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Pseudomonas Vaccine

I. Preparation and Assay[1]

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After implantation of permanent electrodes in cats, we have often observed that cutaneous infection by *Pseudomonas aeruginosa* develops at the sites of implantation.

The promising therapeutic and prophylactic results obtained from vaccination of the cats encouraged us to make a more detailed and a more comprehensive study, to prepare an effective vaccine against this type of suppurative process.

P. aeruginosa IICB 59 was used in all experiments. This strain, isolated from a burned patient, was of the smooth, green-pigmented, cytochrome oxidase-positive type. When injected by the intraperitoneal route, approximately 1.2×10^8 cells were required to kill 100% of the mice in 24 to 48 hr; the concentration of this LD_{100} was determined by plate count.

Pseudomonas for vaccine preparation was grown on nutrient agar (Difco) for 24 hr at 37 C; the colonies were then suspended in 0.5% phenol-saline. After standing overnight at 37 C (temperatures of 60 and 65 C were tried with negative results), the suspension was tested for sterility, and the bacterial density was adjusted to approximately 9×10^8 cells per milliliter. Neither pathological symptoms (including pyrogenicity) nor anatomopathological lesions appeared after the use of this vaccine. An increase of 10 times in the vaccine concentration failed to provoke any signs of toxicity.

Twenty-five mice of both sexes, weighing 15 to 20 g, were vaccinated subcutaneously with doses of 0.2 ml each; this procedure was repeated at 48-hr intervals for a total of three doses. After 5 weeks, all the vaccinated and six nonvaccinated mice were challenged with one LD_{100}; within 24 to 48 hr, all nonvaccinated animals died, showing, without exception, peritoneal gelatinous edema, abscesses at the sites of inoculation, and invasion of the spleens by *Pseudomonas*. The vaccinated mice all survived and were kept under observation for 5 months; no ill effects were apparent. The anatomopathological examination showed neither organic anomalies nor lesions at the sites of inoculation.

After 1 month, a new lot of 29 mice was vaccinated to repeat the test.

Other groups of 25 and 27 vaccinated mice were inoculated with two or five times the LD_{100} (i.e., 2.4×10^8 or 6×10^8 instead of 1.2×10^8 cells). Results of these experiments are shown in Table 1.

TABLE 1. *Protection of mice vaccinated with Pseudomonas aeruginosa IICB 59 against challenge with the same strain*

Mice	No. of deaths/no. challenged		
	LD_{100}	2 LD_{100}	5 LD_{100}
Vaccinated....	0/54	7/27	14/25
Control.....	12/12	9/9	9/9

TABLE 2. *Absence of protection of mice vaccinated with Pseudomonas aeruginosa IICB 59 against challenge with one LD_{100} of other P. aeruginosa strains*

Mice	No. of deaths/no. challenged			
	Strain 59	Strain 154	Strain 157	Strain 167
Vaccinated..	0/10	10/10	10/10	10/10
Control.....	3/3	3/3	3/3	3/3

Agglutination titers were determined by the method of Christie (Australian J. Exptl. Biol. Med. Sci. **26**:425, 1948). Nonvaccinated mice were negative. At 1 month after vaccination, the mice had titers ranging between 1:20 and 1:40; 3 months later the reaction was negative but the immunity persisted. Nonvaccinated mice surviving sublethal doses of live *Pseudomonas* had titers of 1:4,000 or more and were immune to one LD_{100}.

The immunity obtained was strain-specific;

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when mice were vaccinated with strain 59 and challenged with one LD_{100} of other strains of *P. aeruginosa*, all of these mice died (Table 2).

The aforementioned characteristics distinguish this vaccine from the toxic, antigenic, and thermostable substance isolated (Hosoya et al., Japan. J. Exptl. Med. **20**:55, 1949) from aged cultures of *Pseudomonas*. This substance transitorily protected guinea pigs against challenge doses of *Pseudomonas*.

Therapeutic and even prophylactic use of vaccines against pathogenic strains of *Pseudomonas* would seem to be of interest in view of the poor results obtained with antibiotic and antiseptic agents.

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