VIRUS-NEUTRALIZING PROPERTIES OF ANTIINFLUENZAL RABBIT IgG WITH REDUCED COMPLEMENT-FIXING AND CYTOPHILIC ACTIVITY

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UDC 576.858.75.097.5

Correlation between the effector functions and virus-neutralizing activity of antiviral immunoglobulin G (IgG) antibodies was investigated in relation to rabbit IgG antibodies against A/PR_8/34 influenza virus. Changes in the effector activity of the antiviral antibodies were produced by reducing the single disulfide bond between the heavy chains in the hinge region of the IgG molecule. Antiinfluenzal IgG, when reduced in this way, retained about 50% of its complement-fixing activity but lost virtually all its ability to be bound to heterologous tissues. Meanwhile the reduced antiinfluenzal IgG, as the results of the delayed hemagglutination test showed, completely preserved its antigen-binding activity. The virus-neutralizing activity of the reduced antiinfluenzal IgG, as estimated in experiments on chick embryos, was indistinguishable from the activity of the native preparation if the dose of virus used in the experiments was 100 EID_{50}. If the dose of virus was increased to 1000 EID_{50} the virus-neutralizing activity of the reduced IgG was less than that of the native preparation. The results are discussed in terms of differences in the structural organization of reduced IgG.

KEY WORDS: IgG antibodies; antigen-binding; virus-neutralizing; effector functions of immunoglobulins; disulfide bonds between IgG chains.

Previous investigations showed that after reduction of the single disulfide bond in the immunoglobulin G (IgG) molecule of antibodies between the heavy chains there is a marked decrease in the complement-fixing and cytophilic activity of the modified antibodies although they retain their ability to bind antigen and to neutralize bacterial toxins [5, 6].

Considering that the investigation of reduced antibodies would shed light on the correlation between their effector functions and specific neutralizing properties, the present investigation was undertaken in order to study the virus-neutralizing activity of reduced rabbit antiinfluenzal IgG antibodies.

EXPERIMENTAL METHOD

Influenza virus strain A/PR_8/34 was used in the experiments after repeated passage through chick embryos. Eluates of the virus obtained from infected allantoic fluid by adsorption and subsequent elution of the virus from formalinized human group O red cells were used as the antigen for the serological tests and for immunization [2].

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N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow. D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 188-191, August, 1977. Original article submitted February 21, 1977.

TABLE 1. Antihemagglutinating Activity of Native and Reduced Antiinfluenzal Rabbit IgG

Batch of antiinfluenzal IgG	Protein concentration, mg/ml	Titer of antiinfluenzal IgG in DHT*	
		native IgG	reduced IgG
I	1,8	160	160
II	7,2	1280	1280
III	7,6	1280	1280

*Reciprocal of dilution of IgG capable of completely inhibiting hemagglutination by 4-8 a.u. of virus.

Antiinfluenzal serum was obtained by immunizing rabbits with the eluate of influenza virus by the scheme described previously [3]. The titer of antihemagglutinins in the serum pool in the delayed hemagglutination test (DHT) was 1:5120; in the biological neutralization test the antiserum, in a dilution of 1:100, neutralized over 10^6 EID_{50} of virus.

IgG was obtained from the pool of antiinfluenzal rabbit sera in two steps. First, the total globulin fraction was precipitated by ammonium sulfate at 35% saturation and pH 7.4. The globulins were then chromatographed on Sephadex G-200 in 0.15 M NaCl, pH 7.8.

The fraction corresponding to the tip of the second peak (7S IgG) was collected, concentrated by ultrafiltration, sterilized by filtration through a Millipore membrane, and kept at 4°C.

The disulfide bond between the heavy chains of IgG was reduced with 0.005 M 2-mercaptoethanol (2ME) as described in [5]. The number of reduced disulfide bonds was estimated by titrating SH groups by Torchinskii's method [7]. The SH groups of the reduced IgG were blocked with 0.01 M sodium monoiodoacetate.

The hemagglutination test (HT) and DHT were set up in a volume of 0.5 ml, using a 5% suspension of human group O red cells.

The complement fixation test (CFT) was carried out by the standard method at 37°C in a volume of 1.25 ml, using 120% complement (native guinea pig serum).

The biological neutralization of influenza virus test was carried out on chick embryos, using 100 and 1000 EID_{50} of virus and various dilutions of IgG. Before infection the mixture of virus and antiinfluenzal IgG was kept for 30 min at 37°C.

The reversed passive cutaneous anaphylaxis test (RPCAT) was carried out as described previously [6].

EXPERIMENTAL RESULTS

After reduction of the antiinfluenzal rabbit IgG with 0.005 M 2ME at 20°C for 2 h, two SH groups were liberated for every mole of protein; i.e., one disulfide bond was broken. As was demonstrated previously, under these conditions the disulfide bond located between the heavy chains in the hinge region of the IgG molecule is broken [4].

A comparative study of three batches of native and reduced antiinfluenzal IgG in the DHT showed that they possessed identical antigen-binding activity (Table 1).

Native and reduced IgG of batch I were tested in the CFT. Reduced IgG was found to have substantially less complement-fixing activity than the reduced protein. Under the conditions chosen for the CFT, to produce complete fixation of the dose of complement used in the test twice as much reduced IgG as unreduced had to be used.

The results of the RPCAT showed that the minimal cutaneous sensitizing dose of unreduced antiinfluenzal IgG was 0.33 ± 0.09 μg, whereas the dose for reduced IgG was not less than 10 μg.

As a result of reduction in the disulfide bond between the heavy chains in the hinge region of the molecule of antiinfluenzal rabbit IgG its antigen-binding activity, determined in vitro, was unchanged. Meanwhile a

TABLE 2. Virus-Neutralizing Activity of Native and Reduced Antiinfluenzal Rabbit IgG

Antiinfluenzal IgG	Dilution	Dose of virus used for infection: 100 EID_{50} number of embryos with reproduction of virus / number of infected embryos	100 EID_{50} titer of virus in HT	1000 EID_{50} number of embryos with reproduction of virus / number of infected embryos	1000 EID_{50} titer of virus in HT
Native	1 : 20	0/4	0	0/4	0
	1 : 40	0/5	0	0/4	0
	1 : 80	0/4	0	0/4	0
	1 : 160	2/4	320	1/4	160
	1 : 320	4/4	320	4/4	2560
Reduced	1 : 20	0/4	0	0/4	0
	1 : 40	0/4	0	0/4	0
	1 : 80	0/4	0	3/3	320
	1 : 160	2/4	320	4/4	160
	1 : 320	4/4	320	4/4	640
Control	—	4/4	2560	4/4	5120

marked decrease in its cytophilic activity and a substantial change in its ability to fix complement in the presence of the specific antigen were observed.

The results of determination of the virus-neutralizing activity of the reduced antiinfluenzal IgG in experiments on chick embryos are shown in Table 2. Clearly the virus-neutralizing activity of the reduced IgG was the same as that of the native preparation if 100 EID_{50} of virus was used in the experiments. However, on increasing the dose of virus up to 1000 EID_{50} the virus-neutralizing activity of the reduced IgG was less than that of the native preparation.

It can be concluded from these results that such highly important effector functions of antibody IgG as ability to be bound to heterologous tissues and to activate the complement system on binding to the specific antigen do not play an important role in the ability of the antibodies to block reproduction of viruses in chick embryos. However, the results do not answer the question of whether these effector functions of antiviral antibodies are essential to their protective properties in vivo in mammals.

Differences between the virus-neutralizing activity of reduced and native antibodies when a dose of 1000 EID_{50} was used to infect the embryos can be explained on the grounds of differences in the structural organization of the reduced IgG and, in particular, of existing information showing an increase in the flexibility of its molecule [3]. In the presence of an excess of antibodies, neutralization of the virus can be postulated to be connected with the covering of the individual virions by antibodies. In the presence of equivalent proportions of antibody and virus or a relative excess of virus, neutralization is due to the formation of rigid lattice structures, similar to the structure of the immune precipitate. In the case of an increase in the flexibility of the antibody IgG molecule following rupture of the disulfide bond in the hinge region the formation of a rigid lattice is less easy, and this reduces the efficiency of virus neutralization. The hypothesis of correlation between flexibility of the molecule of bivalent antibodies and the efficiency of virus neutralization was put forward previously by Gutman and Kul'berg [1].

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