INCREASING THE SPECIFIC ACTIVITY OF ANTI-INFLUENZA SERUM BY ANTIGLOBULIN ANTIBODIES AND THEIR BIVALENT F(ab')$_2$-FRAGMENTS

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γ G-antibodies against rat globulin, added to a combination of influenza virus and rat type-specific antibodies, potentiated the neutralizing action of the antiviral antibodies by 8-16 times in the delay-of-hemagglutination test and also in the virus-neutralization test in chick embryos. Antiglobulin antibodies by themselves did not inhibit the virus. If the concentration of type-specific and antiglobulin antibodies remained constant, the potentiating action of the latter was unchanged with an increase in the infecting dose of the virus by 100-1000 times. The potentiating action is decisively dependent on the integrity of the antiglobulin antibody molecules, for their bivalent F(ab')$_2$-fragments have considerably lower activity. It is concluded that the mechanism of action of antiglobulin antibodies is connected with an increase in the effectiveness of 3-dimensional screening of the virus particles through the formation of immune aggregates with a rigid reticular structure.

The analysis of the mechanism of the virus-neutralizing action of specific antibodies is an important task in the study of immunity to viruses. The recently described phenomenon of increased activity of virus-neutralizing antibodies as a result of the addition of antiglobulin serum to the formed immune complex is very interesting in this connection [4, 5, 10-12]. The investigation of the mechanism of this phenomenon provides an approach to the explanation of the process of steric screening of virus particles in the immune complex. It can be postulated that the virus particles are most effectively screened by the formation of aggregates with a rigid reticular structure.

The action of antiglobulin antibodies and their bivalent F(ab')$_2$-fragments on the complex of influenza virus with rat type-specific antibodies was investigated.

EXPERIMENTAL METHOD

The γG-globulin fraction of rabbit serum against rat immunoglobulin, isolated by ion-exchange chromatography on DEAE-Sephadex as described previously [2], was used in the work. The preparations did not contain natural antiviral inhibitors. According to the results of the quantitative-precipitations test [3] 1 ml of γG-globulin contained 0.84 mg antibodies against rat γ-globulin.

Bivalent F(ab')$_2$-fragments of antiglobulin antibodies were obtained with the aid of pepsin by a modified Nisonoff's method [2, 11]. The F(ab')$_2$-fragments retained their ability to precipitate rat γ-globulin but they did not react with antibodies against the Fc-fragment of rabbit γG-globulin. Before the hemagglutination-inhibition test and influenza virus neutralization in chick embryos, strains of type A (PR-8) and A1 (Em$_1$) virus were incubated for 30 min at 22°C with serial 1:2 dilutions of the corresponding rat monospecific sera. The antiglobulin antibodies or F(ab')$_2$-fragments were then added to all tubes in a volume of 0.5 ml and the samples allowed to stand at 37°C for 45 min. The subsequent procedures and the reading of the reactions were carried out by standard methods.

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TABLE 1. Dependence of Titer of Specific Anti-influenza Antibodies on Concentration of Antiglobulin Antibodies

Monospecific serum	Antiglobulin antibodies (in mg)	Influenza virus used in test: strain	Influenza virus used in test: dose	Titer of antibodies in monospecific serum	Titer of antibodies in monospecific serum after addition of antiglobulin antibodies
Hemagglutination inhibition test[1]					
Against strain Em_1	—	EM_1	8	1:2 560	—
» »	0,14	EM_1	8	—	1:20 480
» »	0,05	EM_1	8	—	1:20 480
» »	0,003	EM_1	8	—	1:10 240
» »	0,0015	EM_1	8	—	1:2 560
Neutralization test[2]					
Against strain Em_1	—	EM_1	100	1:320	—
» »	0,14	EM_1	100	—	1:5 120
» »	—	EM_1	10 000	1:320	—
» »	0,14	EM_1	10 000	—	1:2 560
» »	—	EM_1	1 000 000	1:80	—
» »	0,14	EM_1	1 000 000	—	1:640
» »	0,05	EM_1	10 000		1:1 280
To strain PR-8	—	PR-8	10	1:640	—
» »	0,14	PR-8	10	—	1:10 240
» »	—	PR-8	10 000	1:320	—
» »	0,14	PR-8	10 000	—	1:10 240
» »	—	PR-8	1 000 000	—	1:10 240
» »	0,14	PR-8	1 000 000	—	1:51 280
» »	0,05	PR-8	10 000	—	1:51 280

Note. Here and in Table 2: 1) dose of virus in a.u. (agglutinating units); 2) dose of virus in ID_{50} (infectious dose of virus for chick embryos).

TABLE 2. Effect of Antiglobulin Antibodies on Titer of Monospecific Rat Sera in Hemagglutination Inhibition and Neutralization Tests

Monospecific serum	Antiglobulin antibodies (in mg)	$F(ab')_2$-fragments of antibodies (in mg)	Influenza virus used in test: strain	Influenza virus used in test: dose	Titer of antibodies in monospecific serum
Hemagglutination inhibition (in mg)					
Against strain EM_1	—	—	EM_1	8	1:640
» »	0,14	—	EM_1	8	1:5120
» »	—	0,1	EM_1	8	1:1280
Neutralization test					
Against strain EM_1	—	—	EM_1	100	1:320
» »	0,14	—	EM_1	100	1:2560
» »	—	0,1	EM_1	100	1:640

EXPERIMENTAL RESULTS

Data showing the effect of antiglobulin antibodies on the titer of monospecific rat anti-influenza serum in the hemagglutination inhibition and neutralization tests are given in Table 1. Addition of antiglobulin antibodies to the virus-specific antibody complex evidently potentiated the blocking action of the latter by 8-16 times. Antiglobulin antibodies by themselves did not inhibit the virus in the absence of specific antibodies. The essential fact was that with the same dose of type-specific and antiglobulin antibodies the potentiating action of the latter in the neutralization test was unchanged if the infecting dose of virus was increased by 100-1000 times. Meanwhile the potentiating action of the antiglobulin antibodies was directly proportional to their concentration.

Since a considerable increase in the infecting dose of virus had no effect on the potentiating action of the antiglobulin antibodies used in the same concentration, it can be concluded that their action is due rather to aggregation of virus particles sensitized by the antibodies than to the more effective screening of the surface of individual particles not associated together. From this point of view it was of great interest to study the action of bivalent F $(ab')_2$-fragments of the antiglobulin antibodies on sensitized virus particles. Bivalent fragments of antibodies, like the undissociated antibodies, can aggregate antigens but, unlike the latter they have lost the inactive Fc-portion of the γ G-globulin molecule [1].

As Table 2 shows, F $(ab')_2$-fragments of antiglobulin antibodies taken in an equimolar concentration relative to the unsplit antibodies have significantly less potentiating action. This at first sight paradoxical effect can be explained on the basis of the structural organization of the bivalent fragments. From data obtained by electron microscopy and other physical methods of investigation it can be accepted that the γG-antibody molecule is Y-shaped, the upper arms consisting of Fab-fragments and the leg of an Fc-fragment [7]. The Fab-fragments can move relative to the Fc-fragment, and the angle between them increases

considerably during the formation of large antigen–antibody aggregates [6-8]. The presence of the Fc-fragment is evidently a factor which restricts to some extent the freedom of rotatory movement of the Fab-fragments which, in turn, may help to increase the rigidity of the reticular structure of the immune aggregates. So far as the $F(ab')_2$-fragments are concerned, both monovalent subunits composing it and joined by a single disulfide bond must possess considerably greater mobility than in the γG-antibody molecule. With this in mind it can be concluded that immune aggregates formed by bivalent fragments will not have the same degree of rigidity of their structural organization as the same aggregates formed with the aid of unsplit antibodies. With respect to the system studied in the present investigation differences in the structure of aggregates formed by antiglobulin antibodies and their $F(ab')_2$-fragments may be manifested as unequal effectiveness of screening of the virus particles, which must be much more marked when the virus particles are packed into a rigid reticular structure.

The results described in this paper can be regarded as a basis for the study of the mechanisms of action of antiviral antibodies. Since indirect 3-dimensional screening of the virus receptors evidently plays an important role in the neutralization of viruses by specific antibodies, the effectiveness of this process largely depends on the structural features of the virus particle and on the character of the macromolecular organization of the specific antibodies and, in particular, of those parts of the molecules which lie outside the zone of their active centers. New approaches are thus provided to the comparative study of the virus-neutralizing action of antibodies belonging to various classes of immunoglobulins and of the mechanism of virus reactivation from neutral mixtures of virus with specific antibodies.

LITERATURE CITED

1. A. Ya. Kul'berg, in: Fundamentals of Molecular Biology (Virology and Immunology) [in Russian], Moscow (1964), p. 208.
2. A. Ya. Kul'berg, Yu. V. Docheva, I. A. Tarkhanova, et al., Biokhimiya, 34, 1178 (1969).
3. E. A. Kabat and M. M. Meyer, Experimental Immunochemistry, Springfield, Ill. (1961).
4. W. K. Ashe and A. L. Notkins, Proc. Nat. Acad. Sci. (Washington), 56, 447 (1966).
5. A. Brown, W. Elsner, E. Zebovitz, et al., Proc. Soc. Exp. Biol. (New York), 130, 718 (1969).
6. A. Feinstein and D. Row, Nature, 205, 147 (1965).
7. N. M. Green, Advances Immunol., 11, 1 (1969).
8. N. E. Hyslop, R. R. Dourmashkin, N. M. Green, et al, J. Exp. Med., 131, 783 (1970).
9. A. Nisonoff, F. C. Wissler, L. N. Lipman, et al., Arch. Biochem. Biophys., 89, 230 (1960).
10. A. L. Notkins, M. Suellen, C. Scheele, et al., J. Exp. Med., 124, 81 (1966).
11. D. D. Porter and A. E. Larsen, Proc. Soc. Exp. Biol. (New York), 126, 680 (1967).
12. E. Zalan, E. Borman, and N. A. Labzoffsky, Arch. ges. Virusforsch., 34, 209 (1971).