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Immunization with *Pseudomonas aeruginosa* High-Molecular-Weight Polysaccharides Prevents Death from *Pseudomonas* Burn Infections in Mice

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High-molecular-weight polysaccharides from the extracellular slime of *Pseudomonas aeruginosa* were evaluated as immunogens in *Pseudomonas* burn infections in mice. Immunization with immunotype 1 or 2 polysaccharides induced a strong immunotype-specific and weak cross-reactive antibody response but protected mice against burn infections caused by either immunotype. Passive protection was provided by rabbit antiserum to immunotype 1 polysaccharide against burn infection by the homologous organism. *Pseudomonas* high-molecular-weight polysaccharides are potentially effective vaccines in burn infections.

High-molecular-weight polysaccharides isolated from culture supernatants or from extracellular slime of *Pseudomonas aeruginosa* appear to share immunotype determinants with the lipopolysaccharide O side chain (7–9). The best-characterized of these polysaccharides are from *P. aeruginosa* immunotypes 1 (8) and 2 (7), designated It-1 and It-2, of the Fisher-Devlin-Gnabasik system (2). The It-1 and It-2 polysaccharides induce type-specific and cross-reactive antibodies in mice and rabbits (4) and are immunogenic in humans (5; G. B. Pier, unpublished data). Antibodies to both polysaccharides are opsonic and provide cross-protection against intraperitoneal challenges in mice (4, 6, 7).

Since patients with extensive burns are particularly susceptible to life-threatening *Pseudomonas* infections, they are prime candidates for specific immunoprophylaxis or immunotherapy. A murine *Pseudomonas* burn infection model (3, 10) closely mimics human *Pseudomonas* burn wound sepsis and avoids some of the unphysiological aspects of massive intraperitoneal challenges. We used this model to evaluate the protective efficacy of the It-1 and It-2 polysaccharide vaccines in *P. aeruginosa* burn infections.

Six-week-old female C3H/FeJ mice (Jackson Laboratories, Bar Harbor, Maine) were immunized on days 0, 5, and 10 with 50-μg intraperitoneal injections of high-molecular-weight polysaccharide obtained from the culture supernatant of *P. aeruginosa* It-1 (8) or It-2 (7) and suspended in normal saline. Control mice received intraperitoneal injections of bovine serum albumin (BSA) according to an identical dose schedule. On day 15, the mice were anesthetized with methoxyflurane (Pitman-Moore, Inc., Washington Crossing, N.J.), subjected to an 11-s alcohol flame burn (2.5 by 2.5 cm), and injected subcutaneously at the burn site with seven 10-fold dilutions of washed log-phase It-1 or It-2 bacteria. Deaths were recorded for 7 days, and the 50% lethal dose (LD_{50}) of bacteria was determined by the method of Spearman-Karber (1). Blood was obtained from the retro-orbital venous plexus of anesthetized mice on days 0 and 15, and serum antibodies reactive with *P. aeruginosa* It-1 and It-2 polysaccharides were quantified by a radioactive antigen-binding assay (4).

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Immunized mice demonstrated an immunotype-specific serum antibody response (Table 1). In addition, there was a small but significant antibody response to It-2 polysaccharide in mice immunized with It-1 polysaccharide, and vice versa (Table 1). The burn injury itself apparently had little or no effect on the immunogenicity of It-1 polysaccharide, as indicated by nearly identical serum antibody responses in recently burned and unburned mice immunized with a single 50-μg intraperitoneal injection of this vaccine (Table 2). Immunization with three 50-μg doses of It-1 polysaccharide protected mice against subsequent It-1 burn infections, as indicated by a greater than 4 log increase in the LD_{50} of the It-1 challenge strain compared with its LD_{50} in BSA-immunized control mice (Table 3). Immunization with It-1 polysaccharide also produced cross-protection against It-2 burn infections, as indicated by a 1 to 2 log increase in the LD_{50} of It-2 organisms. Similarly, immunization with It-2 polysaccharide protected against both It-2 and It-1 burn infections, as evidenced by 2 to 3 log and 4 to 5 log increases in LD_{50}, respectively (Table 3). It-2 immunization appeared to provide somewhat greater protection against both homologous and heterologous challenges than did immunization with It-1 polysaccharide.

The intravenous administration of 0.2 ml of rabbit antiserum to It-1 polysaccharide (8) 18 h before burn infection resulted in a 2 to 3 log increase in the LD_{50} of the It-1

TABLE 1. Serum antibody responses of C3H/FeJ mice to immunization with *P. aeruginosa* It-1 and It-2 high-molecular-weight polysaccharides

Immunization[a]	Serum antibody concn (μg/ml)[b]			
	It-1		It-2	
	Day 0	Day 15	Day 0	Day 15
It-1	4.1 ± 0.4	35.2 ± 9.3[c]	3.8 ± 0.2	4.4 ± 0.2[c]
It-2	4.0 ± 0.2	5.2 ± 0.5[c]	10.5 ± 8.4	64.1 ± 26.4[c]

[a] Fifty micrograms were injected intraperitoneally on days 0, 5, and 10.

[b] Radioactive antigen binding assay; mean ± standard deviation; five mice per group.

[c] Significant increase compared with preimmunization level; $P \leq 0.02$ based on *t*-test on paired samples.

TABLE 2. Effect of burn injury on serum antibody responses of C3H/FeJ mice to immunization with *P. aeruginosa* It-1 high-molecular-weight polysaccharide[a]

Group	Serum antibody concn (μg/ml)[b]	
	Day 5	Day 10
Unburned	10.3 ± 5.8	10.8 ± 4.8
Burned	9.3 ± 5.6	11.2 ± 6.4

[a] Groups of 10 mice received 50 μg of It-1 polysaccharide intraperitoneally 24 h after undergoing an 11-s flame burn (2.5 by 2.5 cm) or no injury.

[b] Radioactive antigen-binding assay. Data expressed as mean ± standard deviation. Antibody levels of unimmunized mice were <2.0 μg/ml.

challenge strain compared with that observed in control mice which had received nonimmune serum (Table 4). Passive protection was somewhat less than that produced by active immunization (Table 3).

Thus, despite limited cross-reactive antibody responses induced in C3H/FeJ mice by *P. aeruginosa* It-1 and It-2 high-molecular-weight polysaccharides, cross-protection was comparable to homologous protection in the mouse burn infection model. The possibility exists that It-2 polysaccharide is somewhat more immunogenic than It-1 polysaccharide, as judged by both antibody levels and degree of protection against bacterial challenges. These findings are similar to those previously reported for CD-1 mice subjected to intraperitoneal infections (4).

Our data establish the efficacy of active immunization with It-1 and It-2 high-molecular-weight polysaccharides and passive immunization directed toward It-1 polysaccharide in *Pseudomonas* burn wound sepsis in mice. To the extent that the murine model reproduces human burn wound sepsis and that antibody responses to It-1 and It-2 polysaccharides reflect those achievable in humans (5; G. B. Pier, unpublished data), effective immunoprophylaxis against *Pseudomonas* burn infections with these and other immunotype-specific high-molecular-weight polysaccharide vaccines appears feasible.

TABLE 3. Type-specific and cross-protection against *P. aeruginosa* burn wound sepsis in C3H/FeJ mice after active immunization with It-1 and It-2 polysaccharides

Challenge strain	Immunization[a]	LD_{50} of challenge strain (log_{10} CFU)[b]
It-1	BSA	3.64
	It-1	8.04[c]
	It-2	8.26[c]
It-2	BSA	5.15
	It-1	6.94[c]
	It-2	7.54[c]

[a] Mice received 50 μg of high-molecular-weight polysaccharide or BSA intraperitoneally on days 0, 5, and 10.

[b] On day 15, seven 10-fold dilutions of washed log-phase bacteria were injected at the site of a fresh 11-s flame burn (2.5 by 2.5 cm). Five mice were used at each dilution, deaths were recorded for 7 days, and the LD_{50} ± 95% confidence interval was determined by the method of Spearman-Karber (1).

[c] Significant protection (nonoverlapping LD_{50} ± 95% confidence interval) compared with BSA-immunized controls.

TABLE 4. Protection against *P. aeruginosa* It-1 burn wound sepsis in C3H/FeJ mice passively immunized with type-specific high-molecular-weight polysaccharide antiserum

Immunization[a]	LD_{50} of challenge strain (log_{10} CFU)
Normal serum	4.0
It-1 antiserum	6.8[b]

[a] Mice received 0.2 ml of normal rabbit serum or It-1 polysaccharide antiserum intravenously 18 h before undergoing an 11-s flame burn (2.5 by 2.5 cm) followed by subcutaneous inoculation of the fresh burn site with seven 10-fold dilutions of the It-1 challenge strain. Five mice were used at each dilution, deaths were recorded for 7 days, and the LD_{50} ± 95% confidence interval was determined by the method of Spearman-Karber (1).

[b] Significant protection (nonoverlapping LD_{50} ± 95% confidence interval) compared with control animals that received normal rabbit serum.

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