SOME FEATURES OF THE TOXIN-NEUTRALIZING ACTION OF ANTITETANUS IMMUNOGLOBULIN AND ITS FRAGMENTS

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The neutralizing ability of antitetanus immunoglobulin (IgG) and its fragments – $F(ab')_2$, Fab', Fd, and L-chains – against free and protagon-bound tetanus toxin was investigated in experiments in vitro. The IgG was obtained from the serum of rabbits immunized with toxoid. With a decrease in the size of the molecule the neutralizing power of the antitoxin against free toxin was reduced. Toxin-neutralizing power calculated per active center was practically identical in the $F(ab')_2$ and Fab' fragments. The neutralizing power of IgG, $F(ab')_2$, and Fab' against toxin bound on protagon increased with a decrease in size of the antitoxin molecule. The possible mechanisms of the effects described are examined.

In connection with the study of the structure of the active center of antibodies and the functional properties of immunoglobulins the problem of immunospecific activity of the various subunits and fragments of the antibody molecule has attracted great attention† [7, 11, 16-19]. In this connection the comparative study of the toxin-neutralizing ability of segments of the antitoxin molecule which differ in size is of considerable interest to both theoretical and practical immunology.

This paper describes a study of the toxin-neutralizing properties of the various subunits and fragments of tetanus antitoxin carried out for this purpose using a medium containing free tetanus toxin and toxin bound on a target substrate (protagon).

EXPERIMENTAL METHOD

The antitoxic serum was obtained from rabbits immunized with purified, adsorbed tetanus toxoid. Immunoglobulin G (IgG) was isolated from the antitoxic sera by the method described by Tarkhanova et al., [11], and the $F(ab')_2$-fragment was obtained by digestion of the IgG with crystalline pepsin (37°C, 8 h, pH 4.1) [21]. The $F(ab')_2$ was purified on CM-cellulose in 0.01 M acetate buffer, pH 5.8 [20]. To obtain Fab' fragments from $F(ab')_2$, the latter was reduced with 0.01 M cystein and alkylated with monoiodoacetic acid. The Fd-fragment and the light chains (L-chains) were obtained by an original modification [11] of Fleischmann's method [17].

Sedimentation analysis of the preparations was carried out in a Spinco Model E ultracentrifuge at 20°C in 0.01 M phosphate buffer, pH 8.6. The speed of centrifugation in a standard cell was 59,780 rpm. The coefficient of diffusion was found by Allison's method [13, 14].

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† The nomenclature of the immunoglobulins and their subunits used in this paper is that recommended by the World Health Organization [15].

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TABLE 1. Toxin-Neutralizing Activity of Preparations of Antitetanus IgG and Its Fragments

Antitoxin preparation	Molecular weight		Toxin-neutralizing activity		Decrease in activity compared with IgG	
	in aqueous solutions	in 8 M urea	i.u./mg	i.u./mmole	per 1 mg protein	per active center
IgG	150 000	—	15	2250	—	—
F (ab')$_2$	100 000	—	8	800	Twice	3 times
F ab'	51 000	—	8	40	"	"
Fd	37 400	21 600	3,5	—	4.3 times	—
L-chain	29 000	20 000	0,1	—	150 times	—

The toxin-neutralizing activity of the antitoxic IgG and its fragments was determined by the standard method of titration in albino mice.

Protagon was obtained from bovine brain by a slightly modified method of Wilson and Cramer [25]. The protagon, suspended in physiological saline (50 mg to 1 ml), was treated with tetanus toxin (1 mg), the mixture was kept for 45 min at 37°C in darkness, and the free toxin was then carefully removed by washing and centrifugation 3-4 times, for 10 min each time, at 6,000 rpm and at 4°C. Different quantities of antitoxin (IgG and its fragments) were added to the washed protagon—toxin complex; the mixture was kept at 37°C for 90 min, and after washing (by centrifugation as described above) the residue was suspended in physiological saline, its pH checked, and injected into mice for titration of its toxicity by the standard method (the method of working with protagon was also described previously [5]).

EXPERIMENTAL RESULTS

When investigated in the ultracentrifuge in aqueous solutions the sedimentation constants of IgG, of the F(ab')$_2$-, Fab'-, and Fd-fragments, and of the light chains were 7S, 5S, 3.5S, 2.86S, and 2.6S, respectively (Table 1). Table 1 also gives the molecular weights of these preparations calculated from the sedimentation constants and the coefficients of forward diffusion determined in 8 M urea [23]. Comparison of the molecular weights of the Fd-fragment and of the light chain, found by determinations in urea, with the values obtained by investigation of the same preparations in aqueous solutions shows that in aqueous solutions 86% of the Fd-fragment of the antitoxin is in the dimer form, while 72% in the light chains is present as the dimer.

The antigenic characteristics of the various preparations and their behavior on immunoelectrophoresis were as described previously [11].

The toxin-neutralizing ability of the antitetanus IgG and its fragments relative to the free toxin is shown in Table 1. In their specific activity (calculated per unit weight and per active center) the F(ab')$_2$- and Fab'-fragments possessed only one-third to one-half of the neutralizing ability of IgG. Since in the preparation of the F(ab')$_2$- and Fab'-fragments no injury takes place to the active centers of the antibodies [22], the observed decrease in toxin-neutralizing activity of the F(ab')$_2$- and Fab'-fragments compared with the original IgG cannot be ascribed to any significant decrease in the affinity of these fragments for the antigenic determinants of the toxin.

In the discussion of this fact it must be remembered that the antitoxin evidently contains no antibodies against the actual toxophore group of the toxin, for in order to obtain the antitoxin, toxoid was used as the antigen, and because of chemical modification toxoid does not possess a toxophore group. In addition, as the result of a comparative investigation of antitoxins obtained by immunization of hens with tetanus toxin and toxoid shows, the toxophore group itself does not contain antigenic determinants [8]. In the light of this fact it is evident that neutralization of the toxophore group, which takes place after interaction between the antitoxin and antigenic determinants located outside the toxophore group of the toxin, takes place through screening of the toxophore group or through a change in the conformation of the toxin molecule so that its toxophore group loses its activity. In both cases not only a specific antideterminant but also other regions of the antitoxin molecule participate in these processes. This can explain the decrease in toxin-neutralizing activity associated with a decrease in size of the antitoxin molecule.

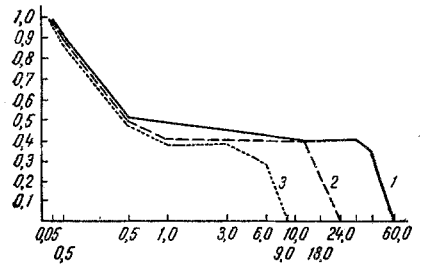

Fig. 1. Neutralizing ability of antitetanus IgG and its $F(ab')_2$- and Fab'-fragments relative to tetanus toxin bound on protagon. Abscissa, amount of antitoxin [IgG, $F(ab')_2$, and Fab'] added to protagon–toxin complex (in i.u.); ordinate, toxicity of residue after treatment of protagon–toxin complex with antitoxin (in MLD). 1) 7S (IgG); 2) 5S [$F(ab')_2$]; 3) 3.5S (Fab').

It is interesting to note that, calculated per active center, the neutralizing ability of $F(ab')_2$ was practically the same as that of the Fab'-fragment. Since, unlike Fab', the bivalent $F(ab')_2$ fragment can produce aggregation of the toxin molecules, their identical toxin-neutralizing activity could mean that the aggregation of the toxin by means of antitoxin does not play an essential role in the screening of the toxophore group.

The decrease in the toxin-neutralizing activity of the Fd-fragment could be due both to destruction of blocking of some of the active centers during the isolation of this part of the antibody molecule, and to the lesser degree of affinity of the Fd-fragment for the determinant groups of the antigen. Meanwhile comparison of the toxin-neutralizing activity of the Fd-fragment of the antitoxin and its light chains, with virtually no ability to neutralize toxin, is further confirmation that it is the Fd-fragment of the heavy chain of the antibody which plays the principal role in the formation of the active center [9-11, 19]. The toxin-neutralizing activity of the Fd-fragment and the light chain was calculated only per unit weight, for as was pointed out above many fragments of IgG and the light chains exist in aqueous solutions in the form of dimers and it is not always possible to decide how many active centers are accessible under these conditions for interaction with the antigen.

Appreciable differences were found when the toxin-neutralizing activity of antitetanus IgG and its fragments was tested against free toxin and against toxin fixed on its target substrate (protagon). Protagon specifically binds tetanus toxin [18, 24] and it can be regarded as the unpurified physicochemical receptor of tetanus toxin in the spinal cord. It will be clear from Fig. 1, which gives the results of this experiment, that the effectiveness of the antitoxin increases from IgG to the Fab'-fragment.

This result can be explained by differences in the accessibility of toxin fixed on protagon for native antitoxin and its fragments. Earlier investigations [5] showed that neutralization of protagon-bound toxin by antitoxin takes place in situ and does not involve detachment from the protagon. Presumably the water-insoluble protagon forms a complex micellary structure in the "crypts" of which the toxin is fixed. Penetration of native antitoxin into these crypts is difficult, whereas a fragment of the molecule with only one-third of its initial size can penetrate into the internal regions of the micelles and neutralize the toxin fixed there. It is evident from the curve shown that a certain quantity of toxin fixed on protagon is neutralized equally easily both by native antitoxin and by its fragments. This part of the toxin is evidently fixed on the surface of the protagon micelles, and the steric factor does not play a significant role in its neutralization.

The results of experiments with tetanus toxin fixed on protagon confirm the view [2, 4] that toxin bound in vivo can be neutralized, even if only partially, by antitoxin, and they strengthen the opinions expressed earlier [2-4, 6] that it would be desirable to test the therapeutic effect of an antitoxin with a smaller molecular size under appropriate conditions.

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