ACTION OF ANTIBODIES PRESENT IN THE SERUM OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND IN THE SERUM OF IMMUNE ANIMALS ON DNA TEMPLATE ACTIVITY

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UDC 616.5-002.525.2-031.81-07:616.153.963.32-097.5-078

The serum of patients with systemic lupus erythematosus and of animals immunized with DNA inhibited RNA synthesis in an RNA-polymerase system. The degree of inhibition of DNA template activity depended on the type of antibodies present in the serum and also on the secondary structure of the DNA used as template in the RNA-polymerase system. The type of the antibodies is determined by their ability to interact with DNAs of different conformations.

After antibodies against DNA had been found in the serum of patients with systemic lupus erythematosus (SLE) [7, 8, 11] the question of the pathogenic role of these antibodies was raised: are they the cause of some of the pathological changes or is their appearance purely the result of the disease, with no effect on its course?

Results showing the ability of antibodies to depress DNA template function have been published [4, 12, 13].

The objective of the present investigation was to study the effect of antibodies present in the serum of patients with SLE and of immune animals on the template activity of DNA.

EXPERIMENTAL METHOD

The RNA-polymerase system which was used was developed by Khesin et al. [6]. The role of template was performed by native double-helical DNA (N-DNA, 40 μg per sample) and thermally denaturated, partially helicized DNA (D-DNA, 12 μg per sample). Uracil-C^{14} (1 μCi per sample, specific activity 45 μCi/g) was added as labeled precursor. Radioactivity was measured on a gas-flow counter (Erlangen-bruck) and expressed in pulses/min.

Intact rabbits were immunized with a complex of single-stranded formalinized DNA (F-DNA) with methylated bovine serum albumin (MBSA) [5].

DNA was isolated from calf thymus [9]. D-DNA and F-DNA were prepared by the method described previously [2].

The titer of antibodies present in the serum was estimated by the passive hemagglutination test (PHT) [3]. The sera of the patients with SLE were classified on the basis of their ability to interact with DNAs of different conformation [2]. For this purpose, the antibody neutralization test (ANT) was used. The sera of type 1 reacted mainly with F-DNA, and to a lesser degree with D-DNA, those of type 2 reacted about

Department of Internal Medicine and Occupational Diseases, I. M. Sechenov First Moscow Medical Institute. Laboratory of Radiation Biochemistry, Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Tareev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 7, pp. 72-74, July, 1971. Original article submitted November 11, 1970.

TABLE 1. Effect of Sera of Patients with SLE and of Immune Rabbits on DNA Template Activity in RNA-polymerase System

Agent tested	Statistical index	Inhibition of incorporation of uracil-C^{14}, %	
		N-DNA	D-DNA
Sera of patients with SLE not containing antibodies against DNA, in remission period	M ± m	0	0
	n	8	8
Sera of patients with SLE containing type 1 antibodies, in subacute phase	M ± m	0	3.6 ± 2.3
	n	5	5
	P		> 0.05
Sera of patients with SLE containing type 2 antibodies, in acute phase	M ± m	40.7 ± 3.32	47.9 ± 3.76
	n	22	6
	P	< 0.001	< 0.001
Sera of patients with SLE containing type 3 antibodies, in acute phase	M ± m	72.6 ± 4.35	26.4 ± 4.72
	n	10	10
	P	< 0.001	< 0.001
Sera of intact rabbits, not containing antibodies	M ± m	0	0
	n	4	4
Sera of immune rabbits containing antibodies in titer of 1 : 640-1 : 10,000	M ± m	57.7 ± 3.12	67.8 ± 3.82
	n	10	10
	P	< 0.001	< 0.001
Pure antibodies isolated from sera of immune rabbits, antibody titer 1 : 80-1 : 240	M ± m	68.5 ± 5.5	68.0 ± 3.26
	n	4	4
	P	< 0.001	< 0.001

equally with F-DNA and D-DNA, and those of type 3 contained antibodies which reacted equally with N-DNA, D-DNA, and F-DNA. Sera from which antibodies had been removed were used in the control tests. The immune and control sera were added to the sample in a volume of 0.1 ml. The fraction of pure antibodies was obtained from the sera of immune rabbits by the Podgorodnichenko's method. F-DNA was adsorbed on nitrocellulose membrane filters. The previously inactivated serum was incubated with the immunosorbent in the proportion of 1 ml serum to 70 μg antigen at 4°C. Exhaustion of the serum ended with a decrease in the antibody titer to 1 : 10. The filters with adsorbed antibodies were washed to remove nonspecific serum proteins with standard salt solution (0.15 M NaCl solution and 0.015 M solution of trisubstituted sodium citrate). The antibodies were eluted with 6 M urea solution for 20 h at 4°C. To remove the urea from the supernatant, it was dialyzed for 48 h against 0.1 M NaCl solution. The titer of the "pure" antibodies thus isolated was determined by the PHT, and the protein concentration estimated by Lowry's method [10]. The purity of the isolated antibodies was checked by immunoelectrophoresis with an antirabbit serum [1]. The arc corresponding to the γ-globulin fraction was clearly visible on electrophoresis.

Bovine serum albumin (BSA), which was added to the sample in an amount equal in protein content, was used as the control for the pure antibodies.

EXPERIMENTAL RESULTS

Tests of the sera of patients with SLE showed that, depending on the type of antibodies present in the serum, they differed in their action on DNA template activity. A relevant factor was whether native or thermally denatured DNA was used as the template in the RNA-polymerase system (Table 1).

The sera of patients with SLE not containing antibodies against DNA did not alter the template activity of the DNA. The sera of patients containing type 1 antibodies, found as a rule in patients in the subacute phase of the disease, did not depress the template activity of N-DNA and inhibited the template activity of D-DNA only very slightly.

Sera containing antibodies of types 2 and 3 were found in patients in the acute phase of the disease. Type 2 sera inhibited RNA synthesis equally ($P > 0.05$) regardless of whether N-DNA or D-DNA was used as the template. The type 3 sera inhibited template activity mainly of N-DNA ($P < 0.001$).

The sera of intact rabbits did not alter the DNA template activity. Unlike the sera of patients with SLE, the sera of immune rabbits inhibited RNA synthesis to a greater degree if D-DNA was used as the template. The pure antibodies isolated from these sera inhibited the template activity of both N-DNA and D-DNA to an equal degree ($P > 0.05$).

These results suggest that the ability of immune sera to inhibit DNA template activity is due to the presence of antibodies against DNA in these sera. The type of the antibodies is of great importance in determining whether the sera of patients with SLE can react with DNA. At a certain stage of the disease, the appropriate type of antibody is evidently produced.

Further investigations are required to study the ability of antibodies to penetrate into biologically active cells, but the results now obtained suggest that in a cell-free system antibodies of a certain type can interact with template DNA and inhibit RNA synthesis. They perhaps block those areas of the DNA molecule on which the enzyme RNA-polymerase "works." This view would be confirmed if it were proved that the antibodies against DNA present in the sera of patients with SLE are not passive "witnesses," but biologically active substances in the body.

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