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ROLE OF PHOSPHORIC ACID RESIDUES IN THE FORMATION OF ANTIGENIC DETERMINANTS OF DNA STRUCTURAL COMPONENTS

A. M. Poverennyi, A. P. Efimova, T. P. Il'ina, and A. S. Saenko

UDC 616.153.962.4-097:/616-008.939.333.2: 616-008.921.83-325

KEY WORDS: antigenic determinant, DNA, phosphoric acid residues, antigen-antibody reaction.

Antibodies to DNA were first found in patients with systemic lupus erythematosus (SLE) and for a long time their appearance was regarded as one of the most important signs of this disease. Subsequently antibodies interacting with various structural components of the nucleus, and carrying under physiological conditons a strong negative (DNA, RNA) or positive (histones) charge, were discovered in patients with SLE. By the use of monocloncal antibodies to DNA, synthesized by hybridomas created on the basis of spleen cells of mice with an autoimmune syndrome, it was found that they react with various macromolecules carrying a negative charge (for example, with proteoglycans) which are structures of cell membranes [3, 4].

These data suggest that the antigenic stimulus for the formation of naturally found antibodies, reacting with DNA, in some cases may be structures carrying a positive or negative charge under physiological conditons.

In the investigation described below the role of phosphoric acid residues was studied in interaction between antibodies induced to structures carrying a positive charge (thymidine) and a negative charge (denatured and UV-modified DNA).

EXPERIMENTAL METHODS

Antithymidine sera were obtained by immunization of rabbits with conjugates of ribosylthymine with bovine serum albumin, prepared by the method in [2]. Antisera to denatured DNA and UV-irradiated DNA were obtained by the method in [6]. As other sources of antibodies, sera from patients with SLE, under treatment in the Institute of Rheumatology, Academy of Medical Sciences of the USSR (Moscow), were used.

Antigens for radioimmunoassay were tritium-labeled thymidine and DNA isolated from *E. coli* W3110 thy^- cells by a modified method [5], with specific activity of 80,000 cpm/μg.

As inhibitors of the antigen-antibody reaction we used thymidine, thymidine triphosphate (TTP, from Calbiochem, USA), thymidyl tri-, hexa-, and octanucleotides – T_3, T_6, and T_8 respectively ("Biochemicals," USA), and also thymine and thymidine dimers, UV-irradiated DNA, and UV-irradiated DNA subjected to hydrolysis down to bases.

Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk.
(Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Nasononva.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 317-319, September, 1986. Original article submitted April 4, 1985.

0007-4888/86/0009-1235$12.50

TABLE 1. Inhibitory Activity (in %) of Thymidine, TTP, and Thymidyl Oligonucleotides in a System of Antithymidine Sera — Thymidine

Inhibitor	Antithymidine sera			
	rabbit No. 1	rabbit No. 2	rabbit No. 3	rabbit No. 4
Thymidine	57	61	60	56
TTP	11	9	13	9
T_3	36	37	31	39
T_6	20	20	22	24
T_8	13	11	16	

Legend. Concentration of inhibitor 10^{-6} M in sample, calculated as thymidine.

TABLE 2. Effect of Thymidine, TTP, and Thymidyl Oligonucleotides on Binding of Antisera to Denatured DNA and Sera of Patients with SLE with Denatured ^{3}H-DNA (% of inhibition)

Inhibitor	Antisera to denatured DNA			Sera from patients with SLE		
	rabbit No. 1	rabbit No. 2	rabbit No. 3	patient No. 1	patient No. 2	patient No. 3
Thymidine	11	13	13	9	11	11
TTP	24	23	24	19	19	20
T_3	19	22	19	17	19	16
T_6	53	48	51	43	45	47
T_8	49	49	50	48	49	47

Legend. Concentration of inhibitor 10^{-4} M in sample calculated as thymidine.

TABLE 3. Interaction of ^{3}H-UV-DNA (500 J/m^2) with Antiserum Specific for UV-DNA in the Presence of Various Inhibitors

Inhibitor	Quantity of inhibitor in sample, M	% of inhibition
Thymine dimers	$2\cdot10^{-5}$	1,6
Thymidine dimers	$2\cdot10^{-5}$	1,7
UV-DNA	$2\cdot10^{-8}$	1,1
	$2\cdot10^{-7}$	37
	$2\cdot10^{-6}$	82,8
Hydrolysate	$2\cdot10^{-8}$	1,2
UV-DNA	$2\cdot10^{-7}$	0,6
	$2\cdot10^{-6}$	3,7

Legend. Quantity of inhibitor in sample expressed as number of dimers (in moles).

Thymine and thymidine dimers were obtained by irradiation of frozen solutions of thymine and thymidine with UV-light (254 nm, 10,000 J/m^2). The quantity of dimers was determined by measuring the change in optical density before and after UV irradiation. DNA (from Sigma, USA) was irradiated with UV-light (254 nm, 500 J/m^2). UV-irradiated DNA was hydrolyzed in 98% formic acid for 2 h at 175°C.

Antigen–antibody immune complexes were isolated by precipitation with ammonium sulfate at 50% saturation [7] or by precipitation on nitrocellulose filters [1].

The quantity of serum causing binding of 50% of labeled antigen was used in the inhibition test.

EXPERIMENTAL RESULTS

The results of investigation of the inhibiting activity of thymidine, TTP, and thymidyl oligonucleotides in the reaction between thymidine and antibodies to it are given in Table 1. The strongest inhibitor effect was produced by thymidine, in the presence of which, binding of labeled thymidine with antibodies was inhibited by 56-61%. TTP inhibitied this interac-

tion by only 9-13%. Thus, the charge of the phosphate residues prevents binding of thymidine, when in the composition of TTP, with antibodies.

Thymidine oligonucleotides also had a much weaker inhibitory action than thymidine. In this case, with an increase in length of the oligonucleotide, the percentage by which they inhibited binding of the antigen with antibodies decreased, and for the octanucleotide varied from 11 to 16.

Table 2 gives data on interaction of thymidine, TTP, and thymidyl oligonucleotides with antisera to denatured DNA and with sera from patients with SLE. TTP (19-24%) had stronger powers of inhibiting binding of antigen with antibodies than thymidine (9-13%). In this case, evidently, the charge of the phosphate group promoted binding of antigen with antibodies. The inhibitory activity of the oligonucleotides was even stronger than that of thymidine.

Table 3 gives data indicating that only thymine dimers, bound with phosphate groups (in the composition of UV-irradiated DNA), can interact with specific antiserum and inhibit its binding with labeled antigen. Under these circumstances dimers, in a concentration of $2 \cdot 10^{-6}$ M (contained in 10 μg of UV-irraidated DNA) inhibited the direct reaction virtually completely (82.8%). The inhibitory activity of the same quantity of dimers in a hydrolysate of UV-irradiated DNA (without phosphate residues) was only 3.7%. Thymine and thymidine dimers obtained during UV-irradiation of the frozen solutions, even in a higher concentration ($2 \cdot 10^{-5}$ M), possessed no inhibitory activity. The fact that their inhibitory powers were weaker than those of dimers of the UV-DNA hydrolysate was probably due to the presence of large quantities of inactive isomers of the dimers.

It can thus be tentatively suggested that structures in the antigen molecule carrying a charge are of great importance for the formation of the antigenic determinant. In cases when no such structures are present in an antigen, their introduction into its analog may prevent interaction with antibodies.

The results suggest that the presence of charged structures in biopolymers, differing significantly in their structure, may determine the presence of cross reactions with antibodies induced by one of them.

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