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Polyclonal and Monoclonal Antibody Therapy for Experimental *Pseudomonas aeruginosa* Pneumonia

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A human immunoglobulin G preparation, enriched in antibodies to lipopolysaccharide (LPS) *Pseudomonas aeruginosa* antigens (PA-IGIV) and murine monoclonal antibodies (MAb) to *P. aeruginosa* Fisher immunotype-1 (IT-1) LPS antigen and outer membrane protein F (porin), were evaluated for therapeutic efficacy in a guinea pig model of *P. aeruginosa* pneumonia. The concentration of antibodies to IT-1 LPS was 7.6 μg/ml in PA-IGIV and 478 μg/ml in the IT-1 MAb preparation. No antibody to IT-1 was detected in MAb to porin. For study, animals were infected by intratracheal instillation of IT-1 *P. aeruginosa* and then treated 2 h later with intravenous infusions of PA-IGIV, IT-1 MAb, or porin MAb. Control groups received intravenous albumin, and routinely died from pneumonia. Both PA-IGIV (500 mg/kg) and IT-1 MAb (≥2.5 mg/kg) treatment resulted in increased survival ($P < 0.01$ to 0.001), and also improved intrapulmonary killing of bacteria. Porin MAb failed to protect from fatal pneumonia. IT-1 MAb treatment produced more survivals than did PA-IGIV treatment but only at dosages of MAb resulting in serum antibody concentrations greater than those achieved with PA-IGIV. PA-IGIV and IT-1 MAb demonstrated in vitro and in vivo (posttreatment guinea pig serum) opsonophagocytic activity for the IT-1 challenge strain. However, the polyclonal preparation required complement, whereas the MAb did not. We conclude that passive immunization with polyclonal hyperimmune *P. aeruginosa* globulin or with MAb to LPS antigens may be useful in the treatment of acute *P. aeruginosa* pneumonia. The relative efficacies of such preparations may be limited, however, by their type-specific LPS antibody concentrations.

Despite the increasing availability of antimicrobial agents with potent activity against *Pseudomonas aeruginosa* in vitro, the mortality associated with *P. aeruginosa* pneumonia remains high (2, 22, 32). Experimental studies have suggested that active immunization with cell wall lipopolysaccharide (LPS) antigen vaccines will reduce mortality from *P. aeruginosa* pneumonia (7, 16, 19). However, the desirability of establishing immune protection more rapidly, and the side effects associated with LPS-containing *P. aeruginosa* vaccines, argue for development of passive immunization strategies (27, 37). Furthermore, the availability of immune serum or globulins would offer the possibility of passive immune therapy for established infection, as well as prophylactic use in high-risk groups.

Several recent developments make feasible the development of immunologic reagents for passive immunization against *P. aeruginosa*. The capacity to render immunoglobulin G preparations suitable for intravenous (i.v.) use allows pain-free and safe administration of much greater amounts of antibody than was previously possible with intramuscular (i.m.) injection of immune serum globulin (8). Furthermore, the development of monoclonal antibodies (MAb) provides methodology for transfer of immunity with extremely small amounts of protein. In selecting a target antigen for passive immune reagents, most studies indicate that antibodies to LPS provide the most effective degree of protection against *P. aeruginosa* (4, 5, 18, 20, 29). Furthermore, recent studies in our laboratory (21) have documented the feasibility of passive immune therapy of experimental *P. aeruginosa* pneumonia by means of a human hyperimmune immunoglobulin G preparation (PA-IGIV) enhanced in LPS antibody activity to this organism (3). In the present study, we compared the therapeutic efficacy of the hyperimmune immunoglobulin G preparation, containing polyclonal antibodies, to that of murine MAb to *P. aeruginosa* antigens, in a guinea pig model of experimental *P. aeruginosa* pneumonia.

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MATERIALS AND METHODS

Animals. Hartley strain guinea pigs (400 g) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Animals were housed in standard cages and fed guinea pig chow (Ralston-Purina, St. Louis, Mo.).

Bacteria. *P. aeruginosa* 220 was employed for experimental pneumonias. This clinical isolate was Fisher immunotype-1 (IT-1) according to the Fisher-Devlin-Gnabasik typing scheme (used throughout this paper) (9). The characteristics of strain 220 have been described fully elsewhere (19). The maintenance and preparation of bacteria for experimental infections (16, 19), and in vitro studies (21, 24), have also been described before.

Antibody preparations. PA-IGIV (lot PR3011), enriched in antibodies against LPS IT-1, -2, -4, and -6, was supplied by Cutter Laboratories, Berkeley, Calif. This preparation was supplied as 5% protein in 10% maltose and was rendered suitable for i.v. infusion by acidification to pH 4.25. Details regarding plasma donor screening and the immunologic properties of this material have been provided elsewhere (3).

Murine MAb directed against *P. aeruginosa* IT-1 and IT-4 LPS antigens, as well as against *P. aeruginosa* outer membrane protein F (porin) (35), were made and characterized by the following methods. BALB/c female mice (Fred Hutchinson Cancer Center, Seattle, Wash.) were immunized intraperitoneally with 10^7 to 10^8 CFU of live *P. aeruginosa* (Fisher IT-1 or IT-4, obtained from Mike Fisher, Parke Davis & Co., Detroit, Mich.) each week for 4 to 6 weeks.

Cell fusions to create the hybridomas were performed as described previously (31). Culture supernatants of hybrid cell lines were screened for the presence of specific antibodies by replicate plating techniques with three different antigens. For antibodies to LPS antigens, overnight cultures of *P. aeruginosa* IT-1 and IT-4 were washed once in phosphate-buffered saline (PBS) and resuspended to an A_{660} of 0.2. Fifty microliters of the bacterial suspension was dispensed into each well of microtiter plates and then centrifuged to pellet the organisms. Excess fluid was expelled, the bacteria were fixed by the addition of 75 μl of 95% ethanol per well, and plates were incubated for 15 min at room temperature. The ethanol was removed, 75 μl of 5% bovine serum albumin (BSA) in PBS was added to each well, and plates were again incubated for 60 min at room temperature to block nonspecific reactions.

Outer membrane protein F (porin) was purified according to the methods of Yoshimura et al. (35). Assay plates were prepared by adding 50 μl of antigen (1.25 μg/ml) per well and permitting the antigen preparation to adsorb for 2 to 14 h at 37°C. Excess antigen was then removed, and the wells were blocked with BSA as described above. Culture fluids from hybridoma cells were incubated in the antigen-coated plates for 45 to 60 min at room temperature. After three washes with PBS–1% BSA, the presence of specific antibody binding was detected by an enzyme-linked immunosorbent assay (ELISA) reaction with biotinylated protein A and the Vectastain ABC procedure (Vector Laboratories Inc., Burlingame, Calif.).

Cells from individual master wells whose supernatants contained antibody to antigens of interest were cloned twice by limiting dilution techniques on mouse thymocyte feeder cell layers. Log-phase clonal cells (10^7) were injected into pristane (Aldrich Chemical Co., Inc., Milwaukee, Wis.)-primed (BALB/c × C57B16)F_1 mice (Jackson Laboratories, Bar Harbor, Maine) for the production of high-titered ascites.

Biochemical characterization of the molecular antigen for each MAb was done by immunoblot (Western blot) analysis (30). Outer membrane preparations from each of the seven Fisher-Devlin-Gnabasik reference immunotypes of *P. aeruginosa* were made by the methods of Tam et al. (33). Samples of each were subjected to polyacrylamide gel electrophoresis (12). Separated molecular entities were transferred from the gel to a nitrocellulose membrane as described before (34), and membranes were reacted with the individual MAb by using previously described methods (30). Immunoblot analysis of the MAb reactive in ELISA with either IT-1 or IT-4 *P. aeruginosa* demonstrated binding only to components present in the membrane preparation of the homologous immunotype. The immunoblot profile was an array of regularly spaced molecular entities (a ladderlike pattern) consistent with that seen in polyacrylamide gel electrophoretic analysis of LPS in the presence of sodium dodecyl sulfate (15). Binding patterns were unchanged if the antigen preparations were pretreated with proteinase K (Sigma Chemical Co., St. Louis, Mo.) before electrophoresis (30).

A similar analysis of the porin MAb showed binding to a 36-kilodalton protein. Unlike the immunotype-specific MAb, the porin MAb was found to bind to the same molecular species in all seven Fisher immunotypes. The immunoblot reaction was completely abolished if the antigen preparation was pretreated with proteinase K. The presence of this protein antigen in the outer membrane preparations, its mass and immunotype distribution, and the earlier descriptions of outer membrane protein F (35) have led us to conclude that this monoclonal specificity is antiporin.

Isotype-specific anti-mouse immunoglobulin reagents (Zymed, South San Francisco, Calif.) were used according to the instructions of the manufacturer to determine the subclass of each murine MAb. IT-1 MAb (Pa9 IIIH10) and porin MAb (PaPor IVE10) were both of the immunoglobulin G2a isotype, and the IT-4 MAb (Pa8 IB5) was immunoglobulin G3 class.

Serologic assays. Concentrations of antibodies against IT-1 and IT-4 *P. aeruginosa* antigens were determined by a radioimmune antigen binding assay previously described in detail (23). The results are reported as the average of duplicate determinations. Opsonophagocytic assays employed guinea pig peritoneal polymorphonuclear leukocytes (PMN) harvested 16 h after i.p. injection of 0.3% shellfish glycogen (Sigma Chemical Co., St. Louis, Mo.) (17). For assays, equal volumes (0.1 ml) of bacteria (5×10^6 CFU) and opsonizing preparation (or control) were mixed and added to 5×10^6 PMN plus 1% fresh nonimmune guinea pig serum (or buffer, as indicated). Reaction mixtures were brought to a final volume of 1 ml with Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.). Triplicate preparations were made for all specimens. Mixtures were incubated at 37°C on a tilting rack (Ames Co., Elkhart, Ind.); samples were removed after 0 and 60 min of incubation and were serially diluted in sterile water for quantitative cultures. Opsonic activity was expressed as the decrease in CFU of *P. aeruginosa* in reaction mixtures during 60-min incubation periods. Cell-free tubes were included in each assay to detect direct killing by serum.

Experimental pneumonia and study design. The methodology for establishing experimental *P. aeruginosa* pneumonia in guinea pigs has been described before (16, 19). Briefly, animals were anaesthetized (intraperitoneal pentobarbital), and their tracheas were isolated by a small midline neck incision. Samples (0.5 ml) of *P. aeruginosa* in isotonic saline were then instilled via a needle into the tracheobronchial tree. Necks were sutured, and animals awakened within 2 to 3 h. Preliminary studies established that 5×10^6 CFU of strain 220 was the minimun inoculum which resulted in uniformly fatal pneumonia in this model. This inoculum was used for lung challenges in this study.

Experiments to compare the therapeutic efficacy of the PA-IGIV preparation versus the MAb preparations involved cohorts of guinea pigs infected with equal inocula of *P. aeruginosa* and then treated 2 h after infection with a single i.v. infusion of antibody preparation. Control groups received infusions of 5% BSA in 10% maltose. Infusion volumes (5 ml) were kept constant for all study groups; the 5% BSA preparation was used for volume supplementation as needed. The methodology for i.v. infusions via the external jugular vein has been described before (21).

For survival studies, animals were observed for four days and were considered long-term survivors if they lived beyond this period (21). In selected studies, animals were sacrificed at timed intervals after infection by intraperitoneal pentobarbital. Lungs were removed surgically, homogenized, and cultured quantitatively, as described before (16, 19). In addition, heart blood was obtained after thoracotomy, and blood was cultured in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (21).

Statistical analysis. Differences in survival from pneumonia were compared by the chi-square analysis with Yates' correction. The paired Student's *t* test was employed for analyses of quantitative bacteriology.

TABLE 1. *P. aeruginosa* LPS antibody concentrations in immunoglobulin G and MAb preparations

Prepn	Antibody concn (μg/ml)	
	Anti-IT-1	Anti-IT-4
PA-IGIV	7.6	44.9
IT-1 MAb	478	<1.0
IT-4 MAb	<1.0	74.0
Porin MAb	<1.0	<1.0

RESULTS

In vitro and in vivo antibody concentrations. The type-specific antibody concentrations for IT-1 and IT-4 *P. aeruginosa* LPS antigens were determined for each of the study preparations. Both preparations of MAb to LPS demonstrated higher antigen-binding activity than did the polyclonal preparation (Table 1). MAb against porin antigen did not demonstrate cross-reactivity with LPS antigens.

Antibody activity was readily detected in guinea pig sera 1 h after infusions of PA-IGIV and MAb preparations, and the concentrations achieved were dose dependent (Table 2). As in the case of humans receiving i.v. immunoglobulin G (25), a rapid drop in antibody concentration was observed as early as 24 h after the infusion of the immunoglobulin G preparation. Similar rapid reductions in circulating antibody concentrations occurred after infusions of the MAb (Table 2).

Survival and clearance. Polyclonal antibody and MAb preparations each enhanced survival from *P. aeruginosa* pneumonia (Table 3). This protection was clearly dose related and for MAb preparations was LPS antigen specific. While type-specific MAb produced survivals superior to those with the polyclonal antibody preparation, this occurred only at dosages of MAb resulting in circulating type-specific anti-LPS antibody concentrations higher than those achieved with PA-IGIV. Dosages of the immunoglobulin G preparation greater than 500 mg of protein per kg were not employed, since this dosage is the maximum dosage of i.v. immunoglobulin G employed in most clinical settings (25).

TABLE 2. Concentrations of antibodies to *P. aeruginosa* IT-1 LPS in serum after a single i.v. infusion of PA-IGIV or murine MAb preparations

Prepn infused and dose (mg of protein/kg of body wt)	Concn (μg/ml) at time after infusion:			
	1 h	1 day	3 days	7 days
PA-IGIV				
100	<1.0[a]	<1.0	ND[b]	ND
250	1.38	<1.0	ND	ND
500	2.69	1.66	<1.0	<1.0
IT-1 MAb				
1.0	1.77	<1.0	<1.0	ND
2.5	4.69	2.65	1.1	<1.0
5.0	6.15	3.70	1.5	<1.0
10	9.34	5.40	2.4	<1.0
20	14.60	ND	ND	ND
IT-4 MAb,[c] 5.0	<1.0	ND	ND	ND
Porin MAb, 5.0	<1.0	ND	ND	ND

[a] Means for three animals.
[b] ND, Not determined.
[c] Antibody concentrations (micrograms per milliliter) against IT4 LPS antigen were 10.45 (1 h), 7.5 (1 day), 3.3 (3 days), and <1.0 (7 days).

TABLE 3. Survival from *P. aeruginosa* pneumonia

Treatment group and dose (mg of protein/kg of body wt)	Mean serum IT-1 antibody concn (μg/ml) 1 h after infusion[a]	No. of survivors/ no. infected (% survival)	*P* vs albumin (control group)
PA-IGIV			
100	<1.0	0/8 (0)	NS[b]
250	1.38	0/8 (0)	NS
500	2.69	8/24 (33)[c]	<0.01
IT-1 MAb			
1.0	1.77	0/6 (0)	NS
2.5	4.65	3/6 (50)	<0.01
5.0	6.15	9/12 (75)	<0.001
20	14.60	6/6 (100)	<0.001
IT-4 MAb, 5.0	<1.0	2/12 (16.7)	NS
Porin MAb, 5.0	<1.0	2/12 (16.7)	NS
Albumin control	<1.0	0/24 (0)	

[a] Values obtained from previous infusion studies (Table 2).
[b] NS, Not significant; $P \geq 0.05$.
[c] Less than the IT-1 MAb 5.0-mg/kg group, $P < 0.05$.

Intrapulmonary killing of *P. aeruginosa* was also compared among the treatment groups. In addition, blood cultures were obtained from all study groups 9 h after infection. By this time, intrapulmonary killing of bacteria was proceeding more effectively among the treatment groups, and by 24 h after infection significant differences in killing were apparent for each antibody-treated group compared with controls (Table 4). Blood cultures were uniformly positive among control animals sampled at 9 h (4/4), but only one treated animal (porin group) had bacteremia.

Absorption study. Several reports have described nonsomatic *P. aeruginosa* antibodies (e.g., anti-exotoxin A) in human immunoglobulin G preparations (26, 28; M. S. Collins, G. C. Tsay, R. F. Roby, and J. H. Dorsey, Rev. Infect. Dis., in press). To determine whether non-LPS antibodies contributed to protective activity in PA-IGIV, we performed absorption studies. The PA-IGIV preparation was absorbed overnight (4°C) with 10^9 CFU of Formalin-fixed and heat-killed (30 min, 100°C) *P. aeruginosa* 220 (24). Survival studies were then conducted with absorbed and nonabsorbed immunoglobulin preparations. To ensure that type-specific antibody was removed by absorption, IT-1 LPS antibody concentrations were determined in preparations before and after absorptions. In addition, sera were collected from guinea pigs 1 h after infusions of absorbed and

TABLE 4. Intrapulmonary killing of *P. aeruginosa* after treatment with immunoglobulin G or MAb preparations

Treatment group and dose (mg of protein/kg of body wt)	Mean ± SEM $\log_{10}$ CFU/ml of lung homogenate at time after infection (h):		
	6	9	24
Albumin	5.02 ± 0.16[a]	5.34 ± 0.07	6.39 ± 0.19
PA-IGIV (500)	4.86 ± 0.30	4.64 ± 0.08	4.32 ± 0.18[b]
IT-1 MAb (5.0)	4.19 ± 0.37	4.78 ± 0.31	3.85 ± 0.61[b]
Porin MAb (5.0)	5.05 ± 0.04	4.97 ± 0.57	4.76 ± 0.25[b]

[a] Dilution factor, ×25; four animals per time point per group.
[b] *P* value versus albumin group, <0.01.

TABLE 5. Effect of absorption on protective activity in PA-IGIV

Treatment group and dose (mg of protein/kg of body wt)	IT-1 antibody (μg/ml)		Opsonin activity in sera[a]	No. of survivors/ no. infected
	Preabsorbed	Postabsorbed		
Albumin	<1.0		0.09 ± 0.02	0/9
PA-IGIV (500)	7.1		1.80 ± 0.13	4/9[b]
PA-IGIV, absorbed (500)	7.1	<1.0	0.64 ± 0.11	0/9

[a] Decrease in CFU (log_{10}) during 60-min incubations (two studied per group).
[b] P value versus albumin control and absorbed group, <0.01.

nonabsorbed globulin and were then assayed for opsonic activity with *P. aeruginosa* 220 as the target organism.

Absorption with killed *P. aeruginosa* removed detectable LPS antibody, as well as protective activity from the PA-IGIV preparation (Table 5). To exclude the possibility that reduced survival among the group treated with absorbed preparation was due to an increased endotoxin content in postabsorbed globulin, the PA-IGIV preparation was assayed for endotoxin in the *Limulus* amoebocyte lysate assay (14). Less than 0.01 ng of LPS per ml was detectable either in pre- or postabsorbed PA-IGIV preparations.

Relative opsonic capacity of antibody preparations. The relative opsonic potency of PA-IGIV and IT-1 MAb preparations and their requirement for complement to enhance opsonic activity were determined with guinea pig PMN. Both antibody preparations effectively opsonized IT-1 *P. aeruginosa*, and the potencies were roughly equivalent over the same dilution range (Table 6). Based upon relative antigen binding activities (Table 1), however, it appeared that the polyclonal preparation was a more potent opsonin. On the other hand, PA-IGIV demonstrated an absolute requirement for complement, while MAb appeared capable of mediating enhanced phagocytic activity without complement. To determine whether the complement dependence of PA-IGIV was limited to assays with guinea pig PMN and sera, or was more generalized, we repeated opsonic assays (two experiments) with human PMN (prepared as described before [13]) and fresh normal human serum (1%). Opsonization with PA-IGIV (diluted 1/2) produced a mean reduction in CFU of 1.18 ± 0.18 (60 min), with 1% serum, and of 0.09 ± 0.01 ($P < 0.02$), without serum.

To ensure that each preparation effectively transferred opsonic activity in vivo, guinea pig sera were collected 1 and 24 h after i.v. infusion of PA-IGIV (500 mg/kg), IT-1 MAb (5 mg/kg), or albumin control. Five guinea pigs were studied per group. The sera were used in opsonin assays involving guinea pig PMN, 1% fresh normal guinea pig serum, and IT-1 *P. aeruginosa*. Each antibody preparation transferred opsonic activity in vivo, with the following mean reductions in CFU: PA-IGIV, 1.98 ± 0.13 (1 h) and 1.06 ± 0.38 (24 h); and IT-1 MAb, 1.12 ± 0.06 (1 h) and 0.74 ± 0.12 (24 h). Mean values for the albumin-treated group were 0.19 ± 0.05 (1 h) and 0.32 ± 0.06 (24 h). All values for treated animals were significantly greater than for the albumin-treated group (range, $P < 0.02$ to 0.01).

DISCUSSION

Despite obvious immunologic dissimilarities between the PA-IGIV preparation and the MAb, comparisons of therapeutic results with these preparations is of interest. Clearly, greater survivals from experimental pneumonia were achieved with type-specific MAb therapy. However, in correlating survival rates with the antibody concentrations achieved in serum, it appeared that the amount of protection per concentration of antibody in serum was similar for both types of preparations. In fact, the major therapeutic advantage for MAb was the capacity to transfer protective antibody levels with 1/100 (or less) the amount of protein required for the polyclonal preparation. It appears that for particularly virulent infections, such as *P. aeruginosa* pneumonia, one limiting feature of passive immune therapy may be the amount of protein which can safely be infused. While greater efficacy for the PA-IGIV may have resulted if higher dosages had been used, most investigators consider 500 mg of protein per kg of body weight to be at or near the maximal dosage for safe passive immunization in humans (25). Thus, for future development of PA-IGIV preparations, it may be advantageous to develop titers in excess of those in the preparation used in this study.

Another potential strategy for improving protection with human polyclonal immunoglobulin G preparations would be to employ immune rather than natural *P. aeruginosa* antibodies, as were used in this study. It has long been known that natural antibodies against *P. aeruginosa* exist in certain normal sera (11). However, several reports have noted superior antigen binding of *P. aeruginosa* antibodies obtained from vaccinated subjects, as compared with natural

TABLE 6. Opsonophagocytic activity in PA-IGIV and IT-1 MAb preparations

Opsonin dilutions	Mean ± SEM decrease in log_{10} CFU			
	PA-IGIV		IT-1 MAb	
	Buffer	Serum[a]	Buffer	Serum[a]
1/2	0.05 ± 0.02[b]	1.35 ± 0.19[c]	1.36 ± 0.21[c]	1.52 ± 0.29[c]
1/4	−0.25 ± 0.10	1.24 ± 0.20[c]	0.79 ± 0.11[c]	1.20 ± 0.27[c]
1/8	0.40 ± 0.17	1.10 ± 0.17[c]	0.62 ± 0.09	0.68 ± 0.11
1/16	0.37 ± 0.09	0.84 ± 0.12[c]	0.46 ± 0.13	0.38 ± 0.09
Diluent only	−0.06 ± 0.09	0.15 ± 0.04	0.18 ± 0.07	0.39 ± 0.17
No cells, 1/4[d]	0.26 ± 0.11	0.23 ± 0.09	0.25 ± 0.16	−0.10 ± 0.05

[a] Fresh guinea pig serum (1%) included in assay preparations.
[b] During 60-min incubations. Negative values indicate bacterial growth.
[c] Greater than diluent only; range, $P < 0.01$ to 0.05.
[d] Assay preparations made without guinea pig PMN, but containing a 1/4 dilution of indicated opsonin.

antibodies (1, 10). Also, as observed in this study and others (1), complement was required for natural *P. aeruginosa* antibodies to function as opsonins. The present study suggests that the capacity to concentrate natural *P. aeruginosa* antibodies by plasma fractionation into immunoglobulin G preparations may partially overcome potential problems of low-affinity natural antibodies. However, it is conceivable that even greater protection could be afforded by an immune polyclonal PA-IGIV preparation.

The potential for use of MAb in infectious diseases has been tempered by a rather long list of concerns (36). For example, certain MAb lack opsonic and complement-fixing activity and exhibit weak epitope binding (6, 36). It was thus encouraging to observe both in vivo efficacy as well as in vitro evidence for opsonizing function of the IT-1 MAb used in this study. An immunoglobulin G2a MAb may have been a fortunate choice of isotype in providing the necessary functional properties for this infection (6). As previously observed by Sawada et al. (29), MAb to LPS antigens provided significantly greater protection against *P. aeruginosa* infection than did MAb to outer membrane protein antigen. While porin MAb improved intrapulmonary killing, this effect was not of sufficient magnitude to ensure survival from pneumonia. Thus, our attempt to use a MAb directed against a commonly expressed *P. aeruginosa* surface epitope (porin) was unsuccessful.

In summary, it appears that passive immunization strategies based upon polyclonal antibody or MAb preparations may have therapeutic usefulness for *P. aeruginosa* pneumonia. During future development of immunologic reagents for use in this kind of setting, consideration should be given to methods for improving the potency of polyclonal products or for producing human rather than murine MAb.

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