THE ANTIGENIC PROPERTIES OF DNA

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The information on the antigenic properties of DNA is contradictory both in the results of several investigations as well as in the interpretation of these results. Results obtained in immunization with purified DNA preparations show that DNA does not possess antigenic properties [6, 10]. However, the results of study of autoantibodies against DNA in lupus erythematosus patients [2] have shown the possibility of formation of antibodies against DNA. Antibodies against DNA were found following immunization with microbial DNA preparations and with microbial suspensions [3, 7, 15], as well as with immunization with tissue DNA [5]. Nearly always in such studies DNA preparations adulterated with proteins were used. It has also been stated that DNA – protein complexes possess antigenic properties [4, 17], but this was denied by several authors [6-11].

Thus from the published results on the problem, it is impossible to come to a conclusion on the antigenicity of DNA. It may be assumed however that molecules as big as those of DNA may be complete antigens, haptens, or semihaptens. In order to elucidate certain aspects of this problem we have following the suggestion of L. A. Zil'ber, and under his supervision, conducted a comparative study of antigenic properties of pure DNA from rat tissues and of the same DNA in complex with a protein (DNP).

EXPERIMENTAL METHOD

DNP was obtained from livers of white rats according to the method of Mirsky and Pollister [14]. The protein in DNP preparations was determined by the Biuret test, nitrogen by the method of Kjeldahl-Conway and phosphorus according to Lowry and Lopez [12]. The ratio N/P varied between 3.5 and 3.7. DNA was obtained by the method of Georgiev [1] from the livers of white rats and chickens and from ascites cells of Zaidell's hepatoma passaged through white rats. The DNA was deproteinized with phenol four times and then (deproteinized) by the method of Sevagh. By the method of Lowry [13] protein in DNA preparations constituted 0-0.03%. The N/P ratio varied between 1.76 and 1.8. The hyperchrome effect was 40%; E(p) was equal to 6500. Rabbits were immunized with rat liver DNA and DNP intraperitoneally at 2 day intervals. DNA preparations were injected 6 times in doses of 15, 20, 25, 30, 35, and 40 mg. DNP was injected in the same doses determined according to their protein content. Passive hemagglutination reaction (PHR) with formalinized tanned sheep erythrocytes, sensitized with DNA was used in modification [2, 9].

RESULTS

The results of study of sera of 6 rabbits immunized with DNA have shown that they did not contain antibodies which were able to react with DNA.

Examination of anti DNP antisera has shown that they contained antibodies which could react with homologous DNA in titers between 1:160 to 1:640 (Table 1). The results of inhibition of PHR have shown that homologous DNA

TABLE 1. PHR of Antisera against DNP of Rat Liver with DNA of Rat Liver and Inhibition of PHR by DNA Preparations of Rat Liver

Antiserum number	Titer in PHR	Titer in inhibition of PHR
13	1:320	1:20
73	1:320	1:20
83	1:640	1:10
84	1:320	1:10
45	1:320	1:10
41	1:160	1:20

TABLE 2. Detection of Antibodies against DNA in Rat Liver DNP Antisera in Inhibition of PHR

Antigen	Antiserum number					
	13	73	83	84	45	41
Rat liver DNA + DNAase	1:10	1:20	1:40	1:20	1:40	1:40
Rat liver DNA + trypsin	1:320	1:160	1:640	1:160	1:160	1:160
Chicken liver DNA	1:320	1:320	1:640	1:320	1:320	1:160
Hepatoma DNA	1:160	1:320	1:640	1:160	1:320	1:160

inhibited the agglutination of sensitized erythrocytes. PHR with normal sera of the same rabbits was negative. The immune sera did not interact with erythrocytes treated with formalin and tannic acid, but not sensitized by DNA.

In order to prove that the sera contained antibodies against DNA we have treated the DNA-sensitized erythrocytes with DNAase. The PHR results of immune sera with DNAase-treated-sensitized erythrocytes are shown in Table 2. DNAase inhibited PHR, but inhibition was not complete.

It could be assumed that the protein impurities which could not be completely removed, played the part of antigens and that their reaction with antiprotein antibodies was manifested in PHR. In order to study this problem we have conducted PHR with trypsin-treated sensitized erythrocytes. As seen from Table 2 trypsin had no effect on the results of the reactions.

Immune sera reacted with DNA of chicken liver and with Zaidell's hepatoma ascites cells in the same titers as with homologous DNA.

Thus our results have shown that when animals were immunized with purified DNA preparations, no antibodies against DNA were produced as evidenced by the negative results of such highly sensitive reactions as PHR with DNA sensitized, formalinized, and tanned erythrocytes.

In the sera of animals immunized with DNP there were produced antibodies which reacted with DNA of a different origin. The specificity of PHR was confirmed by its inhibition by DNA. The fact that the DNA portion of DNP and not the protein was responsible for the production of antibodies, as revealed in PHR, was confirmed by the fact that the effect of DNAase on DNA-sensitized erythrocytes inhibited the agglutination of erythrocytes, while trypsin had no effect.

Thus when rabbits were immunized with DNP preparations they produced antibodies against DNA in their blood serum; this would indicate that DNA possesses at least semi-hapten properties. In considering the data which show that purified DNA preparations did not elicit antibody production, while DNA bound to protein did, it is necessary in our opinion to keep in mind that DNA introduced into an organism may be destroyed by DNAase which is present in most tissues of higher animals in sufficient quantities. The destruction of DNA by DNAase has been confirmed not only in vitro, but also in vivo [16]. These data are in accord with a proposition [8] that DNA is not able to stimulate the production of antibodies, because it is hydrolyzed by DNAase immediately after its introduction into the body. From the above it is impossible to deduce why only protein-bound DNA molecules stimulate antibody production. It may be that the protein protects the DNA molecules from the action of DNAase, and thus permits DNA to manifest its antigenic properties. It may also be assumed that DNA possesses the properties of either a hapten or a semi-hapten and in such a case the protein would play the part of a carrier. Whichever way it may be, protein bound to DNA creates conditions under which DNA is able to elicit antibody production. Some authors

[18, 19, 20] obtained antisera of very low titers following immunization of animals with DNA. It may be supposed that in these studies the small amounts of protein bound to DNA created conditions under which antibodies in low titers could be produced.

SUMMARY

Immunization with purified DNA preparations failed to stimulate the production of antibodies. Immunization with DNP preparations stimulated the production of antibodies to DNA. The DNA molecule is capable of stimulating the production of antibodies only when it is associated with protein.

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