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IMMUNOGENICITY AND THYMUS-DEPENDENCE OF POLYMERIZED *Clostridium perfringens* α-TOXOID

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By condensing the α-toxoid of Clostridium perfringens type A with glutaraldehyde a polymer of the α-toxoid with molecular weight 450,000-600,000 was obtained. Experiments on guinea pigs showed that the immunogenicity of both the monomer and the polymer of the α-toxoid, when used in the adsorbed form is practically identical. On immunization with unadsorbed antigens the primary response to the polymer was 3 times greater than the immune response to the monomer. Polymerization of the α-toxoid did not change its thymus dependence.

KEY WORDS: α-toxoid; polymer; immunogenicity; thymus dependence

A central place in the problem of the prevention of gas gangrene caused by Clostridium perfringens type A is occupied by the development of methods of obtaining highly immunogenic toxoid preparations. There are reports in the literature that the immunogenic properties of the natural antigens of aggregations of molecules can be enhanced by their covalent-bonding by chemical bridges [4, 8-10]. One method of polymerizing proteins is by condensing them with glutaraldehyde.

The object of this investigation was to obtain a polymer of the α-toxoid of Cl. perfringens by using glutaraldehyde and to study its properties.

EXPERIMENTAL METHOD

The α-toxoid was obtained by detoxication of the principal isocomponent of the α-toxin of Cl. perfringens type A strain BR6K No. 28. The toxin was concentrated by precipitation at the isoelectric point with increased ionic strength of the culture fluid and fractionated by the batch method on DEAE-cellulose, equilibrated in 0.005 M KH_2PO_4 solution [1]. Under these conditions the principal component of the α-toxin, accounting for 95% of the total activity of the α-toxin in the culture fluid, possesses negative adsorption. The α-toxin was

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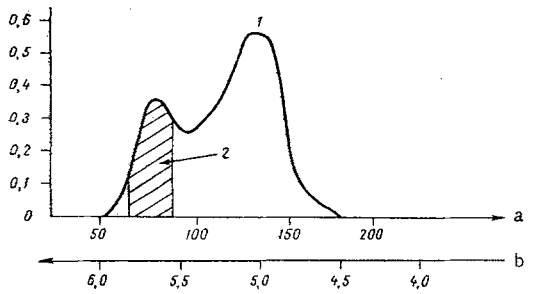

Fig. 1. Fractionation of polymer of Cl. perfringens α-toxoid on column with Sepharose 6B. Abscissa: a) volume of fraction (in mg); b) molecular weight of polymerized protein (in log); ordinate, protein content determined on spectrophotometer from E_{280} nm; 1) protein content; 2) zone of activity of toxoid.

detoxicated by Ito's method [7]. The resulting toxoid was freed from formaldehyde by precipitation at pH 3.5-3.8 in medium containing 20% NaCl. The toxoid was polymerized in 0.01 M acetate buffer, pH 8.0, with a protein concentration of 10 mg/ml. To the solution of toxoid at room temperature a 2.5% solution of glutaraldehyde was added drop by drop, with constant mixing, until the ratio of toxoid to glutaraldehyde was 1 : 50. The polymerization reaction took place for 1 h. The resulting polymer was chromatographed on a column (60 × 1.9 cm) with Sepharose 6B in 0.01 M acetate buffer, pH 8.0. The column was calibrated by macroimmunoglobulin (molecular weight 900,000) and human serum albumin (molecular weight 69,000).

The immunogenicity of the monomer and polymer of Cl. perfringens toxoid was studied in immunization experiments with inbred mice and guinea pigs. Guinea pigs weighing 250-300 g were immunized in groups of 8-10 animals by two subcutaneous injections, at an interval of 35 days, of the preparations, adsorbed on aluminum hydroxide, in doses of 10 and 5 fixation units (f.u.) consecutively. Blood was taken on the 30th day after the first injection of the preparation and on the 12th day after the second injection. When unadsorbed antigens were used, the second injection was given after 30 days. The antibody level was determined by the toxin neutralization test. Mice were immunized with 2 f.u. toxoid subcutaneously, as 2 injections at an interval of 30 days. Blood was taken on the 20th and 30th days after the first injection of the preparation and on the 10th day after the second injection.

The blood antibody level of the inbred mice was determined by the passive hemagglutination test (PHT) in blood sera from each mouse (0.05-0.025 ml) heated to 56°C for 30 min. Formalinized, tanninized sheep's red cells, sensitized with highly purified Cl. perfringens toxoid, were used as the diagnostic agent. The formalinized red cells were obtained by the method of Csismas [5], they were treated with tannin for 10 min at room temperature, and sensitized for 18-20 h at 37°C, using a sensitizing dose of 5-7 f.u. toxoid to 1 ml of a 2.5% suspension of red cells. The antibody titer was expressed at $-\log_2$ of the last dilution of serum to give a positive reaction.

The thymus-dependence of the antigens was studied on thymectomized (CBA × C57BL/6)F_1 mice. These animals were irradiated with γ rays in a dose of 850 R and were given an injection of syngeneic bone marrow (B mice). Before immunization, some of these B mice also received syngeneic T cells (B+T mice).

EXPERIMENTAL RESULTS

The polymer of Cl. perfringens α-toxoid, obtained by condensation of the monomer with molecular weight 50,000, was fractionated on a column with Sepharose 6B. The results are given in Fig. 1.

During preparation of the antigen polymer (Fig. 1) a process of selective polymerization took place: Proteins with molecular weight 450,000-600,000 possessed the properties of the α-toxoid. Impurities accompanying the α-toxoid were polymerized into products with molecular weight 100,000-450,000. The resulting polymer of the α-toxoid gave one precipitation zone in agar with hyperimmune antiperfringens serum. The yield of the polymer was about 90% and the specific activity up to 300 f.u./mg protein nitrogen. A similar phenomenon of selective polymerization also was observed during aggregation of the phospholipase C (α-toxin) of Cl. perfringens, but the yield of toxoid under these circumstances did not exceed 30%.

TABLE 1. Immunogenicity of Monomer and Polymer of Cl. perfringens α-Toxoid for Guinea Pigs

Antigen	Dose, f.u.	Primary response, i.u./ml	Secondary response, i.u./ml
Monomer:			
adsorbed	10+5	3,02 (1,91–4,37)*	8,32 (5,01–13,8)
unadsorbed	10+5	0,37 (0,26–0,54)	7,41 (3,89±14,13)
"	20+10	0,48 (0,35–0,66)	9,12 (5,75–14,45)
Polymer:			
adsorbed	10+5	2,51 (1,82–3,47)	11,22 (8,01–15,70)
unadsorbed	10+5	0,44 (0,30–0,63)	4,37 (2,88–6,61)
"	20+10	1,32 (1,00–1,74)	10,96 (5,50–21,88)

*Confidence intervals at 95% level of probability.

TABLE 2. Thymus Dependence of Monomer and Polymer of Cl. perfringens α-Toxoid in Experiments on Mice

Antigen	Mice	Titer of antibodies in $-\log_2$ units (M ± m)	
		20th day	30th day
Monomer of α-toxoid	Intact	6,0±0,94	7,9±0,89
	B mice	0	0
	B + T mice	5,5±0,60	7,3±0,45
Polymer of α-toxoid	Intact	5,7±0,75	7,4±0,57
	B mice	0	0
	B + T mice	4,2±0,01	6,5±1,20
Polymer of phospholipase C	Intact	6,9±0,57	7,7±0,50
	B mice	0	0
	B + T mice	6,0±0,57	5,9±0,71

The results of the study of the immunogenic properties of the monomer and polymer of Cl. perfringens α-toxoid for guinea pigs are given in Table 1. They show that after injection of the adsorbed antigens, no difference between them as regards immunogenicity could be detected.

After a single injection of double doses of the unadsorbed preparation a threefold increase in immunogenicity was found during the primary response to the polymer: 0.48 i.u./ml for the monomer and 1.32 i.u./ml for the polymer. During the secondary response the difference between the immune response to injection of the monomer and polymer virtually disappeared (9.12 and 10.96 i.u./ml respectively).

The results of a study of the thymus-dependence of the test antigens in experiments on mice are given in Table 2.

As Table 2 shows, the monomer of Cl. perfringens α-toxoid is a thymus-dependent antigen, for in the absence of T cells, no antibody production took place in the B mice in response to immunization with the toxoid. Injection of syngeneic T cells along with the toxoid monomer into B mice led to partial recovery of their ability to give an immune response (the group of B+T mice). Similar results also were obtained by immunization of mice with polymers obtained from the toxoid or phospholipase C. Polymerization of α-toxoid by condensation with glutaraldehyde did not change the thymus dependence of the antigen.

The investigation showed that during a single injection of unadsorbed monomer and polymer of Cl. perfringens α-toxoid into guinea pigs the immunogenicity of the polymer was 3 times higher than that of the monomer. Under the same conditions the immunogenicity of the adsorbed antigens was virtually equal. The effect of the higher immunogenicity of the unadsorbed polymer was evidently attributable to the longer duration of circulation of the aggregated molecule in the body. The ability to increase the immunogenicity of the toxoid threefold as a result of its polymerization may be interesting from the standpoint of obtaining a substance for use in the preparation of homologous human plasma.

In the process of polymerization of the α-toxoid no change took place in its thymus dependence. It is impossible at present to give an unequivocal interpretation of these results. The writers know of another example of a change in the thymus dependence of a natural antigen (flagellin) during its polymerization. Polymerization of the antigen under these circumstances took place spontaneously, without the use of "cross-linking" agents [2, 3, 6, 11]. However, the possibility cannot be ruled out that not all natural antigens change their

thymus dependence during polymerization. Possibly the molecular weight of the polymer obtained in the present experiments is too low to change the thymus dependence of the antigen. The methods of polymerization used in this investigation did not allow stable polymers of the toxoid with higher molecular weight to be obtained. It is also possible that in order to change the thymus dependence of Cl. perfringens toxoid other "cross-linking" agents must be used rather than glutaraldehyde.

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PREPARATION OF A MOUSE ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS AND THE STUDY OF ITS ACTION ON ROSETTE-FORMING CELLS

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A method of obtaining antiserum against isologous aggregated mouse immunoglobulins (MAAS) is described. This serum blocks the antigen-binding receptors of immune rosette-forming cells in vitro. MAAS was injected into mice immunized with sheep's red cells. By comparison with immunized mice receiving normal isologous serum, rosette-forming B cells were absent at the peak of the primary response in the spleen of the mice receiving MAAS. The number of antibody-forming cells was not reduced under the influence of MAAS.

KEY WORDS: rosette-forming cells; aggregated immunoglobulins; B cells; primary immune response

Data indicating that during the primary immune response changes take place in the supramolecular organization of the receptors of B lymphocytes were obtained previously. This effect was manifested in the fact that receptors of rosette-forming B cells (RFC) during the first days after immunization of mice with sheep's red cells could be blocked by means of rabbit antiserum against aggregated mouse immunoglobulins (RAAS). Subsequently the RFC were insensitive to the action of RAAS, which likewise did not affect the spontaneous RFC present in the spleen of the mice before immunization [1]. These results, pointing to the appearance of new antigenic determinants in the antibody-like receptors of the immune B lymphocytes, showed that, in principle, it is possible to use antibodies against aggregated immunoglobulins in order to block these immune B lymphocytes in vivo in the early stages of the immune response, so that an overall assessment could be made of the functional role of the immune RFC in various forms of immune response.

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