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Functionally Active Monoclonal Antibody That Recognizes an Epitope on the O Side Chain of *Pseudomonas aeruginosa* Immunotype-1 Lipopolysaccharide

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A murine monoclonal antibody (MAb) was prepared against *Pseudomonas aeruginosa* immunotype-1 (It-1) lipopolysaccharide (LPS). The MAb bound It-1 LPS in the enzyme-linked immunosorbent assay and in the immunodiffusion and immunoblotting assays, agglutinated and opsonized It-1 bacteria, and protected against challenge with live It-1 organisms in a murine burn infection model. All of these activities were immunotype specific. Correlation of the opsonic and protective properties of the MAb with its recognition site on the LPS O side chain confirmed that such immunotype-specific determinants are important targets for protective antibodies in *Pseudomonas* disease. The functional equivalence of this MAb and polyclonal antibodies from hyperimmune plasma underscores the therapeutic potential of single MAbs which recognize critical determinants in the LPS O side chain.

Pseudomonas aeruginosa causes common and often life-threatening infections in humans. Lipopolysaccharide (LPS) is a major structural component of the outer cell membrane of *P. aeruginosa* and plays an important virulence role based on its antiphagocytic and endotoxic properties (27). *P. aeruginosa* LPS, like that of other gram-negative bacteria, is a complex macromolecule composed of the biologically active lipid A moiety, covalently attached core oligosaccharide, and distally linked polysaccharide O side chain (19). The latter consists of repeating oligosaccharide subunits, the variable structure of which determines individual O-antigenic types or immunotypes of *P. aeruginosa* (39). Immunotype-specific antibodies, which are thought to recognize antigenic determinants in the O side chain of LPS, are acquired through natural exposure to *P. aeruginosa* or immunization with LPS-containing vaccines (40). Functionally, these antibodies act as opsonins, increasing the efficiency of phagocytosis and killing of *P. aeruginosa* by host phagocytes (41). As a result, immunotype-specific antibodies appear to serve a critical protective function in the infected or potentially infected host (29).

Current concepts of the specificity and function of antibodies that react with *P. aeruginosa* LPS are largely based on studies done with polyclonal antibodies. Hybridoma technology has made it possible to produce monoclonal antibodies (MAbs) with exquisite specificity for single epitopes within the LPS macromolecule. Using this methodology, we prepared a murine MAb which recognizes a determinant on the O side chain of LPS from *P. aeruginosa* Fisher immunotype 1 (It-1), the immunotype most commonly implicated in human disease (3). This MAb has opsonic and protective activities comparable to those of polyclonal type-specific antibodies, a finding which underscores the immunoprophylactic and therapeutic potential of single MAbs directed toward appropriate epitopes on the exposed O side chain of *Pseudomonas* LPS and perhaps other LPSs.

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MATERIALS AND METHODS

LPSs. *P. aeruginosa* It-1 LPS (Fisher-Devlin-Gnabasik system [12]), purified by hot phenol-water extraction (38) and gel filtration chromatography, was obtained from List Biological Laboratories, Campbell, Calif. Trichloroacetic acid-extracted LPSs from Fisher immunotypes 2 through 7 (15) were obtained from M. Fisher, Parke, Davis & Co., Detroit, Mich.

Preparation of MAbs. Female BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were immunized with four weekly intraperitoneal (i.p.) injections of 10^8 heat-killed *P. aeruginosa* It-1 organisms (obtained from M. Fisher, Parke, Davis). Four days after the final immunization, the spleens were removed aseptically and dissociated into a single-cell suspension. Approximately 5×10^7 spleen cells were fused with 5×10^7 non-immunoglobulin-producing Sp 2/0-Ag 14 myeloma cells (33) (American Type Culture Collection, Rockville, Md.; CRL 1581) by using 4,000-molecular-weight polyethylene glycol (50% [wt/vol] in water) as previously described (8). After fusion, the cells were washed and suspended in 80 ml of medium containing hypoxanthine, aminopterin, and thymidine. This mixture was dispensed in 100-μl portions into 96-well tissue culture plates (Costar, Cambridge, Mass.) previously seeded with 3×10^5 BALB/c spleen cells. The cultures were incubated in a 10% CO_2 atmosphere at 37°C and fed with hypoxanthine- and thymidine-containing medium on day 7. The supernatants from viable cultures were screened by enzyme-linked immunosorbent assay (ELISA) for antibody (see below) on days 14 and 21. Hybridomas from positive wells (i.e., optical density > 0.8) were cloned by limiting dilution in 96-well culture plates containing 3×10^5 splenic feeder cells per well. Positive clones were recloned three times, grown in large-scale culture in serum-free medium,

and adapted as mouse ascites tumors in pristane-primed BALB/c mice. The MAb designated PIC9.1.1 was affinity purified from ascites fluid on a protein A column (Bio-Rad Laboratories, Richmond, Calif.) (6), and the purified material was suspended in saline at a concentration of 1.54 mg/ml.

ELISA. Hybridomas were screened by ELISA by using as the solid-phase antigen heat-killed *P. aeruginosa* It-1 adsorbed to glass immunoassay cuvettes (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Samples containing 150 μl of bacterial suspension at a density of 1.5×10^8 cells per ml in 0.01 M phosphate-buffered saline (PBS) plus 0.02 M $MgCl_2$ were added to the cuvettes and incubated overnight at 25°C. The cuvettes were then washed with PBS containing 0.05% Tween 20, and 100-μl portions of hybridoma culture supernatant were added. The cuvettes were then incubated at 25°C for 1 h and washed again. Subsequent additions included goat anti-mouse immunoglobulin G (IgG)-peroxidase conjugate (Cooper Biomedical, Inc., West Chester, Pa.) diluted 1:10,000 in PBS-Tween 20 and incubated at 25°C for 1 h, 2,2′-azino-di-3-ethylbenzthiazoline sulfonate substrate (6) (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) incubated for 45 min, and 2 mM sodium azide to stop the reactions. Absorbance was read at 420 nm with a manual enzyme immunoassay reader (Gilford), and positive reactions (optical density > 0.8) were confirmed.

Subsequent binding studies were performed by an ELISA which used polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) coated with 10 μg of purified *P. aeruginosa* LPS (immunotypes 1 through 7) per ml (28). After overnight incubation at 4°C, the LPS suspension was removed and the wells were washed five times with PBS-Tween 20. Samples (50 μl) of serial fourfold dilutions of antibody were added to the wells, incubated for 30 min at 4°C, and washed five times with PBS-Tween 20. The final additions, separated by PBS-Tween 20 washes, were 50-μl portions of rabbit anti-mouse IgG-M-A (Zymed, South San Francisco, Calif.) diluted 1:500 and incubated for 30 min at 4°C, 50-μl portions of goat anti-rabbit IgG-alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, Mo.) diluted 1:250 and incubated for 30 min at 4°C, and 100-μl portions of *p*-nitrophenylphosphate substrate (Sigma 104; Sigma) (1 mg/ml in 10% diethanolamine [pH 9.8]) incubated for 60 min at 25°C. Absorbance was read at 405 nm in a Titertek multiscan micro-ELISA spectrophotometer (Flow Laboratories, Inc., McLean, Va.).

Immunodiffusion assay. Immunodiffusion analysis was performed with a plastic matrix superimposed on 1% agarose (4). Ascites fluid diluted 1:20 was reacted with LPS from Fisher immunotypes 1 through 7, each at a concentration of 500 μg/ml.

Bacterial agglutination. Strains representing all 17 *P. aeruginosa* serotypes of the International Antigenic Typing System were incubated overnight on tryptic soy agar at 32°C. The bacteria were removed from the plates with sterile cotton swabs and mixed with sterile normal saline to produce a turbid suspension (McFarland 1 standard; approximately 2×10^8 organisms). The suspension was mixed with serial dilutions of antibody on a glass slide and observed for clumping.

Immunoblots. LPSs were electrophoresed on a 14% sodium dodecyl sulfate (SDS)-polyacrylamide slab gel with a 4% stacking gel and a 0.1% SDS-Tris-glycine running buffer system (18, 20). The gel was fixed overnight in 40% ethanol–5% acetic acid and silver stained (37). LPSs from an identical companion gel were electrophoretically transferred to nitrocellulose sheets by using a current of 150 mA in Tris-glycine buffer (25 mM Tris and 192 mM glycine [pH 8.3] in 5 liters of water) for 18 h (36). Blocking with 3% liquid gelatin (Hipure; Norland Products, Inc., New Brunswick, N.J.) in Tris buffer with saline (TBS) was performed for 30 min at room temperature, and the sheets were cut into strips and incubated with antibody in TBS-Tween 20 with gelatin for 2 h at room temperature. The nitrocellulose strips were then washed in TBS-Tween 20 and incubated sequentially with affinity-purified rabbit anti-mouse IgG-M-A (1 μg/ml; Zymed), affinity-purified goat anti-rabbit IgG-horseradish peroxidase conjugate (1 μg/ml; Bio-Rad), and 4-chloro-1-naphthol substrate (Bio-Rad).

Radioactive antigen binding assay. Antibody binding to LPS-related immunotype-specific high-molecular-weight polysaccharides of *P. aeruginosa* Fisher immunotypes 1 through 7 (24) was quantified by a radioactive antigen binding assay with intrinsically labeled ^{14}C-high-molecular-weight polysaccharides (7, 23). Percent binding was converted to micrograms of antibody bound per milliliter by a standard curve prepared from human sera.

Opsonophagocytosis. Granulocytes isolated from human blood by dextran sedimentation and Ficoll-Hypaque density centrifugation (2) were added to washed mid-log-phase bacteria in the presence of appropriately diluted antibody and newborn rabbit serum (NRS) as a complement source (10). Immediately before use, NRS was absorbed with each test bacterium to remove antibody activity against the strain. Reaction mixtures, which were added to the wells of polystyrene microtiter plates, contained 1×10^6 granulocytes and 1×10^4 bacteria in a total volume of 100 μl. The control wells in each experiment contained granulocytes alone, NRS alone, and granulocytes plus NRS. Samples (10 μl) were withdrawn from the reaction mixtures at time zero and after 120 min of incubation at 37°C with vigorous shaking. The samples were diluted in 0.1% bovine serum albumin in sterile distilled water to lyse the granulocytes and were plated on tryptic soy agar for enumeration of bacteria (CFU). The percentage of bacteria killed was calculated by the following formula: $100 \times [1 - (\text{number of bacteria at 120 min})/(\text{number of bacteria at 0 min})]$.

Chemiluminescence. As previously described (34), the chemiluminescence assay was performed with 0.1 ml of whole mouse blood (diluted 1:5), 0.1 ml of mouse serum, 0.1 ml of antibody (various dilutions of mouse ascites fluid), 0.7 ml of bacteria (1×10^8 organisms), 0.9 ml of Hanks balanced salt solution, and 0.1 ml of 200 μM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). The reactions were performed at room temperature in dark-adapted polypropylene scintillation vials, and counts per minute were determined every 20 min for 2 h in a liquid scintillation spectrophotometer. Maximum chemiluminescence was calculated by substracting background counts per minute from peak counts per minute.

Mouse burn infections. As previously described (35) and modified (22), 6-week-old female Swiss Webster mice were burned and infected 3 h after a single intravenous (i.v.) injection of affinity-purified MAb suspended in 0.2 ml of sterile pyrogen-free saline. Groups of five mice were challenged with 10-fold dilutions of washed bacteria from an overnight culture suspended in 0.5 ml of saline and were inoculated subcutaneously at a fresh burn site. Deaths were recorded for 7 days, and 50% lethal doses (LD_{50}s) were calculated by the Spearman-Karber method (9). The significance of differences between groups was determined by a two-tailed test with Z scores derived from a normal distribution curve (16).

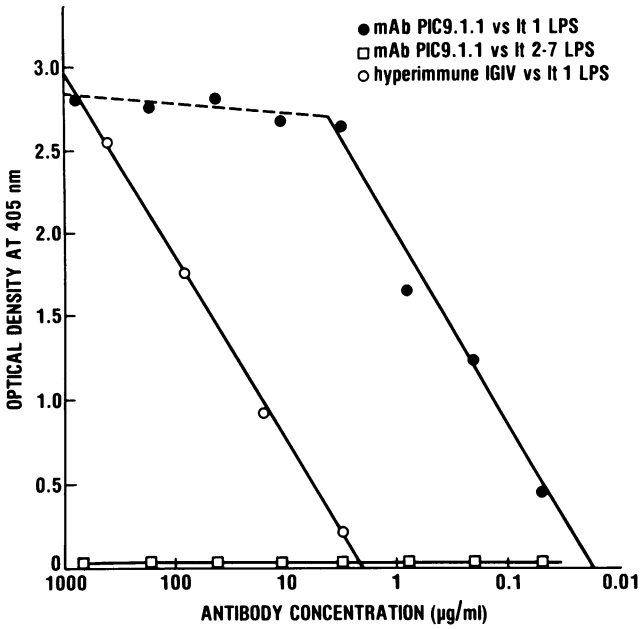

FIG. 1. ELISA reactivity of MAb PIC9.1.1 with purified LPSs from *P. aeruginosa* immunotypes 1 through 7 compared with reactivity against It-1 LPS of IGIV prepared from the pooled plasma of individuals previously immunized with a heptavalent *P. aeruginosa* LPS vaccine. Datum points represent the means of duplicate determinations.

LPS-induced mouse lethality. D-Galactosamine hydrochloride (30 mg; Sigma) was administered i.p. in combination with various doses of purified freshly sonicated *P. aeruginosa* It-1 LPS dispersed in 0.5 ml of sterile pyrogen-free water (13). In protection experiments, mice received an i.v. injection of antibody (or normal saline) before or after LPS challenge. Five mice were included in each treatment group, and deaths were monitored for 5 days.

RESULTS

The hybridoma clone designated PIC9.1.1 was isolated after a fusion in which cultures in 80 of 576 inoculated wells grew and reacted by ELISA with purified *P. aeruginosa* It-1 LPS. Twelve of these cultures produced antibody on repeated passage, and the highest antibody producer (PIC9.1.1) was cloned three times as described above. The MAb produced by this clone belonged to the IgG1 subclass, as determined by a standard capture ELISA using commercially available IgG subclass-specific reagents (HyClone Laboratories, Logan, Utah). The PIC9.1.1 MAb bound It-1 LPS in the ELISA at an antibody concentration as low as 100 ng/ml but demonstrated no reactivity with the LPSs from the remaining six Fisher immunotypes (Fig. 1). The binding curve produced by the PIC9.1.1 antibody in the ELISA paralleled that generated with human immune globulin for i.v. use (IGIV) prepared from the pooled plasma of individuals previously immunized with a heptavalent *Pseudomonas* LPS vaccine which included Fisher It-1 LPS (15) (Fig. 1). As expected, the MAb produced binding equivalent to that of the IGIV (equal optical densities in the ELISA) at approximately 1/100 the antibody concentration. The PIC9.1.1 antibody also demonstrated It-1-specific activity in immunodiffusion tests against purified *P. aeruginosa* LPSs and agglutinated only It-1 bacteria among the 17 serotypes of the International Antigenic Typing System (data not shown).

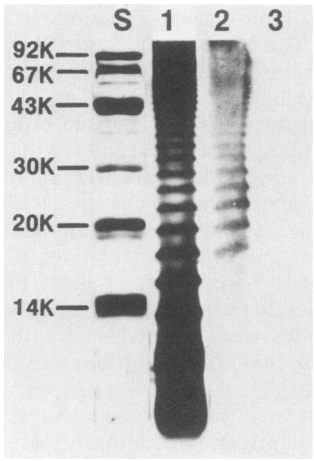

FIG. 2. Immunoblot analysis of MAb PIC9.1.1. Purified *P. aeruginosa* It-1 LPS was electrophoresed in a 14% SDS-polyacrylamide slab gel, which was then silver stained (lane 1). LPS from an identical companion gel was electrophoretically transferred to a nitrocellulose membrane which was then incubated sequentially with MAb PIC9.1.1, secondary and tertiary peroxidase-conjugated antibody probes, and chloronaphthol substrate (lane 2). Lane 3 contained It-3 LPS, with which the MAb did not react, and lane S contained molecular-weight standards.

A number of molecular species are detectable when smooth LPS is subjected to electrophoresis under reducing conditions on SDS-polyacrylamide slab gels (14). Fast-migrating species on such gels represent different variants of the lipid A-core oligosaccharide complex. Slower-migrating species represent LPS molecules which contain a complete core structure and covalently attached O side chains. These larger species appear as regularly spaced bands on silver-stained 14% SDS-polyacrylamide gels (37), with adjacent bands representing LPS molecules differing from one another by a single oligosaccharide subunit in the O side chain. An immunoblot of electrophoretically separated It-1 LPS, developed with the PIC9.1.1 MAb, demonstrated a series of bands corresponding to the regularly spaced, slower-migrating species observed on a silver-stained companion gel containing the It-1 LPS (Fig. 2). In contrast, activity corresponding to the faster-migrating core-lipid A complex detectable on the silver-stained gel was clearly absent on the immunoblot. Moreover, immunoblots of LPSs from Fisher immunotypes 2 through 7 developed with the PIC9.1.1 antibody showed no activity corresponding to either fast- or slow-migrating structures. These data indicate that MAb PIC9.1.1 recognized an epitope on the O side chain of It-1 LPS and that this interaction was type specific. This interpretation was supported by the reactivitiy of the MAb in a radioactive antigen binding assay with a high-molecular-weight polysaccharide thought to represent an antigenic form of the type-specific LPS O side chain (24, 25). Antibody PIC9.1.1 bound to the It-1 polysaccharide (antibody binding, 489 μg/ml) but did not bind to the polysaccharides derived from immunotypes 2 through 7 (data not shown).

The opsonic activity of the PIC9.1.1 MAb was documented by granulocyte-mediated chemiluminescence and bactericidal assays. Ascites-derived protein A-purified MAb at a concentration of 0.15 μg/ml elicited a peak response of $>10^5$ cpm in the whole-mouse-blood, luminol-enhanced chemiluminescence system (Fig. 3). Opsonization in this assay was type specific, as evidenced by the failure of the antibody

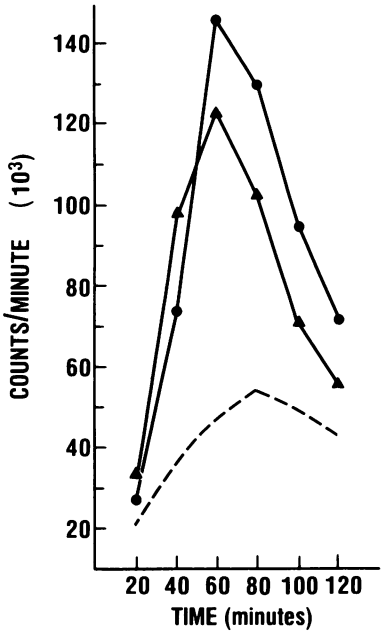

FIG. 3. Enhancement by MAb PIC9.1.1 of granulocyte-mediated chemiluminescence. Reaction mixtures containing *P. aeruginosa* It-1 organisms (10^8 CFU), diluted fresh whole mouse blood, MAb, and luminol were incubated at room temperature, and counts per minute were measured at 20-min intervals in a liquid scintillation spectrophotometer (see the text). The broken line represents background counts per minute determined in the absence of antibody. Purified MAb, obtained from mouse ascites fluid, was used at a final concentration of 0.15 μg/ml (●) or 15 ng/ml (▲). The assays were run in duplicate, and the data shown are means. Reaction mixtures containing the MAb, whole mouse blood, luminol, and each of the six other Fisher immunotypes (2 through 7) all elicited <55,000 cpm.

to elicit activity above the background level (55,000 cpm) in the presence of bacteria representing Fisher immunotypes 2 through 7. The results of the chemiluminescence assay correlated with those obtained in a human granulocyte-mediated bactericidal system. Under the conditions of the latter assay, PIC9.1.1 at a concentration of 1.5 μg/ml promoted 75% killing of the *P. aeruginosa* It-1 test strain in the absence of complement and >99% killing when a complement source was included (Fig. 4). In contrast, an It-3 organism (PA86) was not killed under similar assay conditions (data not shown). The PIC9.1.1 antibody thus mediated type-specific opsonization, phagocytosis, and killing of *P. aeruginosa* It-1 by complement-dependent as well as -independent mechanisms and did so at relatively low antibody concentrations.

Pseudomonas burn wound sepsis in mice has proven to be a useful model in which to assess the immunoprophylactic and therapeutic activities of specific antibodies. The PIC9.1.1 MAb, evaluated in this model at a dose of 110 μg per mouse (5.5 mg/kg), provided significant protection against live challenges with two representative It-1 organisms (Table 1). This protection was reflected in a >2,000-fold increase in the LD_{50} for the PA220 challenge and a 26-fold increase in the LD_{50} for the It-1 prototype strain (significant differences in both instances compared with controls). Titration of this protective activity against PA220 challenge revealed significant protection at an antibody dose as low as 11 μg per mouse or 550 μg/kg (Table 2). Moreover, the protection induced by the MAb against this challenge strain at a dose of 110 μg per mouse was almost equivalent to that provided by human *P. aeruginosa* hyperimmune IGIV administered at a dose of 10,000 μg per mouse (Table 1). In comparison, the PIC9.1.1 antibody provided no significant protection against challenge with an It-3 organism, indicating the immunotype specificity of the protective activity of the antibody.

To assess the possible antiendotoxic properties of PIC9.1.1 in an in vivo model, the antibody was administered to galactosamine-sensitized mice before, concurrent with, or after lethal doses of LPS. Data from a representative experiment in which the MAb was administered 2 h before LPS challenge (Table 3) indicate that the antibody exerted no protective effect. This finding was confirmed in additional experiments in which the MAb was administered 18 h before, 4 h after, and at the same time as LPS (data not shown).

DISCUSSION

Several recent reports (30–32) document the preparation of type-specific MAbs against *P. aeruginosa* LPSs. The first of these studies described four murine MAbs specific for LPS from the relatively rare Homma 7 (Fisher It-3) serotype (32). Although the epitopes recognized by these MAbs were not identified and their opsonic properties were not evaluated, the antibodies were highly protective in murine infection models. More recently, the same group of investigators reported the preparation of a human MAb that reacts with Homma type 5 (Fisher It-2) LPS (31). This antibody also appeared to protect against mouse infections, although it did not agglutinate type 5 bacteria, opsonic activity was not documented, and the antigenic determinant recognized by the antibody was not identified. Another very recent report (30) described seven murine MAbs that react with various combinations of *P. aeruginosa* immunotypes; two of these antibodies apparently recognize single immunotypes, Fisher

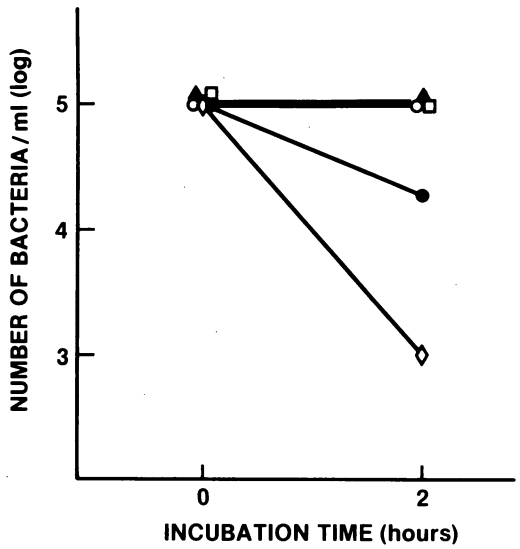

FIG. 4. Human granulocyte-mediated opsonophagocytosis of *P. aeruginosa* It-1 in the presence of MAb PIC9.1.1. Reaction mixtures contained 10^6 granulocytes, 10^4 CFU of the PA220 test strain, the MAb at a final concentration of 1.5 μg/ml, and previously absorbed NRS as a complement source in a total volume of 100 μl (see the text). Viable bacteria were enumerated before and after incubation with vigorous shaking at 37°C for 2 h. The data shown represent the means of duplicate samples. Symbols: ▲, granulocytes alone; ○, NRS alone; □, granulocytes plus NRS; ●, granulocytes plus MAb; ◇, granulocytes, MAb, and NRS.

It-5 and It-1. Both antibodies were opsonic for the homologous strains used in their preparation, and both appeared to protect against i.p. challenge with the same organisms in mice. However, the It-1-specific antibody exerted little or no protective activity against homologous challenge in a rat burn infection model, even upon repeated dosing. Moreover, the location of the epitope recognized by this antibody within the It-1 LPS macromolecule was not clearly documented on the basis of immunoblotting or other data.

The PIC9.1.1 MAb described here reacted with *P. aeruginosa* It-1 LPS in the ELISA and in the immunodiffusion and immunoblotting assays, it agglutinated and opsonized It-1 bacteria, and it protected against challenge with live It-1 organisms in a murine burn infection model. All of these activities appeared to be immunotype specific. Moreover, the reactivity of the PIC9.1.1 antibody with It-1 LPS alone among the seven antigenically distinct LPSs of the Fisher immunotyping system indicates that this antibody recognizes a unique epitope in the It-1 O side chain rather than a determinant in the core oligosaccharide or lipid A moiety shared among different *Pseudomonas* immunotypes (21). This conclusion is also supported by the demonstrated binding of the PIC9.1.1 antibody to a high-molecular-weight polysaccharide thought to represent an immunogenic form of the O side chain of It-1 LPS (25) and by its reactivity on immunoblots only with slower-migrating, O-side-chain-containing molecular species (37).

The definitive structure of the repeating oligosaccharide subunit of the O side chain of *P. aeruginosa* It-1 LPS was recently elucidated (17). The composition of this tetrasaccharide is apparently identical to that of the It-1 high-molecular-weight polysaccharide of Pier (T. A. Knirel, personal communication) with which the PIC9.1.1 MAb reacts. It can therefore be concluded with reasonable certainty that the MAb recognizes some portion of this unique tetrasaccharide structure (17):

where D-GalNAcAN is 2-acetamido-2-deoxy-D-galactouronamide, D-GalNFmA is 2-formamido-2-deoxy-D-galacturonic acid, D-QuiNAc is 2-acetamido-2,6-dideoxy-D-glucopyranose (*N*-acetylquinovosamine), L-Rha is L-rhamnopyranose, and OAc is acetate.

Correlation of the opsonic and protective properties of the PIC9.1.1 MAb with its recognition site on the O side chain of It-1 LPS confirms the previously held view, developed through studies with polyclonal antibodies, that such immunotype-specific determinants in the hypervariable region of LPS represent major target sites for protective antibodies in *Pseudomonas* disease (40). Moreover, the inability of the PIC9.1.1 MAb to prevent LPS-induced lethality in D-galactosamine-sensitized mice suggests, but does not prove, that the protection conferred by the antibody in the burn infection model was mediated by opsonic rather than anti-endotoxic mechanisms. This interpretation is consistent with the concept that antibodies (like PIC9.1.1) directed against determinants in the O side chain of LPS act primarily as opsonins, whereas those recognizing epitopes in the lipid A-core region possess endotoxin-neutralizing properties. Presumably, both activities confer protection in acute infec-

TABLE 1. Protective activity of PIC9.1.1 MAb in *P. aeruginosa* burn infections in mice

Challenge strain (Fisher immunotype) and pretreatment[a]	LD_{50}[b]	Fold increase in LD_{50}[c]	*P* value[c,d]
PA220 (1)			
Saline	4.1×10^3		
MAb	1.0×10^7	2,439	<0.01
Hyperimmune IGIV	6.1×10^7	14,878	<0.01
It-1 (1)			
Saline	7.8×10^2		
MAb	2.0×10^4	26	<0.05
PA86 (3)			
Saline	2.5×10^1		
MAb	9.9×10^1	4	NS

[a] Antibody or saline was administered in a 0.2-ml volume 2 h before challenge. The MAb (PIC9.1.1) was affinity purified on protein A-Sepharose from mouse ascites fluid and suspended in saline at a concentration of 550 μg/ml (110 μg per mouse). The IGIV (lot T6686; Parke, Davis) was prepared from pooled plasma obtained from individuals previously immunized with a heptavalent *P. aeruginosa* LPS vaccine (see the text); lyophilized antibody was reconstituted in pyrogen-free water at a concentration of 50 mg/ml (10 mg per mouse).

[b] Expressed as CFU, as determined by the Spearman-Karber method (see the text).

[c] Compared with normal saline control.

[d] *P* values were determined from Z scores derived from a normal distribution curve. NS, Not significant.

TABLE 2. Relationship between dose of MAb PIC9.1.1 and survival in *P. aeruginosa* burn infections in mice

Antibody dose (μg per mouse)[a]	LD_{50}[b]	*P* value[c]
0	5.6×10^2	
1	3.5×10^3	0.19
11	3.5×10^6	<0.001
55	8.9×10^6	<0.001
110	8.9×10^6	<0.001
220	2.2×10^7	<0.001
440	1.4×10^7	<0.001

[a] The MAb was suspended in 0.2 ml of normal saline and injected i.v. 2 h before live challenge. Control animals received 0.2 ml of saline.

[b] Expressed as CFU, as determined by the Spearman-Karber method (see the text).

[c] Compared with control animals. *P* values were determined from Z scores derived from a normal distribution curve.

TABLE 3. Effect of PIC9.1.1 MAb on LPS-induced lethality in galactosamine-sensitized mice[a]

MAb dose (μg) (no. of mice in challenge group)	No. of mice killed with LPS dose (μg) of:			
	0.1	1	10	100
0 (10)	5	10	9	9
25 (5)	3	5	5	4
50 (5)	1	5	5	5
100 (5)	3	4	5	5
200 (5)	4	4	5	5

[a] Mice received MAb injected i.v. in 0.2 ml of normal saline 2 h before i.p. injection of LPS mixed with 30 mg of D-galactosamine suspended in 0.5 ml of pyrogen-free water. Deaths were recorded for 5 days.

tions, although this is better documented for opsonization. It must be cautioned, however, that the galactosamine-enhanced model is a highly artificial one, the relevance of which is yet to be established in relation to endotoxin effects in human disease.

Our present findings with MAb PIC9.1.1 indicate that a single MAb which recognizes a suitable epitope in the LPS macromolecule can mediate protective activity against infection roughly equivalent to that of hyperimmune polyclonal antibodies derived from the whole plasma of individuals immunized with an LPS vaccine (1, 11, 26). This observation has both theoretical and practical implications. On the theoretical side, it reduces concern over the inherent functional limitations of MAbs based on their homogeneity and restricted specificity for particular molecular sequences on single bacterial virulence factors (5). On the practical side, the functional equivalence in a *Pseudomonas* infection model of MAb PIC9.1.1 and hyperimmune IGIV underscores the therapeutic potential of single MAbs which recognize critical determinants in the LPS O side chain. It is reasonable to conclude from these results that the PIC9.1.1 MAb, or comparable human MAbs, will prove clinically useful in *P. aeruginosa* It-1 infections, as will analogous MAbs which recognize epitopes on the LPS O side chains of heterologous *P. aeruginosa* immunotypes and other pathogenic gram-negative bacteria bearing LPS on their surface.

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