ANTIBODIES TO SINGLE-HELICAL DNA IN THE SERA OF HEALTHY PERSONS AND LABORATORY ANIMALS

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A few years ago antibodies against DNA were found in the sera of patients with systemic lupus erythematosus. Using a micromodification of the complement fixation reaction suggested by Grossman and co-workers [7], several workers [8-10] found that preparations of DNA denatured by boiling are serologically more active than native DNA. By means of serological reactions developed by the present authors – the passive hemagglutination reaction (PHR), the passive hemaglutination inhibition reaction (PHIR), and the antibody neutralization reaction (ANR) – the existence of two types of antibody against DNA was demonstrated [4]. One type is directed against formalinized denatured DNA, the other against native DNA. A link was thus established between the serological activity of DNA and its macrostructure.

In the present investigation evidence was obtained of the existence of antibodies (normal) in the sera of healthy persons and laboratory animals against single-helical DNA.

EXPERIMENTAL METHOD

Nucleic acids were isolated from the tissues of animals and from bacterial cells. DNA was isolated from hens' erythrocytes by a method involving separation of the nucleoproteins on the basis of differences in their solubility in salt solutions of different concentration and on phenol deproteinization [6]. The N/P ratio in the preparation was 1.62, and the molecular melting point 86°. The bacterial DNAs were located from Pseudomonas pyocyanea and Corynebacterium pyogenes by precipitation with procollagen [3]. The N/P ratio in the preparation from C. pyogenes was 1.74 and its molecular melting point 82°. The N/P ratio in the preparation from P. pyocyanea was 1.68 and its molecular melting point 97°. RNA was isolated from rabbit's liver by Georgiev's method [1]. High-polymer RNA precipitated by 1 M NaCl solution was used in the experiments. No DNA could be detected in 1 mg of the preparation by Dische's diphenyl method.

The method of treatment of the DNA by heat and formalin and of obtaining the formalinized sheep's erythrocytes sensitized to DNA was described earlier [4]. The serum of a patient with systemic lupus erythematosus agglutinated the sensitized erythrocytes in dilutions of 1:8000-1:32,000. The serum was heated for 30 min at 56° before the investigation, and the formalinized sheep's erythrocytes were then added in a dose of 0.2 ml of a 50% suspension of erythrocytes per ml whole serum. After shaking, the serum was allowed to stand for 18 h at 4°. and the supernatant fluid was then separated from the residue. This treatment was necessary because many of the blood sera contained heterophilic antibodies against sheep's erythrocytes in low dilutions. The γ-globulin was isolated from the individual sera by methanol in the cold [6]. Paper electrophoresis was used to verify the purity of the γ-globulin.

In the first place the sera of the healthy persons and laboratory animals were investigated in the PHR in dilutions of 1:10-1:640. The solvent was a 1% solution of normal rabbit serum previously shown to be free from antibodies against DNA. The PHIR was carried out with those sera which were found to contain antibodies against DNA. For this purpose dilutions of serum were prepared, starting from 1:5, in volumes of 0.2 ml in seven rows. To the dilutions of the serum in row 1, the solvent was added in a volume of 0.2 ml, in row 2 a solution of native DNA in a concentration of 10 μg/ml and a volume of 0.2 ml was added, in row 3 a solution of formalinized DNA in a concentration of 10 μg/ml, in row 4 DNA solution in a concentration of 10 μg/ml treated with DNAase in a concentration of 100 μg/ml, in row 5 a solution of native DNA in a concentration of 50 μg/ml, in row 6 a solution of formalinized RNA in a concentration of 50 μg/ml, in row 7 a solution of RNA in a concentration of 50 μg/ml treated with RNAase in a concentration of 100 μg/ml. The experiments were carried out in polystyrene plates with wells. The plates were shaken and incubated for 30 min at 37°, after which one drop of DNA-sensitized erythrocytes was added to each well.

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TABLE 1. Activity of Sera of Healthy Persons and Laboratory Animals in the PHR

Test object	No. of individuals	Distribution of activity of sera in the PHR by titer						
		0*	1:10	1:20	1:40	1:80	1:160	1:320
Man	587	263	77	132	82	26	7	—
Guinea pig.	130	27	7	21	35	26	11	3
Albino rat	65	20	—	—	1	2	15	27
Golden hamster. .	52	42	4	3	3	—	—	—
Rabbit.	33	14	5	12	2	—	—	—
Albino mouse . . .	4	1	1	1	—	1	—	1

* Sera active in dilutions below 1:10 are shown in the column.

TABLE 2. Relationship between Age of Guinea Pigs and Titer of Antibodies against Single-Helical DNA

Wt. of guinea pigs (in g)	Distribution of animals according to titer of antibodies in the PHR						
	0	1:10	1:20	1:40	1:80	1:160	1:320
230	11	11	4	6	9	1	—
650	—	—	1	1	1	7	3

EXPERIMENTAL RESULTS

In every case the positive result of the PHR was specific: agglutination of the erythrocytes in the PHIR was inhibited only by the formalinized DNA, and the preparations of RNA, the native DNA, and the DNA treated with DNase gave no such effect (Table 1).

Hence, the majority of the sera of the animals and human subjects contained antibodies against single-helical DNA in some titer or other. It should be added that in the sera of the healthy persons and animals the antibodies could be found only with the aid of very highly active batches of sensitized erythrocytes, which had not previously been available.

Experiments with 30 sera from different species of animals showed conclusively that for neutralization of 1-2 serum units, 0.015-0.06 mg of formalinized DNA of varied origin was required, whereas the native DNA or the RNA preparations were inactive even in a dose of 40 μg.

In all probability the titer of the antibodies against single-helical DNA varied with age. Results indicating this were obtained with guinea pigs, whose age bears a definite correlation with body weight (Table 2).

In the PHR and ANR with plague antiserum and erythrocytes sensitized with capsular antigen of Pasteurella pestis, the addition of formaliinzed DNA had no effect on the results of the reaction. This confirms the specificity of the serological methods used.

It might be expected that antibodies against single-helical DNA are formed by the lymphoid tissue, but extracts of the spleen and lymph glands were inactive in the PHR, evidently on account of the liberation of certain DNA derivatives into the solution. The γ-globulin from the positive sera possessed the same activity as the original serum. Heating the serum for 30 min at 60° did not affect its activity, but heating to 70° rendered it inactive.

An unexpected discovery was the neutralizing effect of free formalin — in its presence the antibodies did not agglutinate the sensitized erythrocytes. Formaldehyde in concentrations of 0.1-0.2% did not affect the activity of the serum; higher concentrations of formaldehyde partially neutralized the antibodies; formalin in concentrations of 2-5% in the blood serum destroyed practically all its activity in the PHR. If such a serum, 1 h after the addition of formalin, was dialyzed against distilled water in the cold, 20-40% of its original activity was restored; however, if the serum was dialyzed 24 h after the addition of the formalin, its activity in the PHR was not restored.

It was previously shown [2, 4] that erythrocytes sensitized with DNA, with complete antigen of Brucella tularensis, and with plague toxin lose their activity after immersion in a 10% formalin solution. The authors' experiments confirmed this fact, but it was found that the loss of activity of the corresponding sera was associated, not with the influence of formaldehyde on the antigen or the antigen-antibody complex, but with its action on the serum antibodies. Meanwhile, high concentrations of formalin did not affect the capsular antigen of P. pestis or antibodies against it. The observations were made on the sera of various species of laboratory animals and on the sera of patients with systemic lupus erythematosus, containing antibodies not only aginst single-helical DNA, but also against native DNA.

Hence, in the blood sera of healthy persons and laboratory animals, antibodies against single-helical DNA were found. These antibodies were specifically neutralized by formalinized DNA and inactivated by formalin.

SUMMARY

Serologic reactions using formalinized erythrocytes sensitized with DNA were used to establish the presence of antibodies to monospiral DNA in the sera of healthy persons and various species of test animals. It was shown on the example of the guinea pig sera that the titer of antibodies increased with the age of animals. The above-mentioned antibodies were neutralized by formaldehyde concentrations of 0.2% and higher.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
