Protection conferred by microbially-supplemented UK and purified PA vaccines

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Summary

The ability of purified Protective Antigen (PA) to induce protective immunity against *Bacillus anthracis* in guinea pigs is demonstrated. When administered together with killed cells of *Bordetella pertussis*, a high degree of protection was conferred against even so-called "vaccine resistant" strains of *B. anthracis* and, furthermore, this protection appeared to be fairly durable. Following a short course of three 50μg doses at 2-week intervals, protection against challenge with the Ames strains of *B. anthracis* was apparent to some extent at all four times examined—1, 3, 6 and 14 months. However, single booster doses at 5 and 11 months conferred solid protection against challenge at 6 and 14 months respectively. In this regard, the purified PA performed better than the licensed UK human vaccine although the latter was then found to contain only < 2.5μg PA per 0.25 ml dose. Antibody analysis confirmed previous observations that antibodies to the lethal factor and edema factor components of anthrax toxin are not essential to protection in the guinea pig and that, while PA is capable of inducing effective protective immunity, the degree of such immunity in an individual cannot be determined from a serological anti-PA titre.

Introduction

Although the nature of the 3-component anthrax toxin and the predominant role of its Protective Antigen (PA) component in the protective action of anthrax vaccines were established in the 1950s and 1960s[6], it has only been in the 1980s, following purification and genetic manipulation of the toxin components, that clear confirmation has been possible of PA as the component in vaccines inducing protective immunity[2,8,9].

The efficacies of the existing vaccines in the field have been demonstrated to an extent[1] but the duration of the protection afforded has not been determined. Also, tests in animals have shown that the human chemical vaccines as constituted have limited protective activities, lower than that induced by live spore vaccines available for use in the USSR[5] but confined to veterinary vaccines in Western bloc countries. However, such tests have further shown that non-specific killed microbial additives, such as Freund's complete adjuvant, *Bordetella pertussis* (as in the human vaccine) or *Corynebacterium ovis* can enhance the protective action of PA in the chemical vaccines to levels equivalent to or exceeding those of the live spore vaccines[3,9].

In attempts at formulating a fully defined subunit vaccine capable of inducing a more comprehensive protective immunity against anthrax with less reactogenicity than the existing vaccines, comparative studies were needed on the protective efficacies and the duration of protection afforded by existing vaccines and by a purified PA vaccine.

Methods and materials

The results of two separate experiments are reported here.

Experiment I was designed to confirm the ability of purified PA to confer protective immunity in guinea pigs, particularly when non-specifically enhanced with the human pertussis vaccine.

Immunised animals received 3 doses (except where otherwise indicated in Tables 1, 2) administered in the rear flanks at 2-week intervals of either (a) hydrophobic interaction chromatography (HIC) purified PA[4], 50 μg/0.3 ml dose, adsorbed onto alhydrogel (Superfos A/S, Vedbaek, Denmark) [unsupplemented group] or (b) the same alhydrogel-adsorbed HIC purified PA pre-mixed 1:1 with Pertussis Vaccine BP (Wellcome Research Laboratories, Beckenham, Kent, UK, batch BA 4346) such that each dose contained 50 μg PA and 1.2 x 10^{10} killed *Bordetella pertussis* cells in a volume of 0.6 ml administered as two 0.3 ml injections, one in

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each rear flank [supplemented group]. Controls receiving just the pertussis vaccine were included. The group sizes and challenge times and doses are given under Table 1.

Experiment II was aimed principally at examining the duration of protection afforded by the supplemented vaccines. The different groups of guinea pigs were immunised 1 to 14 months before a target challenge date (Table 2). Three doses of alhydrogel-adsorbed HIC purified PA (*ca.* 50 μg/dose) supplemented with the pertussis vaccine (1.0×10^{10} cells/dose) were administered in total volumes of 0.5 ml (0.25 ml in each rear flank) at 2-week intervals as in Experiment I. The UK vaccine (product license 1511/0037, Department of Health and Social Security, London, batch 123 G2) was mixed 1:1 (v/v) with the pertussis vaccine and the mixture administered in 3 doses of 0.5 ml (0.25 ml in each rear flank) at 2-week intervals.

A new batch of pertussis vaccine (VC1447) was used for the 6, 3 and 1 month groups and, after dose 3, all animals received 0.15 ml Freund's complete adjuvant in a rear flank. Half of the 6 and 14 months groups received a single booster dose 5 and 11 months respectively after dose 3 - i.e., 1 and 3 months respectively before challenge. It was not possible to prepare sufficient purified PA in advance for the whole experiment and three separate batches were used for the four vaccination sessions. The PA was either purified from culture by the method of Quinn *et al*[4] or was HIC-purified material kindly supplied by Dr. S. H. Leppla.

The oldest group, originally targeted for challenge at 18 months, showed signs of aging (hair loss, anorexia and wasting in a proportion of the animals) and had to be challenged at 14 months and thus separately from the other groups. Challenge was predominately with the Ames strain of *B. anthracis* but small supplementary groups of guinea pigs were included to give preliminary indications on what protection can be achieved against challenge with the New Hampshire (NH) strain, a second "vaccine resistant" strain.

Two guinea pigs from each group were bled 2 days before challenge and the sera tested for antibodies to PA and also to the lethal factor (LF) and edema factor (EF) components of anthrax toxin by the inhibition ELISA method described elsewhere[8,9]. LF for the ELISA tests was prepared by the method of Quinn *et al*[4]; the EF was kindly supplied by Dr. S. H. Leppla but, quantities being limited, in Experiment II, only 8 selected serum samples were tested for anti-EF antibodies.

Table 1. Protection conferred by purified PA supplemented with the human killed whole cell *Bordetella pertussis* vaccine.

*Vaccine	Challenge at (weeks after last dose)	Pre-challenge titers (PA/LF/EF)	Challenge strains	
			†Vollum	‡AMES/NH
PA alone	2	65600/-/-	5/6[83%]	1/12[8.3%]
PA alone (5 doses)	4	5.2×10^5/-/n	n	3/3
PA + *B. pertussis*	2	65600/-/-	6/6[100%]	7/12[58%]
	6	n	n	6/8[75%]
B. pertussis only	2	-/-/-	0/6[0%]	0/12[0%]

* Except where otherwise stated, 3 dose administered at 2-week intervals. PA at 50 µg/dose.
† "vaccine sensitive". Challenge dose of 900 spores. Figure shows no. surviving/no. challenged [% survival]
‡ "vaccine resistant". Challenge dose 850 spores.
n = not done

Results and discussion

The ability of PA to induce protective immunity predicted previously[9] and partially demonstrated with an engineered strain of *B. sublitis*[2], is confirmed. As previously demonstrated with the existing chemical vaccines[9], microbial supplementation (Table 1) markedly enhanced protection with 1 of 12 (8.3%) surviving Ames and NH strain challenge 2 weeks after receiving 3 doses of PA alone *versus* 7 of 12 (58%) surviving among those which received PA supplemented with the pertussis vaccine. The protection appeared not to have declined 4 weeks later.

Total performance in the supplemented UK human vaccine in Experiment II (32 of 52 [61.5%] surviving) and the purified PA vaccine (34 of 48 [70.8%] surviving) did not differ greatly (Table 2). Protection was apparent to some extent at all times but was clearly less in the unboosted animals at 14 months. There was no obvious explanation why a higher proportion that had received the UK vaccine succumbed at 3 months than at 6 months (unboosted group).

Of significance is the complete protection afforded in the supplemented PA groups that had received a booster dose. In this respect, the purified PA performed better than the UK vaccine with 100% survival 3 months after the 11 month booster. However, subsequent ELISA antigen analysis indicated that the UK vaccine only possessed < 25 μg/0.25 ml dose.

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Table 2. Degree and duration of protection conferred by the UK and purified PA vaccines supplemented with the human killed whole cell *Bordetella pertussis* vaccine.

Challenge with	Immunization with	* Survivors in group Time between last dose of short course and challenge					
		1 month	3 month	6 month		14 month	
				†UB	‡B	†UB	§B
AMES	Unvaccinated	0/4 (-/-/nd)	0/2 (-/-/nd)	0/6 (-/-/nd)		0/6 (-/-/nd)	
1300 spores in 14 month group	UK + *B. pertussis*	7/8 (16400/4096/nd)	3/8 (2048/512/nd)	5/6 (2048/1024/nd)	5/5 (8192/4096/16400)	3/9 (4096/4096/nd)	5/8 (16400/32800/8192)
3000 spores in other groups	PA + B. *pertussis*	7/8 (1.3×10^5/-/-)	6/8 (32800/-/-)	4/6 (65600/-/-)	6/6 (1.3×10^5/-/-)	3/6 (32800/-/-)	7/7 (2.6×10^5/-/-)
	PA + *B. pertussis* (1 dose)	2/3 (4096/-/nd)					
NH	Unvaccinated	**0/2	0/2				
3000 spores	UK + *B. pertussis*	2/4	2/4				
	PA + *B. pertussis*	3/4	1/3				

* mean anti-PA/LF/EF pre-challenge titres in parenthesis under the survival figures. The anti-PA/LF titres are the means of duplicate readings on sera from 2 guinea pigs; anti-EF titres are single readings on sera from one guinea pig.
† Unboosted. ‡ boosted with a single dose at 5 months. § boosted with a single dose at 11 months.
** anti-PA/LF/EF titres as under Ames survivor equivalents above. nd = not done.

Preliminary evidence was also supplied with small groups of animals that PA alone could elicit good protection if administered in a sufficient number of doses (Table 1) and that a single dose of supplemented PA may confer significant protection (Table 2).

The results of antibody analysis confirmed the previous observation[9] that antibodies to LF and EF are not essential to protection in the guinea pig; high degrees of protection were evident in the complete absence of antibodies to those toxin components. On the other hand, there is no change in the previous conclusions[3,8,9] that, while PA is capable of inducing effective protective immunity, the degree of that immunity in an individual cannot be directly assessed from measuring the serological anti-PA titer.

Non-specific microbial stimulation of protection indicates involvement of some cellular immune mechanisms-more than that simply produced by inciting a coincidental inflammatory response since, apparently unlike the live spore vaccine[7], the performance of the UK and US vaccines were not enhanced by saponin[9]. Furthermore, the enhanced protection appears to be fairly durable with two-thirds of the unboosted guinea pigs immunised with the supplemented PA surviving at six months and half surviving at 14 months. In the latter group, the single booster at 11 months resulted in 100% protection 3 months later. The choice of *B. pertussis* as the microbial additive for these studies from amongst others shown to be effective[9] was based on the availability of killed cells of *B. pertussis* in the form of a vaccine already licensed for human use.

Further tests are needed to determine optimum dose sizes and schedules in relation to protection against challenge with a range of strains, various challenge sizes and challenge by the respiratory and oral routes.

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