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OPERATION GRANBY: THE EFFECT OF CO-ADMINISTRATION OF THE PERTUSSIS VACCINE ON SPECIFIC ANTIBODY TITRE DEVELOPMENT TO THE ANTHRAX VACCINE IN MAN

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SUMMARY

Sera collected from UK military personnel vaccinated against anthrax during Operation GRANBY in the Gulf region in 1990/91, were analysed at CBDE. The results of the analysis showed that co-administration of pertussis with the primary dose of anthrax vaccine had no significant effect on the titre of specific antibody ultimately achieved. However, there was evidence to suggest that pertussis served to shorten by weeks the time period required to achieve a substantial antibody titre.

KEYWORDS

1. ELISA
2. ADJUVANTISATION OF ANTHRAX VACCINE
3. ANTIBODY TITRE AS INDICATOR OF PROTECTIVE EFFECT

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1. INTRODUCTION

The human vaccine for anthrax accepted for use in the UK consists of alum-precipitated Protective Antigen (PA). This formula, adopted from that developed by the Michigan Department of Public Health in the 1960's [1], seeks to induce a high titre of PA-specific antibody, thus preventing the complexing of PA with Lethal Factor and formation of the aptly-named Lethal Toxin.

In recent years, however, severe deficiencies in the protective capacity of this vaccine against virulent strains of anthrax have been indicated from experimental animal studies [2, 3, 4, 5, 6]. Adjuvantising formulae have thus been sought, amongst which is the incorporation of killed Bordetella pertussis, the causative organism of whooping cough, into the vaccination regime [7, 8]. The adjuvantising potential of killed mico-organisms such as Mycobacterium tuberculosis, M butyricum, Cornybacterium parvum, and B pertussis has long been recognised [9] and is attributed to a non-specific 'shut-down' of draining lymph nodes due to the additional particulate load. The effect of the 'shut-down' is to prolong the encounter of lymphocytes with specific immunogenic material draining from the vaccination site, and to expand the number of stimulated lymphocytes involved in an immune response with enhanced cell-mediated immunity. The results of animal studies have indicated that the efficacy of the human anthrax vaccine can be augmented by the inclusion of killed B pertussis [10].

When the need to vaccinate UK troops arose during the outbreak of hostilities in the Gulf conflict during the period August 1990 to April 1991, the decision was made to co-administer the pertussis and anthrax vaccines. Many of the troops were later also vaccinated against plague. Blood samples were collected during the vaccination schedule by the RAMC in order to monitor the development of an antibody response and enable estimation of the degree of protection conferred.

This study is concerned with the retrospective analysis of these serum samples to determine the benefit derived from contemporaneous vaccination with anthrax and pertussis. To this end, samples derived from two studies have been analysed: in the first, every individual was primed with pertussis concomitantly with anthrax; whereas in the second, only 30% of individuals received pertussis with the anthrax prime.

2. MATERIALS AND METHODS

Vaccines used in the study

a. Anthrax

The human vaccine licensed for use in the UK and supplied by the Public Health Laboratory Service to the Department of Health was used in both studies. In study 1, two doses (0.5 ml/dose, intra-muscularly) of the vaccine were given to all individuals with a mean time interval of 21 days between doses. In study 2, the first two doses were given with the same time interval as for study 1, but 6/94 individuals received a third dose approximately 1 month later.

b. Pertussis

In study 1, Pertussis vaccine BP (Wellcome Research Laboratories, Beckenham, Kent) was administered simultaneously with both the priming and booster doses of anthrax. Both vaccines were injected into the same muscle mass. In study 2, 30% (25/94) of individuals received pertussis with the anthrax prime but of these, only 8/25 (32%) were administered pertussis and anthrax booster doses concomitantly.

c. Plague

In both studies, individuals received a single dose of plague vaccine, consisting of killed, virulent *Y pestis* organisms (Cutter Labs Inc) in 0.5 ml volume intra-muscularly. This was generally administered simultaneously with the anthrax booster dose.

Sera

Serum samples were received from one of two sources: either from 205 General Hospital, Riyadh (for study 1); or for study 2 from Queen Elizabeth Military Hospital, Woolwich (QEMH). In this first case, samples were derived in the field from healthy soldiers on active duty in the Gulf region during the period January-March 1991. Samples were supplied in two batches: (a) 85 samples taken 7-24 days after the anthrax booster (and therefore at 4 to 7 weeks into the immunisation schedule) and accompanied by comprehensive vaccination histories; (b) 37 samples, with no attendant information, but supplied approximately 1 month later. For the second study, samples were collected at QEMH from 94 individuals (male and female) evacuated there with injuries and from medical personnel. Full vaccination histories were supplied and samples had been taken 2-3 months after the final anthrax booster dose.

In either case, whole blood samples were collected into vacutainer tubes, allowed to clot, frozen and delivered to the laboratory at CBDE where the serum was separated and analysed.

Method of analysis

A standard Enzyme-Linked Immunosorbent Assay (ELISA) protocol was used to analyse samples for their content of antibody specific for PA [7]. Briefly, purified PA was coated to microtitre plates (Dynatech) at 5 ug/ml in carbonate/bicarbonate antigen binding buffer pH 9.6, supplemented with 0.5% w/v BSA and 0.5% v/v Tween 20, overnight (37 C) until dry. Plates were then soaked for 5 minutes in PBS-0.02% Tween 20, before washing (x3) in the same buffer. Blocking was effected with 4% BSA in PBS with 0.02% Tween 20 (BPBST) (1 h, 37 C) and followed by 3 further washes in PBS-Tween. Doubling dilutions of serum samples were then added to the plates and incubated (1 h, 37C). Unbound antibody was then removed by washing (x3). Anti-human IgG labelled with horse radish peroxidase (Sera-Lab) was then added in BPBST at a working dilution of 1:3000 and incubated on the plates (1h, 37C). Plates were subsequently washed (x5), before addition of the substrate 2,2'-azino-bis(3-ethylbenzthiazolene 6 sulphonic acid) diamonium (ABTS) and measurement of the optical density per well at 405 nm. The titre was estimated to be the maximum dilution of serum at which the OD exceeded background by 0.1 units. Known high and low-titre samples were included on each plate to control the assay.

STATISTICAL ANALYSIS

The Kolmogarov-Smirnov 2 sample test 2 tailed (12) was applied to analyse the results for statistical significance.

3. RESULTS

Analysis of batch (a) sera from study 1, taken at 4-7 weeks into the immunisation schedule, showed that 18% of the population had not started to respond well, with a titre lower than 1:256. The titre distribution for 1b sera, collected a further one month into the schedule, differed significantly, showing a clear shift upwards and with no individual having a titre less than 1:256. The distribution of individuals in 1a according to titre is shown in Figure 1 and contrasted with that for study 1b.

Similarly, the titre distribution for study 2 shows a significant shift upwards when compared with that for study 1a (Figure 2). However there was no significant difference between studies 1b and 2 on the basis of distribution of vaccinees to titre (Figure 3).

Within Study 2, whether or not pertussis was co-administered with the anthrax prime had no significant effect on the ultimate distribution of vaccinees to titre (Figure 4). Similarly, a second dose of pertussis caused no significant increase in the ultimate titre achieved by recipients.

Within study 1a also, no significant effect on titre could be discerned from the administration of either plague vaccine, or of a second dose of pertussis, or of plague instead of pertussis.

The intensity of vaccination of individuals in study 1 who spent longer in the Gulf than those of study 2, was understandably greater: for example, 84% of the population in study 1 were given a second dose of pertussis compared with 27% in study 2; 92% in study 1 received the plague vaccine (often simultaneously with the second anthrax and pertussis doses) compared with 46% in study 2; 98% in study 1 received a second anthrax dose compared with 70% in study 2.

4. DISCUSSION

In such a study where military activities of necessity disrupt the collection of sequential samples from individuals at pre-determined times in the immunisation schedule, many confounding factors operate to affect the results achieved. In general terms, the batch (a) samples in study 1, collected early in the immunisation schedule (7-24 days after the anthrax booster dose) revealed a relatively immature immune response, with an apparently considerable percentage of non-responders. By comparison, samples in batch (b) collected a further one month into the immunisation schedule, although from an entirely different population, showed a much more mature immune response with an 11-fold lower incidence of poor responders (titres <1:256), and approximately double the incidence of high responders (titres >1:4096).

Similarly, samples gained from study 2 gave valuable information on titre distributions in a population even later in the schedule, when sufficient time had elapsed for a full immune response to develop. In study 2, it was also possible to make a direct comparison between two treatment groups within the population. The first group, who received the anthrax primary dose adjuvantised with pertussis, ultimately did not develop a significantly different distribution of titre compared with the second, who were given the anthrax prime alone. However, individuals in study 1b, all of whom were primed with pertussis plus anthrax, achieved comparable titres to those seen in study 2, but in a time period at least 9 weeks earlier than that for study 2. Thus the effect of pertussis may be to induce a faster immune response to the antigen with which it is administered, although this can only be conjecture without assay of study 2 sera taken concomitantly with sera from 1b.

In either study, administration of a second dose of pertussis had no significant effect of ultimate titre achieved.

A very few individuals in both studies received a dose of the plague vaccine with the anthrax priming dose, and most in study 1 (a) received plague with the anthrax and pertussis boosters. Although it is difficult to quantify the exact effect of the

plague vaccine under these field trial conditions, it could also be expected to have an adjuvantising effect on the anthrax schedule.

Large individual differences in antibody titre in response to vaccination occur, caused not only by innate factors such as an individual's genetic make-up but also by extrinsic factors impinging on him to cause stress, and up- or down-modulate his general well-being and health. It is debatable, therefore, just how useful it is to monitor serum antibody titre only. Certainly, serum antibody titre is not an absolute indicator of the degree of protection conferred by vaccination and ideally should be considered in relation also to indices of cell-mediated immunity. But the latter are difficult to achieve under field trial conditions because of the pre-requisite for freshly-prepared lymphocytes as substrate for the tests. However, the titre of specific antibody measurable in serum does give some indication of the maturity of the specific response to an immunisation schedule, especially if determined across large well-matched (for gender and age) populations such as these.

In conclusion, adjuvantisation of the primary anthrax dose with pertussis, although not affecting the absolute titre of specific antibody ultimately achieved, may serve to accelerate the early production of specific antibodies.

COMPARISON OF TITRE DISTRIBUTIONS IN STUDY 1a versus 1b

Titre	Log titre	N	%CF	N	%CF	S.D
		Study 1 (a)		Study 1 (b)		
1:16	4	3	3.5	0	0	
1:32	5	3	7.0	0	0	
1:64	6	0	7.0	0	0	
1:128	7	10	18.8	0	0	
1:256	8	11	31.8	1	2.7	p<.05
1:512	9	2	34.1	0	2.7	p<.01
1:1024	10	5	40.0	8	24.3	
1:2048	11	17	60.0	0	24.3	p<.005
1:4096	12	24	88.2	20	78.4	
1:10000	13	9	98.8	6	95	
1:20000	14	1	100.0	1	97	
1:40000	15	0	100.0	1	100	
		85		37		

KEY
N = no. of individuals
%CF = % cumulative frequency
S.D. = significance of difference

FIGURE 1

COMPARISON OF TITRE DISTRIBUTIONS IN STUDY 1a versus 2

Titre	Log titre	N	%CF	N	%CF	S.D
		Study 1 (a)		Study 2		
1:16	4	3	3.5	0	0	
1:32	5	3	7	0	0	
1:64	6	0	7	0	0	
1:128	7	10	18.8	0	0	
1:256	8	11	31.8	1	1.07	p<.001
1:512	9	2	34.1	5	6.45	p<.005
1:1024	10	5	40	14	21.5	p<.1
1:2048	11	17	60	17	39.8	p<.1
1:4096	12	24	88.2	16	57	p<.001
1:10000	13	9	98.8	36	95.7	
1:20000	14	1	100	4	100	
		85		93		

KEY
N = no. of individuals
%CF = % cumulative frequency
S.D. = significance of difference

FIGURE 2

COMPARISON OF TITRE DISTRIBUTIONS IN STUDY 1b versus 2

Titre	Log titre	N	%CF	N	%CF	S.D
		Study 1 (b)		Study 2		
1:16	4	0	0	0	0	
1:32	5	0	0	0	0	
1:64	6	0	0	0	0	
1:128	7	0	0	0	0	
1:256	8	1	2.7	1	1.1	NSD
1:512	9	0	2.7	5	6.5	
1:1024	10	8	24.3	14	21.5	
1:2048	11	0	24.3	17	39.8	
1:4096	12	20	78.4	16	57	
1:10000	13	6	95	36	95.7	
1:20000	14	1	97	4	100	
1:40000	15	1	100	0	100	
		37		93		

KEY
N = no. of individuals
%CF = % cumulative frequency
S.D. = significance of difference

FIGURE 3

EFFECT OF PERTUSSIS WITH ANTHRAX PRIME (STUDY 2)

Titre	Log titre	N	%CF	N	%CF	S.D
		(a)pertussis with anthrax prime		(b)no pertussis with anthrax prime		
1:128	7	1	4	0	0	
1:256	8	0	4	0	0	
1:512	9	2	12	3	4.5	
1:1024	10	2	20	12	22.7	
1:2048	11	4	36	11	39.4	
1:4096	12	6	60	9	53	
1:10000	13	9	96	28	95.5	
1:20000	14	1	100	3	100	
		25		66		

KEY
N = no. of individuals
%CF = % cumulative frequency
S.D. = significance of difference

FIGURE 4

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