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New Immunotype Schema for *Pseudomonas aeruginosa* Based on Protective Antigens

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The development of an antigen schema for *Pseudomonas aeruginosa* based on challenge protection in mice as distinguished from serological tests in vitro was described. For 342 cultures, seven groups of cross-protective homogeneity were defined.

The serological heterogeneity of *Pseudomonas aeruginosa* has been repeatedly demonstrated, and a number of serotype schema have been formulated by various investigators since 1926 (1–7, 9–11). These previous studies were based on serologic reactions in vitro, usually whole-bacterial cell agglutinations, for classification purposes. In the course of a program to develop a polyvalent anti-infective immunizing preparation, it became apparent that protective immunity was not specifically related to in vitro-derived schemata. This conclusion was reached after a series of tests were done on 10 strains representative of the serogroups I through X of Verder and Evans (12). Killed whole-cell vaccines were made of each of these 10 strains, and these were injected in mice. A cross-challenge series of tests was subsequently carried out, wherein groups of mice previously injected with a given strain of vaccine were challenged with each of the 10 strains. Results of these experiments revealed a considerable immunologic crossover and nonspecificity; a representative set of data on the 10 groups among these 10 strains is presented in Table 1. A somewhat similar observation was also made by Markley and Smallman, who used a few challenge strains (8). The occasional low levels of homologous strain immunity seen here was unexpected and may merit further study.

The principal inference made from this experiment was that the specific agglutinogens in *P. aeruginosa* were not reliable indicators of the protective antigens, and, indeed, they may be wholly different. In order to help resolve the matter, we undertook the development of an antigen schema based upon challenge protection, as distinguished from serologic tests in vitro. To accomplish this, 342 cultures of *P. aeruginosa*, from human pathologies, were obtained from various hospital laboratories around the United States. A whole-cell killed vaccine (0.5% phenol, 60 C for 60 min) was prepared from each culture as a suspension of approximately 10^9 cells/ml. Groups of mice were given a single subcutaneous injection of each vaccine, undiluted, and were then challenged within 1 week with an estimated 100 LD_{50} of culture intraperitoneally administered in a 5% hog gastric mucin adjuvant. The LD_{50} values for mucin-adjuvant challenge strains were based on survival 5 to 7 days after challenge, and ranged from approximately 10^2 to 10^5 cells per mouse. Challenge with 100 LD_{50} resulted in a rapidly developing systemic infection and death in 12 to 24 hr for most of the animals. Strains were also selected for such challenges from preliminary tests which indicated specificity of protective immunity. It was assumed that if a group of vaccines induced a high level of resistance against challenge by a given strain, then the strains used for these vaccines and the challenge strain all had a common and dominant protective antigen. Strain vaccines not inducing protection against a given strain were then tested against another challenge strain.

An example of an early series of tests of this sort is given in Table 2. In this series, killed whole-cell vaccines were made from 16 different strains of clinical origin and groups of mice were injected with each. After 1 week, all mice were challenged with strain 1. The high level of resistance induced by vaccines made from strains 7 and 11 suggested that they shared a grouping with strain 1. In a second test employing strain 2 for challenge, a similar grouping was inferred for strains 5, 9, and 10 with strain 2. Challenge with strain 3 indicated that strains 6, 8, 12, 14, and 15 had a common grouping. Finally, challenge with strain 4 served to suggest its relation for a protective antigen with strain 6. Thus, four protective immunotypes were postulated in this example.

The application of this procedure to 342 cultures of *P. aeruginosa* led to the definition of seven

TABLE 1. *Results of cross-immunization cross-challenge tests in mice among ten P. aeruginosa serogroups of Verder and Evans*[a]

Challenge strain	Serogroup no.	Approximate degree of protection from vaccine made from strain									
		359	FR-31	2108	Mills	1 M	58F	2915	T488	6370	G2312
359	I	++	+	++	+	++	++	+++	+++	++	+++
FR-31	II	+	+	+	+++	+	+	++	++	+++	++++
2108	III	0	++	+++	++	+	++	++	0	+	0
Mills	IV	++	+	+	++	0	+	+	0	+	+
1 M	V	+++	++	+++	++	++++	++++	+	+++	+	+
58F	VI	+++	+	+++	+	+++	++++	+	++	++	++++
2915	VII	+	+++	+	+	+	0	+++	++	++	+++
T488	VIII	+	+	+	++	+	+	0	++++	+	0
6370	IX	+	+	0	++++	+	+	0	+	+++	+
G2312	X	0	+	+	+++	++	+++	+	+	0	+

[a] Approximate degree of protection for each group is expressed as per cent survivors represented by one of the following symbols: 0, 0 to 7; +, 8 to 27; ++, 28 to 47; +++, 48 to 67; and ++++, > 68.

TABLE 2. *Example of screening test results for indicating common protective immunologic groups*

Strain used for vaccine	Per cent survivors after challenge by strain no.			
	1	2	3	4
1	100	ND[a]	ND	ND
2	0	100	ND	ND
3	0	ND	100	ND
4	0	0	0	100
5	0	100	ND	ND
6	0	10	83	100
7	90	20	ND	ND
8	0	0	100	ND
9	10	100	ND	ND
10	0	100	ND	ND
11	100	0	ND	ND
12	20	0	100	ND
13	20	0	17	17
14	0	10	100	ND
15	10	20	100	ND
16	0	10	0	0

[a] Not determined.

groups (immunotypes) of cross-protective homogeneity. Among the 342 cultures, 21.8% were identified as type 1, 17.7% as type 2, 20.1% as type 3, 10.3% as type 4, 8.6% as type 5, 5.5% as type 6, 10.0% as type 7, and 6.0% as unclassified. A retrospective test indicated virtually no identity between these seven serotypes and those of the Verder and Evans schema (12). Because of this, it is likely that little or no resemblance is to be expected for our seven immunotypes and those defined by various previously cited in vitro-based methodologies.

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