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Pseudomonas Vaccine

III. Evaluation of a Polyvalent Vaccine

CARMEN L. DE FAJARDO AND HORACIO F. LABORDE

Instituto de Investigación de Ciencias Biológicas, Laboratorio de Microbiología, Montevideo, Uruguay

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A previous communication (H. F. Laborde and C. L. de Fajardo, J. Bacteriol. **90**:290, 1965) reported the absence of protection in mice inoculated with a phenol-killed *Pseudomonas aeruginosa* vaccine when these mice were challenged with other pathogenic *P. aeruginosa* strains. This failure to protect mice persisted (*unpublished data*) when vaccines were prepared by methods similar to those described for *Salmonella* immunization (M. Kawakami et al, J. Bacteriol. **92**:1585, 1966).

This paper reports the preparation and assay of a polyvalent vaccine (PVV) constituted by a pool of killed pathogenic *P. aeruginosa* strains. These strains were isolated from human patients and were of the smooth, green-pigmented, cytochrome oxidase-positive type. Characteristics of mice used and of the cell culture, LD_{100} determination, and virulence maintenance have been described (H. F. Laborde and C. L. de Fajardo, J. Bacteriol. **93**:508, 1967). The following strains were used for vaccine preparation: 480, 49-IH, 9465, 6018, 4037, and 7887 from the Instituto de Higiene, Montevideo, Uruguay, and 59, 154, and 157 from our own collection. *P. aeruginosa* 72 F1 was used for some challenge tests. Sera from immunized and control animals were assayed by a rapid agglutination test similar to one used for brucellosis (R. B. H. Gradwohl, *Clinical Laboratory Methods and Diagnosis*, The C. V. Mosby Company, St. Louis, 1948). Antigens were prepared by suspending live *P. aeruginosa* in physiological saline, and the density was adjusted to equal that of the McFarland no. 10 standard.

Vaccine preparation. PVV was prepared by suspending 15 mg of wet cells of each strain in 5 ml of 0.5% phenol saline. After standing 24 hr at 37 C, the suspension was adjusted with physiological saline to a concentration of 10 mg/ml, and the resulting vaccine was tested for sterility.

Two sets of experiments were done: (i) vaccination with PVV alone and (ii) vaccination with PVV supplemented by a booster inoculation of live or killed *Pseudomonas*.

Vaccination with PVV alone. The vaccine was administered to mice by subcutaneous injection of three 0.2-ml doses at 2-day intervals. No signs of toxicity were observed. After 5 weeks, vaccinated and nonvaccinated mice were challenged with 1 LD_{100} of strains not included or included in the vaccine.

TABLE 1. *Protection of mice vaccinated with PVV against challenge with 1 LD_{100} of Pseudomonas aeruginosa strains included or not included in the vaccine*

Mice	Challenge test					Agglutination test			
	No. of deaths/no. challenged					Antigen 49-IH	Antigen 9465	Antigen 2-IH	Antigen 72F1
	Strain 59	Strain 157	Strain 154	Strain 9465	Strain 72F1[a]				
Vaccinated.....	2/10	3/28	3/10	2/10	8/10	3,200	1,800	1,600	320
Control.....	3/3	3/3	3/3	3/3	3/3	—	—	—	—

[a] Strain not included in the vaccine.

The PVV established a good degree of immunity only against strains included in the vaccine and afforded almost no protection against strains not included in the PVV. Vaccination evoked a detectable serum antibody response in virtually all animals (Table 1).

Vaccination with PVV plus booster inoculation of live or killed Pseudomonas. Three lots of new mice were inoculated subcutaneously with three doses at 2-day intervals of the same type of PVV used previously. Thirty days later, these lots (labeled A, B, and C) were reinoculated as follows: A, with a 0.2-ml subcutaneous dose of PVV; B, with 1 LD_{100} (0.2 ml of a McFarland 2 saline suspension of live bacteria) of a *Pseudomonas* contained in the vaccine; C, with one intraperitoneal

sublethal dose (1 SLD, consisting in 0.2 ml of a saline suspension of live bacteria equivalent in opacity to McFarland tube no. 1) of a *Pseudomonas* included in the vaccine. No signs of toxicity were observed. After 20 days, the three lots of mice were challenged with 1 LD_{100} of strain 72F1, a strain not present in the PVV (Table 2).

TABLE 2. *Protection of mice (with secondary vaccination) against challenge with 1 LD_{100} of Pseudomonas aeruginosa 72F1, a strain not included in the vaccine*

Lot	Vaccination		Challenge test		Agglutination test[a]
	Primary	Secondary	No. of deaths/no. challenged		
			Vaccinated	Control	
A	PVV	PVV	10/17	3/3	3,200
B	PVV	1 LD_{100} of 157[b]	1/28	3/3	3,200
C	PVV	1 SLD of 4037[b]	5/24	3/3	3,200

[a] Results were the same with antigens 2-IH, 72F1, 49-IH, and 9465.
[b] Strains included in the PVV.

The booster inoculation of viable organisms resulted in moderate to good protection against challenge with a strain of *Pseudomonas* not included in the vaccine. Simultaneous agglutination tests show that all vaccines provoked a high serum agglutination response against strains included or not included in the vaccination schedule.

From a therapeutic viewpoint, a polyvalent vaccine might be useful, when prepared with pathogenic *P. aeruginosa* strains isolated from burn and surgical wards. High and lasting serum agglutination titers seem to be a guide to use in evaluating the status of immunity in vaccinated patients exposed to *Pseudomonas* infection.

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