FACTORS MODULATING ANTIBODY FORMATION AGAINST *Clostridium perfringens* TOXOID IN MAMMALS

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The problem of obtaining a preparation for the prophylaxis of gas gangrene due to *Clostridium perfringens* type A in man has existed for decades. During this period effective nutrient media have been developed for the culture of this microorganism, and highly toxigenic strains have been bred. However, the immunogenicity of the toxoids used to prevent gas gangrene is not sufficient to produce immunity to the infection.

A fundamental difference in the technology of preparation of *Cl. perfringens* toxoid is detoxication of the α-toxin in the culture fluid followed by one-stage purification of the resulting toxoid (commercial preparation). Meanwhile it has been shown that a more immunogenic preparation can be obtained if the α-toxin is purified before toxication [1, 6, 8, 9]. Experiments with immunization of guinea pigs and rabbits have shown that the immunogenicity of the preparations increases with an increase in the purity of the α-toxin used for detoxication. Under the same experimental conditions, when inbred mice were immunized, less highly purified preparations proved to be most effective. The conditions of immunization for each species of animal were optimal [4].

This paper presents data on the further study of the immunogenic properties of *Cl. perfringens* toxoid preparations obtained from purified α-toxin for animals of diferent species and man.

EXPERIMENTAL METHODS

Method of obtaining preparations of experimental *Cl. perfringens* type A toxoid from purified α-toxin and of studying their immunogenic properties in animals were described [1, 6]. The immunogenicity of the toxoid was tested on a group of 35 persons. For primary immunization two injections were given at intervals of 30-34 days. The toxoid was injected subcutaneously below the left scapula in a volume of 1 ml (30 fixation units). Revaccination was carried out 1 year after the end of primary immunization, with the same dose and the same batch of toxoid. Blood was taken 12-14 days after the second injection of the preparation and revaccination. The titer of perfringens antitoxin in the human and guinea pig sera was determined in the toxin neutralization test *in vitro* with lecithovitellin emulsion and *in vivo* in albino mice. The geometric mean of the titers and the standard error were calculated.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the immunogenicity of toxoid prepared from a primary concentrate of α-toxin in experiments on guinea pigs was almost twice as high as the immunogenicity of the commercial preparation. The immunogenicity of toxoid obtained by detoxication of a more highly purified toxin, as a result of fractionation of proteins of the primary concentrate on DEAE-cellulose, in experiments on the same animals (mean of 15 experiments) was 4.5 times higher than the immunogenicity of the commercial preparation (purified toxoid).

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TABLE 1. Immunogenicity of Preparations of *Cl. perfringens* Toxoid for Guinea Pigs and Mice (after two immunizations)

Preparation	Guinea pig	Mice	
	i.u./ml	passive hemagglutination test (PHT)	i.u./ml
Commercial toxoid (primary concentrate of crude toxoid)	1,12 (1,06—1,38)	15,8±0,17 (15,45—16,15)	11,7 (8,70—14,40
Toxoid obtained from primary concentrate of α-toxin	2,20 (1,50—3,62)	16,5±0,35 (15,77—17,23)	7,5* >7<8
Toxoid obtained from α-toxin purified on DEAE-cellulose (purified toxoid)	5,10 (3,89—6,76)	13,7±0,24 (13,2—14,2)	4,0* >3<5

*In all experiments level of perfringens toxoid was within the specified limits.

TABLE 2. Effect of "Ana-Eluate" on Immunogenicity of *Cl. perfringens* Toxoid (mean results)

Preparation	Guinea pigs (i.u./mg)			C57BL/6 mice	DBA/2* mice
				30th day after first injection	
	first injection	second injection‡	revaccination	toxoid Cl. periringens (M ± m)	toxoid Cl. oedematiens (M ± m)
Purified toxoid	0,32 (0,18—0,56)	1,04 (0,73—1,48)	3,98 (3,30—4,74)	5,7±0,33 (4,95—6,45)	10,5±0,32 (9,28—11,72)
Purified toxoid + "ana-eluate"†	0,11 (0,07—0,18)	0,28 (0,14—0,58)	1,41 (0,67—2,97)	6,8±0,52 (5,65—7,95)	11,7±0,36 (10,67—12,53)
Commercial toxoid	0,23 (,019—0,35)	0,43 (0,35—0,55)	0,96 (0,56—1,56)		

*Results of PHT ($-\log_2$).

†Immunizing dose contained 100 mg of "ana-eluate."

‡Depending on time of year and batch of animals, absolute values of titers could differ fivefold between one experiment and another. These particular experiments were carried out in spring, when the absolute values of the titers were lowest.

A comparative study of the properties of purified toxoid — nontoxicity, sorption, stability on keeping, degree of purity, and yield — showed that it satisfies the requirements for vaccine preparations [1]. This version of toxoid was tested on a group of subjects none of whom had previously been immunized with *Cl. perfringens* toxoid. The initial level of perfringens toxoid in the subjects' blood stream did not exceed 0.1 i.u./ml, i.e., the lower level of reliable determination by the method used. Analysis of the results of this test showed that the mean titer after the second injection of toxoid was 6.5-7.5 times higher than the initial level. The geometric mean of the titers after revaccination remained at the same level as after the second injection of the toxoid — 0.78 and 0.74 i.u./ml of perfringens toxoid. The preparation gave rise to few reactions.

The extreme lack of data in the literature on the immunogenic properties of *Cl. perfringens* toxoid preparations in man [7, 10] and also the absence of information on immunogenicity of the commercial toxoid make it somewhat more difficult to interpret the results.

A basic distinguishing feature of the test toxoid is that the maximal immune response can be achieved during immunization with it after the second dose of primary immunization and not after late revaccination, as is characteristic of the preparations described by Adams [7] and Tytell, et al. [10], which were prepared by detoxication of α-toxin in the culture fluid. The much higher level of perfringens antitoxin (7-14 times higher) obtained in the present investigation as a result of double primary immunization with purified toxoid can be taken as evidence of the higher immunogenicity for man, as also for guinea pigs and rabbits, of *Cl. perfringens* toxoid preparations obtained by detoxication of previously purified α-toxin.

One of the causes of the higher immunogenicity of the purified toxoid may be that during fractionation of the proteins of the primary concentrate of α-toxin on DEAE-cellulose, a factor lowering the immunogenicity of the toxoid is separated. To test this hypothesis, proteins of the fraction adsorbed on DEAE-cellulose were eluted by increasing concentrations of buffer solution and were treated under the same conditions as the α-toxin. After removal of formaldehyde and salts and testing for nontoxicity and absence of α-toxoid, this fraction, which we called the "ana-eluate," was adsorbed on aluminum hydroxide.

The effect of the "ana-eluate" on immunogenicity of the purified toxoid was studied in guinea pigs and mice. The basis for the experiments on mice consisted of the results of the experiments (Table 1) showing that immunogenicity of *Cl. perfringens* toxoid preparations for animals of this species decreased with an increase in the degree of purification of α-toxin used to prepare the toxoid. In comparative experiments mice were immunized with serologically pure *Cl. oedematiens* toxoid [5]. Mice of genotypes with a low reaction to these antigens [2, 3] were used for immunization.

It will be clear from Table 2 that addition of the "ana-eluate" to the purified toxoid significantly lowered the titers of perfringens antitoxin in the guinea pigs both after primary immunization and after revaccination. The same "ana-eluate" led to a twofold increase in the perfringens and oedematiens antitoxin levels by the 20th day after immunization of mice with the corresponding preparations.

It can thus be concluded that the culture fluid of *Cl. perfringens* contains a factor (or factors) which, under optimal experimental conditions, lowers antibody production in some species of animals but activates it in others. This last phenomenon is evidently nonspecific, for a similar effect also was obtained on immunization with *Cl. oedematiens* toxoid.

A further study of this phenomenon would seem to be desirable.

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