ANTIGENIC PROPERTIES OF DNA IN THE COMPOSITION OF AN ARTIFICIAL DNA-PROTEIN COMPLEX

(UDC 576.893.4.097.2:591.111.8-2)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 6, pp. 59-61, June, 1966
Original article submitted December 25, 1964

It has been discovered that DNA, in a complex with protein or polysaccharide, possesses antigenic properties [3, 6, 7, 11, 12]. However, some authors have been unable to obtain antibodies against DNA by immunization of animals with desoxyribonucleoprotein (DNP) [5, 9].

In an earlier investigation, it was shown that the antigenic properties of DNA cannot be exhibited by immunization with purified DNA preparations, but antibodies against DNA are formed by immunization with natural DNP [2].

In the present investigation, carried out at the suggestion and under the direction of L. A. Zil'ber, the possibility of formation of antibodies against DNA artificially combined with protein was investigated.

EXPERIMENTAL METHOD

DNA was obtained from the liver of albino rats, chicken liver, and the cells of a Seidel ascites hepatoma by Georgiev's method [1]. The preparations were deproteinized by treatment with phenol four times and by Sevag's method several times. Protein was detected in the preparations by Lowry's micromethod [10] in an amount of less than 1%. The N/P ratio varied from 1.76 to 1.8.

The hyperchromic effect was 40% and the value of Ep 6500. The artificial complexes of DNA from rat's liver and ox serum albumin (DNA-OSA) and DNA from rat's liver and horse serum α-globulin (DNA-HSG) were obtained by the formula given by Tongur, Diskina, and Spitkovskii [4], with certain modifications. The artificial complexes contained 65-70% DNA and 30-35% protein.

The immunization of the rabbits with DNA and DNA-protein preparations and the passive hemagglutination reaction (PHR) with formalinized, tanninized sheep's erythrocytes, sensitized with DNA, were carried out by the methods described previously [2].

EXPERIMENTAL RESULTS

The results of the study of the sera of rabbits immunized with DNA from rat's liver in the PHR showed that these sera did not contain antibodies capable of reacting with the DNA of rat's liver (Fig. 1). Agglutination of the DNA-sensitized erythrocytes by the sera in a dilution of 1:10 cannot be regarded as specific, for in these dilutions the sera also agglutinated unsensitized erythrocytes. Normal sera of the same rabbits agglutinated the erythrocytes in the same titers. The study of the sera of rabbits immunized with DNA-OSA and DNA-HSG preparations in the PHR with erythrocytes sensitized by DNA from rat's liver showed that the tested sera agglutinated DNA-sensitized

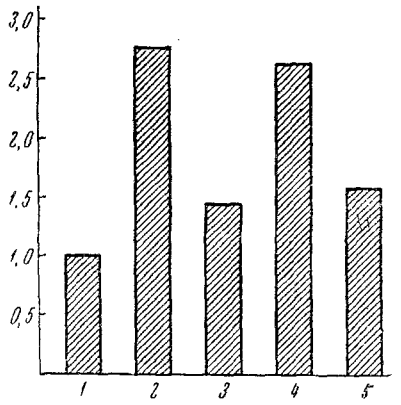

Fig. 1. Immunological activity of rabbit sera against DNA of rat's liver in a DNA-protein complex in the PHR with DNA of rat's liver. Data for six sera against DNA, 3 against DNA-OSA, and three against DNA-HSG. Along the axis of abscissas: 1) sera against DNA; 2) against DNA-OSA; 3) IPHR of sera against DNA-OSA with preparations of DNA from rat's liver; 4) sera against DNA-HSG; 5) IPHR of sera against DNA-HSG with preparations of DNA from rat's liver; along the axis of ordinates (here and in Fig. 2) — log of reciprocals of titer of sera.

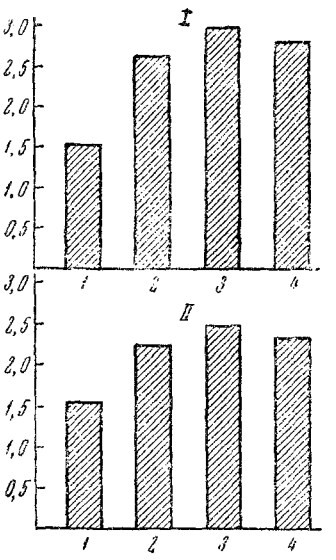

Fig. 2. Study of the specificity of antibodies against DNA in rabbit sera against DNA-protein complex in the PHR. I — Sera against DNA-OSA; II — against DNA-HSG. Along the axis of abscissas: 1) action of DNAase; 2) action of trypsin; 3) PHR with DNA of chicken liver; 4) with DNA of Seidel's ascites hepatoma.

erythrocytes in dilutions of 1:320 to 1:640 (Fig. 1). Normal sera of the same rabbits gave a negative PHR. The immune sera did not agglutinate erythrocytes not sensitized with DNA. The results of the reaction of inhibition of passive hemagglutination (IPHR) showed that DNA inhibits the agglutination of sensitized erythrocytes (Fig. 1).

To prove that the test sera contained antibodies against DNA, the action of DNAase on sensitized erythrocytes was used. Sensitized erythrocytes, treated with DNAase, were added to both immune and normal sera of the same rabbits. The normal sera did not react with erythrocytes treated with DNAase. The results of the PHR of the immune sera with DNA-sensitized erythrocytes treated with DNAase demonstrate that DNAase depressed the PHR (Fig. 2).

It was considered that the DNA preparations contained slight traces of protein, firmly bound with the DNA molecule. This protein, however, could stimulate the production of antiprotein antibodies, and its interaction with these antibodies could affect the PHR. To detect the possible role of protein contaminants, the PHR was studied with DNA-sensitized erythrocytes treated with trypsin. As is clear from Fig. 2, trypsin did not affect the results of the reaction.

The PHR was also studied with erythrocytes sensitized with DNA of chicken liver and of cells of Seidel's ascites hepatoma. The immune sera reacted with these DNA preparations in the same titers as with the DNA of rat's liver (Fig. 2).

The results thus show that purified DNA preparations, incapable of causing antibody formation after immunization of animals, acquire this property after attachment of the DNA to heterologous protein, as revealed by the PHR with formalinized, tanninized erythrocytes, sensitized with DNA. DNA preparations inhibited the PHR with DNA-sensitized erythrocytes.

The DNA in the composition of an artificial DNA-protein complex, and not the admixtures of homologous protein, was responsible for the production of antibodies detected by the PHR. This is confirmed by the fact that the action of DNAase on DNA-sensitized erythrocytes inhibited agglutination, while the action of trypsin had no effect on its result.

The negative results obtained by immunization of animals with DNA preparations suggest that well-purified DNA preparations do not contain contaminants causing antibody formation detectable by the PHR. In any case, so little of these comtaminants was present that the negligible amount of antibodies formed in response to them could not be detected even by such a highly sensitive reaction as the PHR with formalinized, tanninized erythrocytes.

The results confirm that antigenic properties can be detected in DNA artificially combined with protein. It may be assumed that DNA, in the antigenic respect, belongs to the semi-haptens. At the same time, it must be remembered that DNA, after introduction into the organism, is immediately destroyed by DNAase, and therefore, as has been suggested [8], it cannot reveal antigenic properties. Meanwhile, when forming a complex with protein, DNA is not destroyed by DNAase and thus stimulates antibody production.

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