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Specificity of early protective responses induced by pseudomonas vaccines

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SUMMARY

A single inoculation of pseudomonas vaccine protected mice against a 1 LD100 challenge by nine different serotypes of *Pseudomonas aeruginosa*, 4 days after vaccination. Three inoculations of the same vaccine, given on consecutive days, protected mice for a longer time, against challenge by a wide range of *Ps. aeruginosa* serotypes, than the single inoculation of vaccine. The amount of vaccine inoculated only marginally influenced the non-specific response. F1 vaccines from eight serotypes of *Ps. aeruginosa* each induced protection against a different range of serotypes.

INTRODUCTION

Failure to protect patients with extensive burns against *Pseudomonas aeruginosa* can result in severe illness, septicaemia and death (Tumbusch *et al.* 1961; Kefalides *et al.* 1964; Jones, Jackson & Lowbury, 1966). Topical chemoprophylaxis has been found effective in preventing colonization of burns, thereby reducing the chance of invasive septicaemia (Lindberg *et al.* 1965; Lowbury & Jackson, 1970), but occasionally fatal infections occur even when the most effective topical chemoprophylaxis has been used (Cason & Lowbury, 1968; Alexander, Fisher, MacMillan & Altemeier, 1969).

Research into alternative methods of preventing invasive *Ps. aeruginosa* septicaemia has shown the value of active immunization (Feller, 1966; Markley & Smallman, 1968; Alexander *et al.* 1969; Jones, 1971). Recent experiments showed that burned mice can be protected against lethal *Ps. aeruginosa* infection by a single injection of vaccine administered 24–48 hr. before infection (Jones, 1971). In the present study, an attempt has been made to determine the degree and specificity of early protective responses induced in mice by a single injection and by three injections on consecutive days of pseudomonas P14F1 vaccine. Experiments were made to test how the size of dose of this vaccine affected the specificity, duration and degree of the protective response. Variations in the specificity of protective responses of mice given single injections of F1 vaccines, extracted from different strains of *Ps. aeruginosa*, are also reported.

MATERIALS AND METHODS

Strains of Ps. aeruginosa *used*

Serotypes 2A, 2A 2B 5C, 3, 5C, 5D, 8, 10, 11, NT (non-typable) and 2/5 (B4) were isolated from patients with burns. Serotypes 1, 6A, 6B, 6C and 9 were isolated from other hospital sources, including tracheostomy sites. Serotypes 2AB and 14 were obtained from Dr M. T. Parker who kindly typed all strains of *Ps. aeruginosa* used in these experiments by serological and phage typing methods.

Vaccines

Pseudomonas P14F1 vaccine was extracted from a culture filtrate of *Ps. aeruginosa* P14, by gel filtration using methods described by Carney & Jones (1968). The same method of extraction was used to obtain F1 vaccines from 1 l. culture filtrates of serotypes 2AB, 3, 5C, 8, 10, NT and strain B4. All vaccines were lyophilized; before injection into mice they were dissolved in physiological saline and sterilized by filtration through a GS Millipore membrane (pore size 0·2 μ).

The vaccines were injected intraperitoneally in 1·0 ml. volumes of saline into 25 g. male Schofield albino mice, by methods described by Jones (1971). The size of dose and frequency of injection of the vaccines is described below.

Infection

In all experiments mice were infected intraperitoneally by injection of 1·0 ml. of a saline suspension of *Ps. aeruginosa*. Groups of mice were challenged with 1 LD100 of *Ps. aeruginosa* at various times after vaccination. Fifteen serotypes of *Ps. aeruginosa* were used for challenging vaccinated mice; the LD100 for these serotypes was determined by i.p. injection into groups of three unvaccinated mice of ten-fold dilutions of saline suspensions ranging from $2{\cdot}1 \times 10^9$ bacteria to $2{\cdot}1 \times 10^6$ bacteria. The numbers of bacteria in the suspensions were estimated from Brown's opacity tubes (Wellcome). Once a dilution of bacterial suspension which killed all the mice had been determined, the numbers of bacteria were reduced by units of the Brown's scale until the smallest number which killed all three mice had been found. Table 1 shows the LD100 of 15 serotypes of *Ps. aeruginosa* for mice determined by this method.

Mice were observed for 48 hr. after challenge, as it was found that mice which survived for this time seldom died later.

In each experiment a control group of three unvaccinated mice received the challenge dose intraperitoneally to confirm its 100% lethality.

Experimental procedures

Specificity of protective responses after a single injection of P14F1 vaccine

Groups of 45 mice were given either 1·0 or 0·01 mg./kg. mouse weight of P14F1 vaccine intraperitoneally. After vaccination, groups of three mice from each of the two groups of vaccinated mice and from a control group of unvaccinated mice were infected intraperitoneally with 1 LD100 of each of 15 different serological types of *Ps. aeruginosa*.

Table 1. *LD*100* *of* 15 *serotypes of* Ps. aeruginosa *for mice*

Serotype of challenge strain of *Ps. aeruginosa*	No. of bacteria injected intraperitoneally into mice
1	$2{\cdot}1 \times 10^8$
2A	$6{\cdot}3 \times 10^8$
2A 2B 5C	$2{\cdot}1 \times 10^8$
3	$2{\cdot}1 \times 10^8$
5C	$4{\cdot}2 \times 10^8$
5D	$2{\cdot}1 \times 10^8$
6A	$6{\cdot}3 \times 10^7$
6B	$6{\cdot}3 \times 10^8$
6C	$6{\cdot}3 \times 10^8$
8	$4{\cdot}2 \times 10^8$
9	$8{\cdot}4 \times 10^8$
10	$4{\cdot}2 \times 10^8$
11	$3{\cdot}2 \times 10^8$
14	$6{\cdot}3 \times 10^7$
NT	$2{\cdot}1 \times 10^9$

* Estimated from Brown's opacity tubes.

Different groups of vaccinated mice were challenged 2, 4, 7 and 14 days after vaccination to see if the protective responses of mice following a single injection of pseudomonas P14F1 vaccine varied at different times after vaccination.

*Investigation of the specificity and degree of early protective responses of mice vaccinated with different amounts of pseudomonas F*1 *vaccine*

Groups of 45 mice were vaccinated with a single injection of 1·0, 0·1 or 0·01 mg./kg. mouse weight of pseudomonas P14F1 vaccine. Four days after vaccination, a time determined by the previous experiment when maximum non-specific protective responses were found, three mice from each of the three groups of vaccinated mice and from a group of unvaccinated control mice were challenged with 1, 5 or 10 LD100 of 15 different serological types of *Ps. aeruginosa*.

*Specificity of protective responses of mice after a triple injection of pseudomonas P*14*F*1 *vaccine*

Groups of 45 mice were inoculated intraperitoneally on each of three consecutive days with 0·1 mg./kg. mouse weight of pseudomonas P14F1 vaccine. On days 2, 4, 7 and 14 after the last injection of vaccine, groups of three mice were challenged with 1LD100 of each of 15 different serological types of *Ps. aeruginosa*.

Specificity of protective responses of mice after single injections of vaccines from different strains of Ps. aeruginosa

Pilot studies, in which groups of mice were given single injections of 1·0, 0·1, 0·01 and 0·001 mg./kg. mouse weight of vaccines made from *Ps. aeruginosa* serotypes 2AB, 3, 5C, 8, 10, B4 and NT, 4 days before 1LD100 homologous challenge, showed that all vaccines except NTF1 would protect mice against homologous

Table 2. *Specificity of protective responses in mice after a single injection of pseudomonas vaccine P14F1*

Serotype of challenge strain	Day of challenge after vaccination: 2 — Vaccine dose (mg./kg.) 1·0	2 — 0·01	4 — 1·0	4 — 0·01	7 — 1·0	7 — 0·01	14 — 1·0	14 — 0·01
1	+	+	+	+	−	−	−	−
2A	−	−	−	−	−	−	−	−
2A 2B 5C	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−
5C	−	−	−	−	−	−	−	−
5D	−	−	+	−	−	−	−	−
6A	−	−	+	+	+	+	+	+
6B	+	+	+	+	+	+	+	+
6C	+	+	+	+	−	−	−	−
8	−	−	+	−	−	−	−	−
9	+	−	+	+	−	−	−	−
10	−	−	−	−	−	−	−	−
11	−	−	+	−	−	−	−	−
14	+	+	+	+	+	+	+	+
NT	−	−	−	−	−	−	−	−

+ = all mice survived; − = one or more mice died.

challenge at a dose of 0·1 mg./kg. mouse weight. A dose of 1·0 mg./kg. mouse weight of NTF1 vaccine was required to protect against the homologous strain.

Groups of 45 mice were vaccinated with 0·1 mg./kg. mouse weight of pseudomonas 2AB, 3, 5C, 8, 10, 14 and B4F1 vaccines respectively and a further group of 45 mice was vaccinated with 1·0 mg./kg. mouse weight of NTF1 vaccine.

Four days after vaccination groups of three mice, from each of the eight groups of vaccinated mice, were challenged with 1 LD100 of each of the 15 different serological types of *Ps. aeruginosa* respectively. Control groups of three unvaccinated mice were injected intraperitoneally with 1 LD100 of each of the 15 different serological types of *Ps. aeruginosa*.

RESULTS

Specificity of protective responses of mice after a single injection of pseudomonas vaccine

Table 2 summarizes results of experiments in which vaccinated mice were challenged on various occasions after vaccination with a range of different serological types of *Ps. aeruginosa*.

Two days after vaccination both doses of vaccine were found to have induced protection in mice against several unrelated serotypes of *Ps. aeruginosa*. The vaccine used at 1·0 mg./kg. mouse weight protected against the homologous strain and four other serotypes; there was no protection against one of these (type 9) with the lower dose of vaccine.

Table 3. *Survival of mice after challenge with* 1, 5 *and* 10 *LD*100 *of different serotypes of* Pseudomonas aeruginosa, 4 *days after a single injection of pseudomonas vaccine P*14*F*1

Serotype of challenge strain	Size of challenge dose: 1 LD 100 Vaccine dose (mg./kg.)			5 LD 100 Vaccine dose (mg./kg.)			10 LD 100 Vaccine dose (mg./kg.)		
	1·0	0·1	0·01	1·0	0·1	0·01	1·0	0·1	0·01
1	+	+	+	−	−	−	−	−	−
2A	−	−	−	−	−	−	−	−	−
2A 2B 5C	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−
5C	−	−	−	−	−	−	−	−	−
5D	+	+	−	−	−	−	−	−	−
6A	+	+	+	+	+	+	+	+	−
6B	+	+	+	−	−	−	−	−	−
6C	+	+	+	−	−	−	−	−	−
8	+	−	−	−	−	−	−	−	−
9	+	+	+	−	−	−	−	−	−
10	−	−	−	−	−	−	−	−	−
11	+	−	−	−	−	−	−	−	−
14	+	+	+	+	+	+	+	+	−
NT	−	−	−	−	−	−	−	−	−

+ = all mice survived; − = one or more mice died.

Mice challenged 4 days after vaccination showed a wider range of protection. In addition to the earlier five serotypes, they were protected against four more by the higher dose of vaccine, but against only one more by the lower dose.

At the 7th and 14th days after vaccination much of the non-specific protection was lost and mice that received either the high or the low dose of vaccine were protected against three serotypes, the homologous type 14, and types 6A and 6B.

Effect of size of challenge dose on the specificity of early protective responses

In this experiment (Table 3) three doses of vaccine, 1·0, 0·1 and 0·01 mg./kg. mouse weight, were used. Three different challenge doses of 1, 5 and 10 LD 100 were given 4 days after vaccination.

It is seen in Table 3 that the largest dose of vaccine protected mice at 4 days against 1 LD 100 of nine serotypes, the same nine that showed similar protection in Table 2 at 4 days. With 5 LD 100 there was protection against only two serotypes – the homologous type and 6A. With 10 LD 100 there was protection against the same two serotypes, but only in mice which had received the two higher vaccine doses.

Table 4. *Specificity of protective responses in mice after three injections of pseudomonas vaccine (P14 F1)*

Serotype of challenge strain	Day of challenge after vaccination			
	2	4	7	14
1	+	+	+	−
2A	+	+	+	−
2A 2B 5C	−	−	−	−
3	−	−	−	−
5C	−	−	−	−
5D	+	−	−	−
6A	+	+	+	+
6B	+	+	+	+
6C	+	+	+	+
8	−	+	−	+
9	+	+	+	−
10	−	−	−	−
11	+	+	+	−
14	+	+	+	+
NT	−	−	−	−

+ = All mice survived; − = one or more mice died.

Specificity of protective responses of mice after three injections on consecutive days of pseudomonas P14F1 vaccine

The protective responses induced in mice by injection of pseudomonas P14F1 vaccine (0·1 mg./kg. mouse weight) on three consecutive days are shown in Table 4.

Two days after the third injection of vaccine mice were protected against 1 LD100 of nine serotypes. Four days after vaccination mice were still protected against nine serotypes, but had lost protection against serotype 5D and gained protection against serotype 8.

Seven days after vaccination mice were protected against eight serotypes and at 14 days after vaccination mice were still protected against five serotypes, having lost protection against only a further four serotypes of *Ps. aeruginosa*.

Specificity of protective responses in mice inoculated with different F1 vaccines

Single injections of F1 vaccines gave complete protection to mice challenged, 4 days after vaccination, with 1 LD100 of their respective homologous strains, i.e. the strains of *Ps. aeruginosa* from which the vaccines had been made (Table 5).

As well as inducing homologous protection the vaccines protected mice against 1 LD100 challenges from one or more unrelated serotypes. All vaccines induced non-specific partial protection over a wide range of serotypes by which some but not all mice were protected.

Table 5 also illustrates the potential usefulness of F1 vaccines made from different strains of *Ps. aeruginosa* in the preparation of multivalent anti-pseudo-

Table 5. *Protection of mice against* Ps. aeruginosa *infections by different pseudomonas F* 1 *vaccines*

Serotype of challenge strain	Mortality in groups of three mice vaccinated with F 1 from one of the following serotypes of *Ps. aeruginosa*							
	14	10	8	5C	3	NT	B4	2AB
14	0	1	2	1	0	0	0	2
10	1	0	2	1	1	2	3	2
8	0	1	0	2	2	3	1	3
5C	2	3	3	0	3	0	0	3
3	1	0	2	1	0	3	3	3
NT	3	2	3	1	3	0	3	3
1	0	1	2	0	2	3	1	1
2A	1	3	3	0	3	0	1	1
2A 2B 5C	3	1	0	0	3	0	2	2
5D	0	3	1	3	3	2	0	2
6A	0	1	3	0	0	1	0	0
6B	0	1	3	0	3	1	2	3
6C	0	1	1	3	3	1	2	3
9	0	3	3	3	3	3	3	0
11	0	3	3	0	3	1	0	2

monas vaccine. The F 1 vaccines made from serotypes 5C, 10, 14 and NT together induce 100 % protective responses in mice against all 15 of the challenge strains of *Ps. aeruginosa* used in these experiments.

DISCUSSION

The early protection induced in mice actively immunized with a single injection of a pseudomonas P14F1 vaccine was found not to be type-specific. Non-specific protection, following a single injection of the vaccine, appeared as early as 2 days after vaccination and was still present 14 days after vaccination. The maximum non-specific response after vaccination occurred about 4 days after vaccination when P14F1 vaccine was found to have protected mice against lethal challenges by 9/15 different serotypes of *Ps. aeruginosa*. The degree of non-specific protection induced by P14F1 vaccine was influenced by the amount of vaccine used and the frequency of vaccination; thus 4 days after vaccination a single injection of 1·0 mg./kg. mouse weight of P14F1 protected mice against challenge by three more strains of *Ps. aeruginosa* than 0·01 mg./kg. mouse weight, and a dose on three consecutive days of P14F1 vaccine protected mice against as wide a range of challenge strains, 2 and 7 days after vaccination, as a single dose of vaccine 4 days after vaccination. Preliminary studies with other F 1 vaccines made from seven unrelated serotypes of *Ps. aeruginosa* showed that protection induced 4 days after a single injection of each vaccine was also not type-specific. The non-specific protective responses induced by the vaccines 4 days after vaccination ranged from complete protection against nine serotypes of *Ps. aeruginosa* by P14F1 vaccine to complete protection against two serotypes by P8F1 vaccine. A potentially useful feature of F 1 vaccines is the speed at which protection develops after vaccination: maximum non-specific

protective responses took only 4 days to appear after a single injection of vaccine. However, the duration of this non-specific protection after a single injection of vaccine was short; for example, 3 days after the maximum non-specific protective response the mice could only resist challenge from three serotypes of *Ps. aeruginosa*, whereas 3 days earlier they had resisted challenge by nine serotypes. In another experiment it was shown that the period of maximum non-specific protection could be extended from 1 to at least 5 days by injecting the vaccine on three consecutive days. The induction of a protracted period of non-specific protection soon after vaccination shows that the F1 vaccines may provide useful anti-pseudomonas vaccines, e.g. for the protection of patients with burns, who may be simultaneously infected with different serotypes of *Ps. aeruginosa*. The experiments show that it is theoretically possible to select vaccines with wide ranges of protective activity, e.g. 5C, 10, 14 and NT, which would induce protection against all the serotypes of *Ps. aeruginosa* used in these experiments. Alexander *et al.* (1969) and Fisher, Devlin & Gnabasik (1969) have shown that combining vaccines, similar in type to F1 vaccines, has no effect on their potential immunogenic properties; combined vaccines were shown to induce protection against a wide range of *Ps. aeruginosa* strains. Previous experiments have also shown that F1 vaccines were as effective in burned mice as in unburned mice (Jones, 1971) and are of low toxicity (Jones, 1969*a*). It is possible to purify F1 vaccines by gel filtration so that only a single line of precipitation is shown by immunodiffusion (Jones, 1969*b*); purification, however, may reduce the range of types against which the vaccine will induce protection and may prove to be an unnecessary procedure.

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