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Polyvalent Antisera to *Pseudomonas* Ribosomal Vaccines: Protection of Mice Against Clinically Isolated Strains

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The preparation of polyvalent antisera to ribosomal vaccines from *Pseudomonas aeruginosa* is described. The ability of these antisera to protect mice by passive immunization against challenge with randomly chosen, clinically isolated strains of *P. aeruginosa* is reported. Significant protection was achieved against 34 of 40 strains tested (85%). Included among these strains against which protection was achieved were four mucoid strains. In addition, the degree of cross-protection attainable by the ribosomal vaccines was investigated. The results obtained indicated that these vaccines are generally serotype specific.

Immunoprophylaxis and immunotherapy for infection with *Pseudomonas aeruginosa* has gained increasing interest in recent years as indicated in the literature (5, 7, 8, 20, 23, 24). There are two primary reasons for this interest. (i) There exist specific populations of patients which have a much higher degree of risk of *Pseudomonas* infection than the general population. These groups include patients with extensive thermal injury, oncology patients on intensive chemotherapy, and patients with cystic fibrosis. (ii) Antibiotics often have only limited effectiveness against *Pseudomonas* infections, and patterns of resistance tend to emerge (18). Different antigenic preparations have been used as potential vaccines for prophylaxis as well as for the production of specific antisera or hyperimmune globulin, which in turn could be used as either prophylaxis or therapy. These antigenic preparations have included lipopolysaccharides (10), cell wall lipid-protein-carbohydrate complexes (19), "slime" polysaccharides (3, 25), "slime" glycolipoprotein (26), "core" glycolipids (4), and endotoxin protein (1), as well as exotoxin A (17, 21, 27). Previous studies from this laboratory have investigated still another type of antigenic preparation as a potential vaccine, that of a ribosomal vaccine (14-16). In those studies, ribosomes were prepared from individual serotypes of *P. aeruginosa*, and their immunogenicity was evaluated against challenge with homologous serotypes of live organisms (15, 16). The present report describes the preparation of polyvalent antisera to *Pseudomonas* ribosomal vaccines and the ability of these antisera to protect mice by passive immunization against challenge with randomly chosen, clinically isolated strains of *P. aeruginosa*.

MATERIALS AND METHODS

Mice and rabbits used, determination of mouse lethal titers of *Pseudomonas* cultures, preparation of ribosomal vaccines, chemical analyses, vaccination of rabbits, and passive mouse protection against laboratory strains were all described previously (15, 16).

Bacteria. Laboratory strains of *P. aeruginosa* were the same as those used before (15). Clinical isolates of *P. aeruginosa* were obtained from the clinical microbiology laboratory of Brooke Army Medical Center after identification through appropriate diagnostic criteria.

Vaccination of rabbits for production of polyvalent antisera. Before vaccination, all rabbits were bled by cardiac puncture to obtain preimmune sera. The rabbits were divided into two groups of eight rabbits each, and each group was vaccinated by subcutaneous injection of an aqueous vaccine preparation at a dosage of 1.0 mg of protein. No adjuvants were used. Ten to 14 days after the initial vaccination the rabbits were given a booster dose of the same vaccine. Fourteen to 20 days after the booster vaccination, the rabbits were again bled by cardiac puncture to obtain the immune sera, which were pooled from the individual rabbits in each group. After this bleeding, the rabbits were subjected to another cycle of vaccination as described above, using vaccines prepared from different serotypes. Vaccines of different serotypes were not mixed or administered concurrently (except as noted below), but rather were administered sequentially. Vaccination cycles were repeated until all available serotypes had been used (five serotypes for group 1, six serotypes for group 2); this covered a period of approximately 8 months.

Passive mouse protection with polyvalent antisera against clinical isolates. Antisera were diluted 1:2 with normal saline, and 0.5-ml aliquots of group 1 antisera were injected intraperitoneally into a group of 40 mice, followed 3 to 4 h later by injection of 0.5-ml aliquots of group 2 antisera into the same mice. The following day groups of 10 of the passively

immunized mice were challenged by injection of an aliquot of whole (live) culture of one of the clinical isolates of *P. aeruginosa* (along with groups of control, nonimmunized mice) as described previously (15) for direct challenge of vaccinated mice. In some experiments the mice were given an additional injection of antiserum (diluted 1:4) from an earlier bleeding from either group 1 or group 1 and group 2 rabbits 1 day before administration of the antisera, as described above. However, no difference was found in the results.

RESULTS

Comparison of vaccination with a combination vaccine versus a monovalent vaccine. Preliminary experiments using the active mode of immunization of mice had indicated that immunization with a combination of vaccines prepared from two different serotypes did not yield as effective protection as immunization with the same two vaccines administered sequentially. Thus, the effect of immunization with vaccines in combination was tested by using the passive mode as follows. One group of rabbits was vaccinated with a combination of vaccines prepared from *P. aeruginosa* types 8 and 12, whereas the second group was vaccinated only with the type 8 vaccine. The immune serum was collected and tested for passive mouse protection. The results are presented in Table 1 and suggest that vaccination with a single vaccine may result in a higher level of passive mouse protective ability in the antiserum than does vaccination with a combination of two vaccines. For this reason vaccines were administered sequentially rather than concurrently for production of polyvalent antisera, as noted above.

Passive mouse protection with polyvalent antisera against challenge with laboratory strains of *P. aeruginosa*. The two polyvalent antisera prepared as described above were tested for mouse protective capacity against the homologous laboratory strains of *P. aeruginosa* used to prepare the vaccines as well as against heterologous strains. The results obtained with homologous strains are given in Table 2 in terms of mouse protective titers of each antiserum, i.e., the highest serial dilution of the antiserum capable of passively transferring significant protection. The results indicate that protection was achieved against all of the serotypes available except types 3 and 9. However, in two other cases (type 2, strain 359, and type 5) protective titers of 1:4 were obtained with antisera from earlier bleedings (2 weeks postbooster), but no protection was obtained with the antisera from the final bleeding (7 weeks postbooster). The results obtained with heterologous strains are shown in Table 3 and indicate that there was significant cross-protection with only one of four serotypes tested. (Earlier experiments using the active mode of immunization with a larger number of serotypes had yielded similar results.)

TABLE 1. *Comparison of vaccination with a combination vaccine versus a monovalent vaccine*

Dilution[b]	Mice survival[a]				*P* value[d]
	Antiserum to combination vaccine[c]		Antiserum to monovalent vaccine[c]		
	No.	%	No.	%	
1:2	3/5	60	4/5	80	
1:4	8/10	80	9/10	90	
1:8	5/10	50	10/10	100	0.01625
1:16	2/10	20	4/10	40	

[a] Mice were scored after 48 h for survivors.

[b] Mice were given 0.5 ml, intraperitoneally, of either antiserum at the stated dilution 3 h before challenge with live organisms (eight 50% lethal doses) of *P. aeruginosa* serotype 8.

[c] Serum was obtained from rabbits after two immunizations with ribosomal vaccines at a dose of 1 mg of protein from each of types 8 and 12 (combination) or type 8 alone (monovalent).

[d] *P* value was calculated by the Fisher exact probability test (28), comparing the survival of the mice given antiserum to combination vaccine with the survival of those given antiserum to monovalent vaccine at the same antiserum dilution.

Passive mouse protection with polyvalent antisera against challenge with clinical isolates of *P. aeruginosa*. The polyvalent antisera were tested for passive mouse protection against randomly chosen clinical isolates of *P. aeruginosa* as described above. Table 4 summarizes these results along with the results of tests using preimmune sera. The data demonstrate that the polyvalent antisera passively protected mice against challenge with the clinical isolates, whereas the preimmune sera afforded no protection at all. In Table 5 the results obtained in similar experiments with the polyvalent antisera, using a much larger number of clinical isolates, are presented. Protection was achieved against 34 of 40 clinical isolates, or 85%. Included among these isolates were four mucoid strains against which the polyvalent antisera also showed excellent protection.

Further experiments with preimmune serum using additional clinical isolates (besides those shown in Table 4) were precluded due to insufficient quantities of this serum from the same group of rabbits. However, in studies performed over a period of 3 years, preimmune sera have been tested from 14 different groups of rabbits, and in only one case was any protective activity against *Pseudomonas* observed.

TABLE 2. *Mouse protective titers of polyvalent antisera against homologous challenge strains of P. aeruginosa*

Antiserum	Vaccine serotype	Elapsed time (wk) since booster vaccinations[a]	Mouse protective titer[b]
Group 1	1	32	1:2
	2 (strain 359)	2	1:4
	2 (strain 359)	7	<1:1
	2 (strain 2243)	12	1:8
	3	16	<1:1
	13	28	1:8
Group 2	5	2	1:4
	5	7	<1:1
	6	21	1:2
	8	32	1:2
	9	27	<1:1
	11	16	1:4
	12	12	1:4

[a] Elapsed time since the booster vaccination with the particular vaccine serotype indicated until the final bleeding of the rabbits for the polyvalent antiserum. Antisera from earlier bleedings were also tested against serotypes which had already been used for vaccination, and the protective titers obtained showed variations of two- to fourfold.

[b] Mouse protective titer is the highest dilution of antiserum giving statistically significant protection determined as described in the footnotes to Table 1. The symbol <1:1 indicates that undiluted antiserum did not protect against the serotype indicated.

DISCUSSION

The results presented in this report describe the preparation of polyvalent antisera to *Pseudomonas* ribosomal vaccines and the protection of mice against challenge with randomly chosen clinical isolates of *P. aeruginosa* by passive immunization with the antisera. Previous reports from this laboratory have described the preparation and properties of *Pseudomonas* ribosomal vaccines (14, 15) and their immunogenicity by active (15) and passive (16) immunization. In these studies protection of mice was determined against challenge with laboratory strains of homologous serotypes. However, to be clinically effective protection must be achieved against a large number of strains found in the clinical setting. This is especially important in the case of passive immunization for patients in need of immediate protection, such as burn patients or immunosuppressed patients. Thus, it was of prime significance to determine if polyvalent antisera could be prepared which would be protective against a high percentage of clinically isolated strains of *P. aeruginosa*. The results obtained demonstrate that the preparation of such antisera is feasible and that the antisera can protect against a relatively high percentage (85%) of a large number (40) of strains tested (Table 5). Of special interest is the fact that the polyvalent antisera also protected against all four of the mucoid strains of *Pseudomonas* tested (Table 5). Mucoid strains of *P. aeruginosa* are isolated with a relatively high frequency from patients with cystic fibrosis (9, 11) and are generally associated with a poorer prognosis (6). Although the role of serum antibody in controlling the growth of *Pseudomonas* in situ in this disease state is not clear (12), the results obtained in this study indicate that further investigation into immunotherapy is warranted.

The ribosomal vaccines used to prepare the polyvalent antisera can be fractionated further into component ribosomal particles (15, 16) and subunits (M. M. Lieberman, G. L. Wright, and

TABLE 3. *Passive protection with heterologous laboratory strains of P. aeruginosa*

Antiserum	Vaccine serotype	Elapsed time (wk) since booster vaccination	Mouse protective titer
Group 1	2-2243 (homologous)	2	1:16
	13 (homologous)	18	1:8
	6 (heterologous)		1:1
	8 (heterologous)		≤1:1[a]
Group 2	2-2243 (heterologous)		≥1:8[b]
	13 (heterologous)		<1:1[c]
	6 (homologous)	11	1:8
	8 (homologous)	22	1:4

[a] No protection obtained at 1:2 dilution of antiserum; not tested with undiluted antiserum.

[b] Protection obtained at 1:8 dilution of antiserum; not tested at greater than 1:8 dilution.

[c] No protection obtained at undiluted antiserum.

TABLE 4. *Passive mouse protection with preimmune and polyvalent immune sera against clinical isolates of P. aeruginosa*

Strain no.	Mice survival[a]				*P* value[b]
	Polyvalent immune serum		Preimmune serum		
	No.	%	No.	%	
230-2	7/9	78	0/10	0	0.00714
282	8/9	89	0/10	0	0.000119
291-2	9/10	90	0/10	0	0.000060
853	6/10	60	1/10	10	0.02709

[a] Mice were scored after 48 h for survivors.

[b] The *P* value was calculated by using the Fisher exact probability test (28).

TABLE 5. *Passive mouse protection with polyvalent antisera against clinical isolates of P. aeruginosa*[a]

Strain no.	Immunized mice survival[b]		Control mice survival[b]		LD_{50} (no.) in challenge inoculum[c]	*P* value[d]
	No.	%	No.	%		
5F	5/10	50	0/10	0	10	0.0162
5R	5/10	50	0/10	0	7.9	0.0162
15	7/10	70	1/10	10	25	0.009526
19-1	10/10	100	0/10	0	2.3	0.0000054
23-B[e]	7/10	70	1/10	10	6.3	0.009526
117	7/9	78	0/10	0	12.5	0.000714
138	5/10	50	0/10	0	8	0.0162
144	0/10	0	0/10	0	6.8	
175	10/10	100	0/10	0	12.6	0.0000054
198	1/9	11	1/10	10	31.5	
225-1	9/10	90	0/10	0	31.5	0.000060
229	8/10	80	1/10	10	12.5	0.002679
230-2	7/9	78	0/10	0	79	0.000714
234	10/10	100	0/10	0	12.5	0.0000054
239	10/10	100	0/10	0	2.2	0.0000054
257-A	10/10	100	0/10	0	2.2	0.0000054
282	8/9	89	0/10	0	6.2	0.000119
291-2	9/10	90	0/10	0	7.9	0.000060
361	10/10	100	0/10	0	19.8	0.0000054
371	9/9	100	0/10	0	20	0.000011
372	10/10	100	1/10	10	4.0	0.000060
535RM[e]	8/10	80	2/10	20	2.0	0.01096
618[e]	9/10	90	0/10	0	6.3	0.000060
655	10/10	100	1/10	10	2.0	0.000060
656	10/10	100	0/10	0	12.5	0.0000054
672	0/10	0	0/10	0	50	
679	9/10	90	1/10	10	6.3	0.000541
718	4/10	40	0/10	0	15.8	0.0433
773	0/10	0	0/10	0	12.5	
802	10/10	100	0/10	0	12.5	0.0000054
803	0/10	0	0/10	0	2.0	
815	0/10	0	0/10	0	31.5	
820	10/10	100	0/10	0	79	0.0000054
845	7/10	70	1/10	10	6.3	0.009526
853	6/10	60	1/10	10	10	0.02709
880	7/10	70	0/10	0	79	0.001548
892	10/10	100	1/10	10	15.8	0.000060
904	7/9	78	0/10	0	14.7	0.000714
935	8/10	80	1/10	10	20	0.002679
942-A[e]	10/10	100	0/10	0	3.1	0.0000054

[a] Significant protection was achieved against 34 of 40 strains tested, or 85%.

[b] Mice were scored after 48 h for survivors.

[c] The number of 50% lethal doses (LD_{50}) in the challenge inoculum was calculated as described previously (15).

[d] The *P* value was calculated by using the Fisher exact probability test (28).

[e] Mucoid strains.

K. M. Wolcott, manuscript in preparation; M. M. Lieberman, D. C. McKissock, and G. L. Wright, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, E95, p. 70). These experiments are being performed to identify the immunogenic principle(s) associated with the vaccines. However, for the practical application of such vaccines only their effectiveness and safety need be demonstrated. The studies described in this report were undertaken with this end in mind. Other investigators have tested *Pseudomonas* vaccines and antisera in clinical settings. Alexander and Fisher (2) used a polyvalent lipopolysaccharide vaccine (10) and demonstrated protective efficacy of the vaccine and hyperimmune globulin in burn patients. However, the vaccine caused significant adverse side effects (2, 22), which imposed severe limitations on its use. Recently, a new type of polyvalent *Pseudomonas* vaccine was described (19) consisting of lipid-protein-carbohydrate complexes extracted from the cell walls of the organism. This vaccine was tested in a controlled clinical trial (13) and was shown to provide significant protection to burn patients against infection with *P. aeruginosa*. The results obtained so far in regard to *Pseudomonas* ribosomal vaccines suggest that further investigation should proceed in both the evaluation of clinical efficacy and safety of the vaccines and the elucidation of the nature of their immunogen(s).

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