstrate the specificity of the reaction observed. As a result of chromatographic fractionation of the autoimmune serum it was shown that the Fab-fragment of the monoclonal antibody to topoisomerase which we obtained interacted only with the IgG-fraction of serum. Thus the response is connected with the variable part of the antibody. As we know, in the case of rheumatoid factor, interaction is linked with the Fc-fragment and the IgM-fraction of serum. Thus the positive reaction of the autoimmune serum which we recorded to monoclonal antibodies is evidently connected with the presence of anti-idiotypic antibodies in it to topoisomerase I. We obtained these antibodies with the help of affinity chromatography. The specificity adsorbed fraction possessed immunologic properties relative to DNA, and it also gave a positive test for monoclonal antibodies to the enzyme. Since anti-idiotypic antibodies must to some extent possess the properties of the primary antigen [6], we tested their DNA-binding ability and compared it with that of native topoisomerase. The results showed that the dissociation constant of topoisomerase — DNA and anti-idiotype — DNA complexes lies between 10^{-10} and 10^{-11} M. We thus showed that autoimmune sera contain anti-idiotypic antibodies to human topoisomerase I.

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