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Research Article

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Protective Activity of Antibodies to Exotoxin A and Lipopolysaccharide at the Onset of *Pseudomonas Aeruginosa* Septicemia in Man

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ABSTRACT Serum antibodies to exotoxin A and type-specific lipopolysaccharide were measured by passive hemagglutination in 52 patients with *Pseudomonas aeruginosa* septicemia. Their comparative protective activities were evaluated by relating the titers of each at the onset of bacteremia to subsequent outcome. High acute serum antitoxin and antilipopolysaccharide titers ($\log_2$ reciprocal mean titers >5) were associated with survival (76% of 17 with high vs. 46% of 24 with low antitoxin titers, $P = 0.05$; 85% of 13 with high vs. 48% of 29 with low antilipopolysaccharide titers, $P = 0.03$). In contrast, neither antibody titer was significantly associated ($P \leq 0.05$) with patients' age or sex, severity of underlying disease, presence of leukopenia, steroid or immunosuppressive therapy. Despite a correlation between acute titers of the two antibodies ($r = 0.33$, $P = 0.06$), they appeared to protect independently and additively. Whereas 75% of 8 patients with high antitoxin titers and only 38% of 16 with low titers survived with low antilipopolysaccharide titers ($P = 0.10$), 100% (6/6), 73% (8/11), and 38% (6/16) survived, respectively, when both, one, or neither antibody was present in high titer ($P = 0.01$). Furthermore, the association between high acute serum antitoxin titers and survival was more pronounced in patients with rapidly fatal underlying disease ($P = 0.06$) and leukopenia ($P = 0.12$) than in more favorable prognostic and immune categories. These data indicate that serum antibodies to exotoxin A and lipopolysaccharide are found in most patients with *P. aeruginosa* septicemia and both are protective. Both antibodies may have therapeutic or prophylactic potential, whereas serum antiexotoxin A antibodies may be particularly beneficial in compromised hosts.

INTRODUCTION

Pseudomonas aeruginosa (PA)[1] infections continue to be a life-threatening problem among immunologically compromised hosts in the hospital environment. Under these circumstances PA has the capacity for tissue invasion that often leads to septicemia and death. In most extensive reviews of gram-negative rod bacteremias, PA has been associated with the highest mortality (1). It is not yet clear what components of PA are responsible for its virulence. Lipopolysaccharide (endotoxin) has been implicated as a pathogenic factor. Circulating antibodies to lipopolysaccharide have been shown to prevent or attenuate some of the adverse effects of endotoxin in experimental animals (2, 3) and to act as opsonins, increasing the efficiency of phagocytosis of bacteria by leukocytes (4, 5). In a study of 182 patients with gram-negative rod bacteremia, including 16 with PA infections (6), high titers of immunoglobulin (Ig)G antibody to the patient's infecting organism were associated with a significant reduction in the frequency of shock and death. In several instances (but not in any of the PA infections), an examination of sera after absorption with lipopolysaccharide purified from the infecting strain revealed

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[1] *Abbreviations used in this paper*: ALPS, antibodies to purified *Pseudomonas* lipopolysaccharides; AT, antitoxin; HI, hemagglutination inhibition; PA, *Pseudomonas aeruginosa*; PHA-ALPS, passive hemagglutination assay for ALPS; PHA-AT, passive hemagglutination assay for AT; SRBC, sheep erythrocytes; TSBD, trypticase soy broth dialysate.

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markedly reduced antibody titers. This suggested the specificity of these protection-associated antibodies for endotoxin. Although a number of attempts to actively or passively immunize animals (7–10) and man (11–14) against PA endotoxin have met with varying degrees of success, no systematic, quantitative study of antibodies to purified *Pseudomonas* lipopolysaccharides (ALPS) has been performed in human infections. Specifically, scarce data exist relating ALPS levels in serum at the onset of bacteremic PA infections to subsequent outcome.

Among a number of extracellular enzymes produced by PA, exotoxin A has received considerable recent attention. Widely produced by clinical PA strains regardless of immunotype (15, 16), it is toxic for tissue cultures (17–20) and lethal for animals (18, 21, 22). It has been purified to homogeneity (21, 23–26), partially characterized (21, 23, 25), and its mode of action delineated in cell-free systems (27–29). This action, which is identical to that of diphtheria toxin, involves the enzymatic transfer of the ADP-ribosyl moiety from nicotinamide adenine dinucleotide to elongation factor 2, resulting in the inhibition of protein synthesis.

In addition to this demonstrated similarity to diphtheria toxin, there are several other lines of evidence suggesting a possible pathogenic role for exotoxin A. Its in vivo production during human infections is suggested by the demonstration of a systemic antibody response to purified exotoxin (30, 31). Its protein synthesis-inhibiting enzymatic activity is expressed in vivo during experimental infections in mice (32, 33). Antitoxin (AT) protects mice against challenge with toxinogenic PA strains, but fails to do so in infections caused by nontoxinogenic organisms (34). Finally, exotoxin is cytotoxic for human macrophages in vitro (20), a finding which might help explain its demonstrated interference with bacterial clearance mechanisms in infected mice (34).

The purpose of this study was to characterize serum antibody responses to exotoxin A and lipopolysaccharide in patients with bacteremic PA infections and to assess the relation between antibody titers to each at the onset of bacteremia and subsequent outcome. By studying the protective activities of their respective antibodies, we hoped to better define the pathogenic roles of exotoxin and endotoxin in serious PA infections.

METHODS

Patient selection and clinical criteria. Selected for study were a consecutive series of 52 patients, 65% of whom were male, with an average age of 54 yr (range 12–89 yr), hospitalized on the medical, surgical, or gynecological services at the University of California Medical Center between 1 May 1973 and 15 February 1977. The criteria for diagnosis were one or more positive blood cultures for PA with signs and symptoms consistent with systemic infection. Criteria used to define the severity of underlying disease were those of McCabe and Jackson (1). Leukopenia was defined as a total leukocyte count $<1{,}000$ cells/mm^2. Death was ascribed to PA septicemia if a patient died within 72 h of a positive blood culture, or if postmortem examination revealed anatomic and(or) cultural evidence of tissue infection.

100 healthy blood donors with a mean age of 24 yr (range 18–48 yr), 93% of whom were males, served as normal controls for serum AT determinations. A separate group of 52 healthy subjects served as controls for serum ALPS determinations.

Blood isolates and culture. Approximately 20 ml of blood was obtained by venipuncture. 5–8 ml was inoculated into each of two culture bottles, one containing enriched trypticase soy broth, and the other thioglycolate broth. Both media contained sodium polyanethol sulfonate as an anticoagulant. Finally, 4–5 ml of the same sample was injected into a tube containing 50 U of heparin. The heparinized blood (obtained simultaneously with the culture sample) was refrigerated within 1 h, and the plasma was separated by centrifugation and stored at −20°C in endotoxin-free glassware.

Identification and immunotyping of clinical PA isolates. Aerobic, oxidase-positive, motile, gram-negative bacilli were confirmed as PA on the basis of the following additional characteristics: oxidation of glucose but not maltose (OF medium, Difco Laboratories, Detroit, Mich.), production of L-arginine dihydrolase but not L-ornithine decarboxylase, production of blue slant and butt plus gas on Seller's medium, and growth in brain heart infusion broth at 42°C (35). PA isolates were immunotyped by slide agglutination (36) using rabbit antisera to purified lipopolysaccharide antigens (37) supplied by M. Fisher (Parke, Davis & Company, Detroit, Mich.).

Preparation of exotoxin A and AT serum. Exotoxin A was purified by affinity chromatography (26) from cultures of PA 103, a high toxin, low protease-producing strain obtained from P. V. Liu (Louisville, Ky.). Briefly, sheep AT IgG produced against previously purified toxin (26) was bound to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.). Filtrates from overnight cultures grown in trypticase soy broth dialysate (TSBD) were concentrated 10-fold and applied directly to an affinity column consisting of the Sepharose-AT ligand. After extensive elution of the column with 0.05 M phosphate buffer, pH 7.4, toxin was released from the ligand with 3 M sodium thiocyanate. The eluted toxin was dialyzed overnight in the cold against phosphate buffer to remove the thiocyanate, assayed for purity and toxicity, and frozen in aliquots at −70°C. Toxin batch 71177, used throughout this study, migrated as a single band on 10% sodium dodecyl sulfate polyacrylamide and disc gels containing 30 μg of protein. This toxin preparation yielded a single precipitin line by immunodiffusion with either AT serum or antiserum to crude PA 103 culture filtrate. A median lethal dose for 20-g female Swiss white mice National Institutes of Health-Naval Medical Research Institute strain was 75 ng. Antisera were prepared in rabbits as previously described (30).

Passive hemagglutination assay for AT (PHA-AT assay). Fresh sheep erythrocytes (SRBC) were sensitized using chromic chloride and coated with toxin (31). Uncoated cells were prepared in the same manner except that toxin was deleted from the sensitizing mixture. Serum samples were heat-inactivated, absorbed with uncoated SRBC, serially diluted, and assayed in microtitration plates using sensitized toxin-coated or uncoated SRBC as previously described (31). PHA-AT titers began with a dilution of 1:2. Titers were expressed as $\log_2$ of their reciprocals, and for statistical purposes, titers <1 were considered to be 0. A positive reference serum (31), negative serum control, buffer controls without serum, and uncoated SRBC with test serum were included in each

assay. Acute and convalescent specimens from each patient were assayed simultaneously.

Passive hemagglutination assay for ALPS (PHA-ALPS assay). Serum antibodies to lipopolysaccharides of the seven Fisher-Devlin-Gnabasik PA immunotypes (38) were assayed by a modification of a published method (39). Fresh SRBC were washed three times in normal saline and resuspended at a concentration of 10% (vol/vol) in phosphate-buffered saline, pH 7.2. This cell suspension was mixed with an equal volume of purified immunotype-specific PA lipopolysaccharide (37) (supplied by M. Fisher, Parke, Davis & Co.) at a final concentration of 50 μg/ml in phosphate-buffered saline and the mixture incubated at 37°C for 30 min. SRBC were then washed three times in normal saline and resuspended at a concentration of 1% (vol/vol) in normal saline plus 1% bovine serum albumin. Uncoated control cells were prepared in similar fashion except that lipopolysaccharide antigen was deleted from the coating mixture. Serum samples were heat-inactivated at 56°C for 30 min, and twofold dilutions performed in normal saline plus 1% bovine serum albumin in microtitration plates. Duplicate serum aliquots were incubated overnight at room temperature with an equal volume of 0.2 M 2-mercaptoethanol (2ME) in normal saline before dilution and assay. An equal 50-μl vol of lipopolysaccharide-coated SRBC, representing the immunotype of the patient's blood isolate, was then added to each dilution well, the plates agitated and allowed to stand at room temperature for 4 h before reading for PHA. PHA-ALPS tests performed on sera before 2ME treatment (total immunoglobulin) began with a dilution of 1:2, and those done on sera treated with 2ME (IgG) began with a dilution of 1:4. Titers were expressed as $\log_2$ of their reciprocals and, for statistical purposes, total immunoglobulin titers <1 and IgG titers <2 were considered to be 0 and 1, respectively. A human hyperimmune, heptavalent reference serum (PEU 26 of 15 August 1969) was included with each assay, and acute and convalescent samples from each patient run simultaneously. PHA-ALPS determinations on 52 healthy subjects were performed by M. Fisher (Parke, Davis & Co.). The assay method used was identical to that described above for ALPS determination on patient samples, and the titers obtained by both laboratories on the reference serum were within one dilution of each other for all seven immunotypes.

Culture preparation and assay for toxin. Several isolated colonies of patients' PA blood isolates grown overnight on blood agar plates were transferred to trypticase soy agar slants, and grown again overnight at 32°C. Growth from these slants was suspended in TSBD plus 10% glycerine, and frozen in small aliquots at −70°C until further use. Before assay, cultures were thawed, inoculated on trypticase soy agar slants, and overnight growth at 32°C suspended in 60 ml of TSBD plus 5 mM nitrilotriacetic acid in 300 ml baffled nephelometer flasks. These cultures were grown for 18 h at 32°C in a rotary shaking incubator at 250 rpm (Psychrotherm, New Brunswick Scientific Co., Inc., New Brunswick, N. J.), centrifuged at 10,000 *g* for 30 min in the cold, and the supernates filter-sterilized.

Toxin was measured by hemagglutination inhibition (HI) (26), employing the microtiter PHA-AT system described above. Four hemagglutinating units of sheep AT serum in 25 μl of veronal-buffered saline plus bovine serum albumin were incubated at 37°C for 1 h with twofold dilutions of unconcentrated culture filtrates in an equal volume of buffer. Toxin-coated SRBC were then added to microtitration wells, hemagglutination read after 4 h, and HI end points related to a simultaneously run toxin standard.

Statistical analyses. The significance of differences in survival between groups of patients with high and low antibody titers was determined with a one-tailed Fisher exact test. Linear regression analysis was performed by the method of least squares, and the validity of fit determined by the correlation coefficient r. The significance of r was computed by a two-tailed Student's t test using the statistic $t = r\sqrt{n-2}/\sqrt{1-r^2}$. Mean antibody titers were compared using a two-tailed Student's t test. The incidence in high and low antibody groups of various prognostic categories, leukopenia, steroid therapy, and administration of cytotoxic drugs was compared using a two-tailed Fisher exact test. Most computations were performed on a programmable calculator using available software (model 65, Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

Primary sites of infection among the 52 patients studied were the urinary tract in 12, the gastrointestinal tract in 8 (perirectal abscess or oropharyngeal abscess), the lung in 7, the skin in 2, and the pelvic organs in 1. The source of bacteremia was not identified in 22 patients, but most of these infections probably originated in the gastrointestinal tract because a PA strain of similar antibiotic susceptibility was isolated from stool. Underlying diseases included leukemia/lymphoma in 18 patients, other neoplastic diseases in 16, cardiovascular disease in 4, collagen vascular disease, chronic urinary tract infection, and peptic ulcer disease in 2 patients each, and other miscellaneous disorders in 8. Overall mortality was 40%. Before bacteremia, 30% had received corticosteroids, 35% cytotoxic drugs, and 35% were leukopenic (<1,000 total leukocytes/mm²). 26 of 31 patients who survived (84%) and 18 of 21 who died (86%) received, within 24 h of initial positive blood cultures, at least one antibiotic to which the infecting organism was sensitive. Determination of serum levels, performed in the majority of patients, documented therapeutic antibiotic concentrations in most.

PA blood isolates from 49 of 52 patients were available for immunotyping and assay of in vitro exotoxin production (Table I). 90% of strains were typable with antisera to the seven Fisher immunotypes and all types

TABLE I

Immunotype Distribution and In Vitro Toxin Production among PA Blood Isolates from Bacteremic Patients

	Nonfatal infections		Fatal infections	
Type	No. strains	No. toxigenic	No. strains	No. toxigenic
1	10	8	9	6
2	6	5	1	1
3	2	2	1	1
4	3	3	2	2
5	0	0	0	0
6	3	1	3	2
7	3	3	1	1
NT*	3	3	2	2
Totals	30	25 (83%)	19	15 (79%)

* Nontypable.

except type 5 were represented. 83% of strains from patients who survived and 79% from patients who succumbed to their infections were exotoxin producers.

PHA-AT was detectable ($\log_2$ of reciprocal titer ≥ 1) in 39 of 41 acute serum specimens from bacteremic patients infected by toxinogenic strains (Fig. 1). The mean AT titer of this group (expressed as $\log_2$ of reciprocal titer) was 5.8 (±3.3 SD). AT was present in 23 of 24 convalescent sera from patients who survived bacteremia caused by toxinogenic strains, and the mean titer was 9.1 (±5.0 SD). 11 of these patients (46%) demonstrated fourfold or greater titer rises, and 3 showed twofold rises. In contrast, the mean acute AT titer of nine patients infected with nontoxinogenic PA strains was 2.9 (±2.2 SD), with no increase in individual titers or in mean convalescent titer (2.5±3.1 SD) among four survivors. Whereas 72% of 100 healthy blood donors had detectable serum AT (Fig. 1), most titers were low, and the mean titer of 2.6 (±2.2 SD) was significantly lower than either the mean acute ($P = 0.001$) or convalescent ($P < 0.001$) titers of the bacteremic patients. The correlation between both acute and convalescent AT titers and in vitro toxin production by infecting strains, as measured by HI (Fig. 2), was further indication that in vivo toxin release bore a direct relationship to toxin measured in vitro.

PHA-ALPS to infecting PA strains was detectable ($\log_2$ of reciprocal titers ≥ 1) in all 42 acute and 25 convalescent sera from patients who survived their bacteremias (Fig. 3); mean acute and convalescent antibody titers were 4.6 (±2.5 SD) and 7.0 (±2.8 SD),

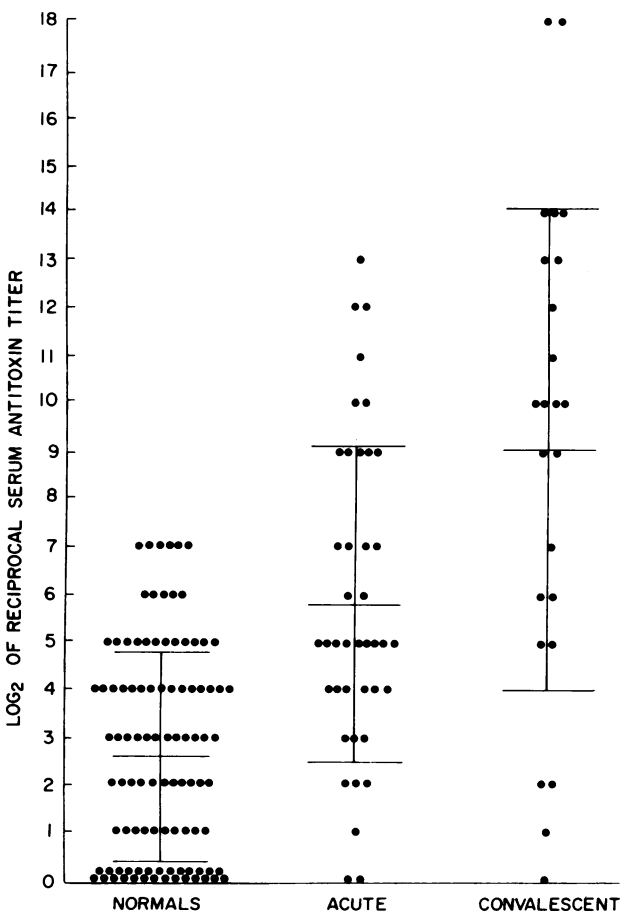

FIGURE 1 Serum PHA-AT titers of 41 patients with PA bacteremia compared with serum titers of 100 healthy blood donors. Only those patients whose infecting PA strains produced detectable exotoxin A in vitro are included. Acute serum samples were obtained simultaneously with initial positive blood culture and convalescent specimens were obtained from 24 survivors 10–14 d later. Brackets indicate mean±SD.

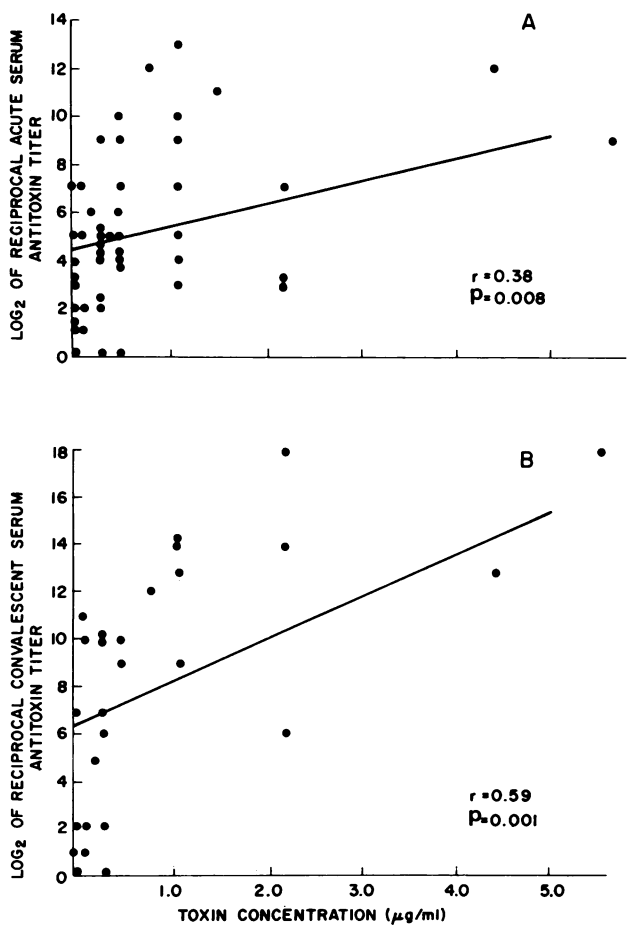

FIGURE 2 Correlation of acute and convalescent serum PHA-AT titers with amount of exotoxin A produced in vitro by blood isolates from patients with PA bacteremia. Toxin was assayed by HI in filtrates from overnight cultures grown in TSBD plus 5 mM nitrilotriacetic acid. Linear regression analysis was by the method of least squares, and P values were obtained by a two-tailed Student's t test with the statistic $t = r\sqrt{n-2}/\sqrt{1-r^2}$. Acute serum AT titers of 47 patients (A) and convalescent titers of 27 patients (B) are plotted against in vitro toxin production by infecting strains.

respectively. ALPS was also present in 58–98% of sera from 52 healthy control subjects, depending on immunotype (Fig. 3), and with the exception of types 2 and 4, mean titers of the controls were lower than either acute or convalescent titers of infected patients. 6 of 23 (26%) survivors of bacteremic infections for whom data were available demonstrated a fourfold or greater rise in ALPS titer to their infecting strains, 8 showed a twofold rise, 5 showed no change, and 4 demonstrated a fall in titer.

Incubation of sera with 2ME resulted in a reduction in PHA-ALPS titers consistent with the previously noted disproportionate contribution by IgM antibodies in the assay system (14). The mean acute ALPS titer of bacteremic patients was reduced by this treatment from 4.6 (±2.5 SD) to 3.1 (±1.6 SD), and the mean convalescent titer from 7.0 (±2.8 SD) to 4.5 (±2.4 SD). However, the correlation between ALPS titers before and after treatment with 2ME was excellent; in the case of acute titers, r was 0.55 ($P = 0.002$), and for convalescent titers r was 0.61 ($P = 0.001$). In view of this close correlation, only ALPS titers of untreated serum samples were subsequently analyzed, except as noted.

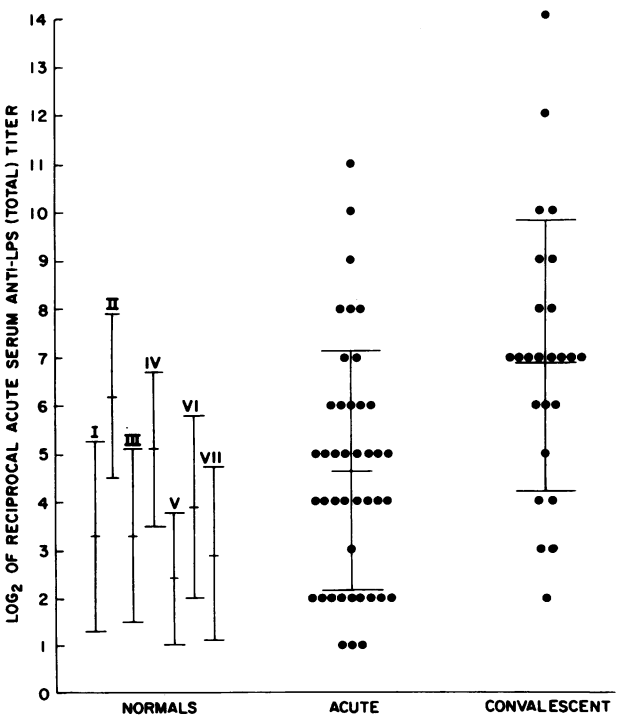

FIGURE 3 Immunotype-specific serum PHA-ALPS titers to infecting strains from 42 patients with PA bacteremia compared with serum titers of 52 healthy subjects (the latter supplied by M. W. Fisher, Parke, Davis & Co.). Acute serum samples were obtained simultaneously with initial positive blood culture, and convalescent specimens were obtained from survivors 10–14 d later. Brackets indicate mean titers±SD and roman numerals refer to the seven Fisher-Devlin-Gnabasik PA immunotypes (38).

As shown in Fig. 4, the correlation between total ALPS and AT titers at the onset of bacteremia was not close ($r = 0.33$, $P = 0.06$). However, when 2ME-treated sera were substituted in the PHA-ALPS assay, the correlation improved ($r = 0.50$, $P = 0.003$). This observation could perhaps be explained by the previous demonstration that the PHA-AT assay measures predominantly IgG antibodies (29), and thus by excluding the IgM component of PHA-ALPS with 2ME, the two assays are made more comparable.

To evaluate the possible protective activities of AT and ALPS, we looked for an association between the titers of each at the time of onset of bacteremia and subsequent survival. In relating AT and survival, 9 of 52 patients whose infecting strains were nontoxinogenic in vitro were excluded from analysis, and 2 patients were eliminated from the analysis of ALPS and outcome because their infecting strains were not available for immunotyping. Five additional patients were excluded because their PA isolates were nontypable, and two were eliminated because of inadequate serum samples. Thus, the analysis of AT and survival considered 41 patients, the analysis of ALPS included 42 patients, and a combined analysis involved 33 patients.

Individual AT titers of patients who survived and those who succumbed to their PA bacteremias are plotted separately in Fig. 5. Titers varied over a wide range in both groups and although the mean acute titer of those who survived (6.2±3.7 SD) was somewhat higher than the mean titer of those who succumbed (5.2±2.6 SD), the difference was not significant ($P = 0.36$). However, when patients were separated into low- and high-titered groups (titers ≤5 and >5) (Table II), survival was clearly greater among those with high AT titers ($P = 0.05$). As indicated in Table III, there were no significant associations between high vs. low AT titers and various host factors which might have contributed to the differential survival rates observed. Furthermore, when survival was examined in relation to AT titer within each of the three McCabe-Jackson prognostic categories, based on the severity of underlying disease (Table IV), there was an indication of protection associated with high titers in the more severe prognostic categories, particularly among those patients with rapidly fatal underlying diseases ($P = 0.06$). Likewise, as shown in Table V, there was a trend toward enhanced survival among patients with high AT titers whether or not they were leukopenic ($P = 0.12$ and $P = 0.23$, respectively).

The data relating survival to ALPS titer were similar to those for AT, but with some important differences. Acute serum ALPS titers were higher (mean titer = 5.5±2.5 SD) and somewhat more widely distributed

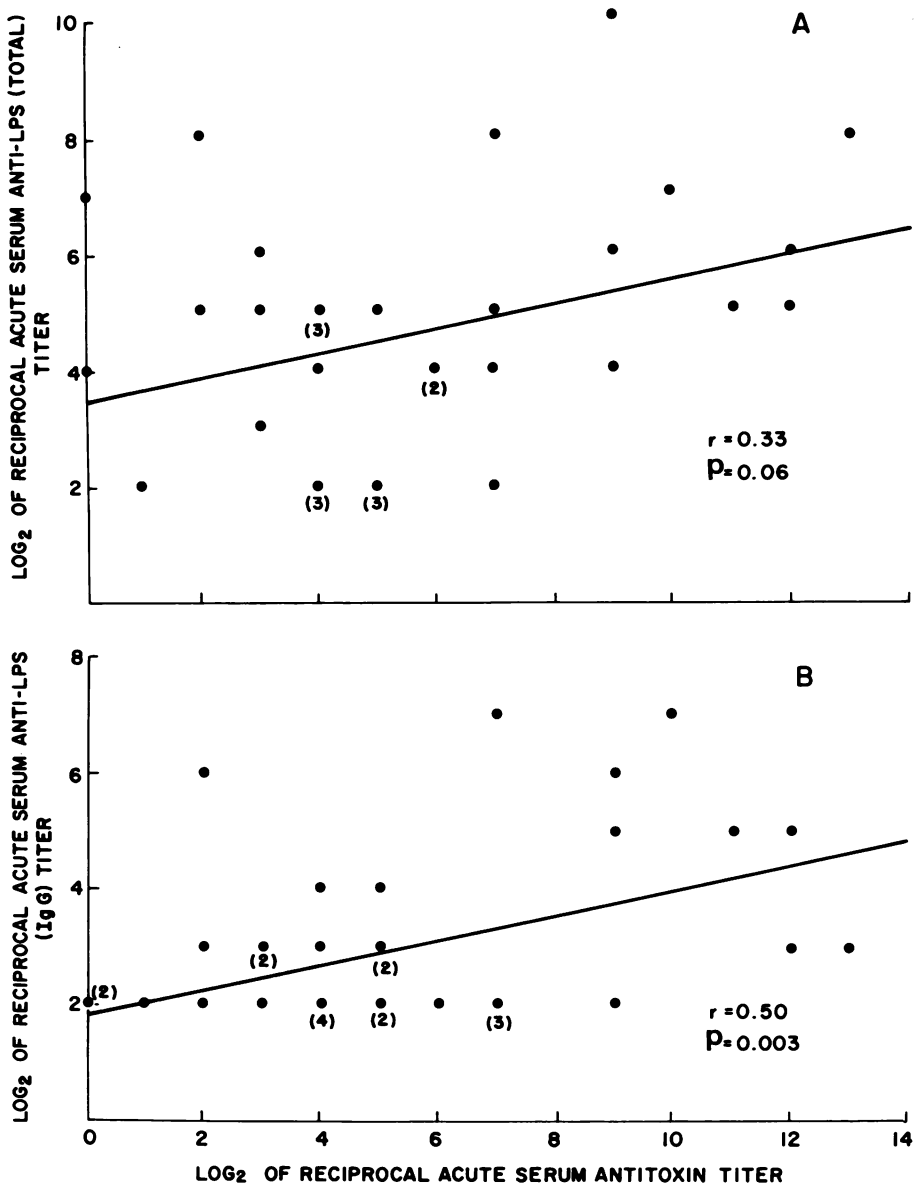

FIGURE 4 Correlation of acute serum PHA-ALPS and PHA-AT titers of 33 patients with PA bacteremia. Linear regression analysis was by the method of least squares, and P values were obtained by a two-tailed Student's t test with the statistic $t = r\sqrt{n-2}/\sqrt{1-r^2}$. Acute serum ALPS titers (A) before treatment with 2ME (total ALPS) and (B) after 2ME treatment (ALPS-IgG), plotted against AT titers of the same specimens.

among those who survived compared with those who died (mean = 3.4±1.9 SD, $P = 0.005$) (Fig. 6). As in the case of AT, patients with high ALPS titers (>5) at the time of their initial positive blood cultures had a higher survival rate than those with low titers ($P = 0.03$) (Table II). However, although not at a statistically significant level, there was a greater disparity in respect to certain host factors between the high- and low-titered groups, including prognostic category, presence of leukopenia, and prior steroid administration, than had been the case with AT (Table III). Despite these findings, there was a trend toward heightened survival in association with high ALPS titers in all three prognostic categories (Table IV). In contrast to AT, there was a stronger association between high ALPS titers and survival among nonleukopenic patients ($P = 0.08$) than among those with low leukocyte counts ($P = 0.40$) (Table V).

Two trends emerged when acute AT and ALPS titers of 33 patients infected with typable, toxinogenic strains

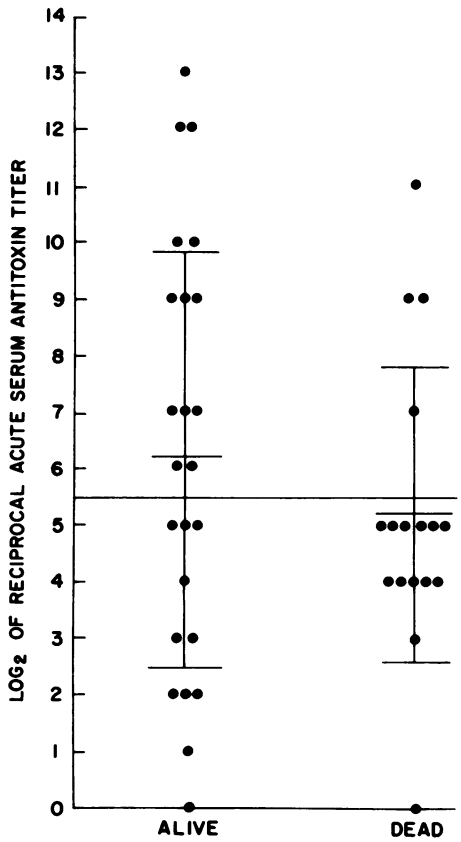

FIGURE 5 Serum PHA-AT titers at onset of PA bacteremia in 24 patients who survived and in 17 patients who subsequently succumbed to their infections. Brackets indicate mean titers±SD, and the horizontal line separates high- and low-titered groups used in statistical comparisons of survival (see text).

were analyzed together (Table VI). First, there was a strong association between high AT titers (>5) and subsequent survival, even when the ALPS titers were low (≤5); and conversely, more favorable survival rates were found among patients with high ALPS titers (>5) irrespective of AT titer. Second, the apparent protective activities of AT and ALPS appeared to be at least additive in that all six patients survived who had high titers of both antibodies at the time of bacteremia. Conversely, the greatest mortality (10 of 16) was observed when both types of antibody were present in low titer and an intermediate mortality rate (3 of 11) occurred when one or the other but not both were found in high titer.

TABLE II
Relationship of Survival to AT and ALPS Titers at Time of PA Bacteremia

Antibody	Titer*	Patients	Survival	P value‡
		n	%	
AT	≤5	24	46	
AT	>5	17	76	0.05
ALPS	≤5	29	48	
ALPS	>5	13	85	0.03

* Log_2 of reciprocal PHA titer.
‡ One-tailed Fisher exact test.

DISCUSSION

Our data confirm that a majority of normal adults have serum antibodies to exotoxin A and one or more of the lipopolysaccharide, immunotype-specific antigens of PA. Significant antibody responses to both commonly accompanied bacteremic infections, even with rapidly fatal underlying disease, leukopenia, and steroid or immunosuppressive drug therapy. Mean acute and convalescent AT titers were higher and titer rises more pronounced among surviving patients with these adverse host factors than among those lacking them (data not shown). In contrast, acute and convalescent ALPS titers were generally lower among patients in these categories, and titer rises less common.

TABLE III
Selected Host Factors in Patients with High and Low AT and ALPS Titers at Time of PA Bacteremia

	AT titer ≤5* (*n* = 24)	AT titer >5* (*n* = 17)	P value‡	ALPS titer ≤5* (*n* = 29)	ALPS titer >5* (*n* = 13)	P value‡
Mean age±SD, *yr*	54±20	46±15	0.18	53±21	48±17	0.42
Male	67%	71%	0.53	62%	77%	0.28
Rapidly fatal disease	38%	29%	0.42	41%	23%	0.15
Ultimately fatal disease	50%	53%	0.55	41%	38%	0.26
Nonfatal disease	13%	18%	0.49	17%	38%	0.14
Leukopenia	38%	35%	0.58	45%	23%	0.16
Steroid therapy	46%	35%	0.36	45%	15%	0.06
Cytotoxic drugs	29%	47%	0.20	38%	23%	0.28

* Log_2 of reciprocal PHA titer.
‡ Two-tailed *t* test used in comparison of mean age. Two-tailed Fisher exact test used in all other comparisons.

TABLE IV

Relationship of Survival to AT and ALPS Titers at Time of PA Bacteremia in Patients with Underlying Disease of Varying Degrees of Severity

Antibody	Titer*	Rapidly fatal			Ultimately fatal			Nonfatal		
		Patients	Survival	P value‡	Patients	Survival	P value‡	Patients	Survival	P value‡
		n	%		n	%		n	%	
AT	≤5	9	22		12	58		3	67	
AT	>5	5	80	0.06	9	78	0.32	3	67	0.80
ALPS	≤5	12	42		12	50		5	60	
ALPS	>5	3	67	0.45	5	80	0.28	5	100	0.22

* Log_2 of reciprocal titer.

‡ One-tailed Fisher exact test.

The demonstration of rising AT titers confirmed our earlier data suggesting that exotoxin is produced in vivo during human infections and induces an antibody response (30, 31). Further, there was a direct relation between the amount of exotoxin produced by blood isolates in vitro and the titer of AT present in patients' sera. That this correlation occurred with acute AT titers suggested antecedent exposure of patients to their infecting organisms. This was most likely due to colonization, particularly of the gastrointestinal tract, before bloodstream invasion (40). AT synthesis might have increased through an anamnestic response in patients with preexisting antibodies. An alternative explanation for high acute AT and ALPS titers is the possibility that infection, or even bacteremia, which may have antedated initial blood cultures, was not promptly recognized.

Because AT and ALPS titers were obtained simultaneously with initial blood cultures positive for PA, we were able to assess the humoral component of host defenses at the onset of bacteremia. Survival after bacteremia was significantly associated with both high acute AT and ALPS titers. Although there was a fair correlation between titers of the two antibodies in acute sera, the relationship of each with survival appeared somewhat independent. This was more apparent for AT, high titers of which appeared particularly to improve prognosis in patients with low ALPS titers. At the same time, the apparent protective activities of the two antibodies appeared additive, as suggested by the

TABLE V

Relationship of Survival to AT and ALPS Titers at Time of PA Bacteremia in Leukopenic and Nonleukopenic Patients

Antibody	Titer*	Leukopenic‡			Nonleukopenic		
		Patients	Survival	P value§	Patients	Survival	P value§
		n	%		n	%	
AT	≤5	9	22		15	60	
AT	>5	6	67	0.12	11	82	0.23
ALPS	≤5	13	38		16	56	
ALPS	>5	3	67	0.40	10	90	0.08

* Log_2 of reciprocal PHA titer.

‡ <1,000 leukocytes (total)/mm^2.

§ One-tailed Fisher exact test.

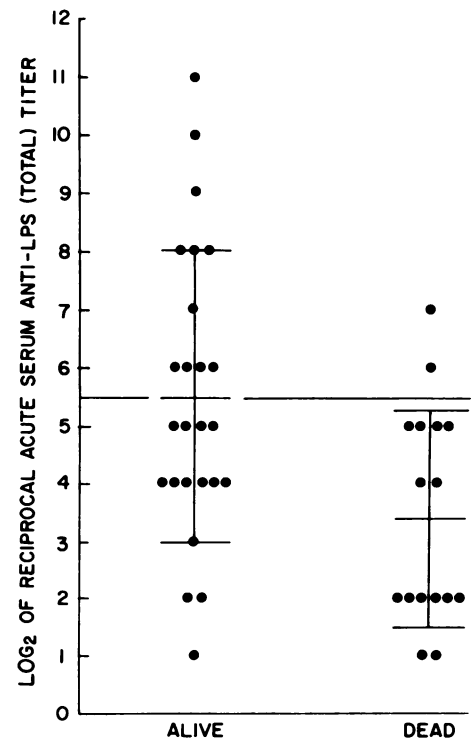

FIGURE 6 Serum PHA-ALPS titers to infecting strains at onset of PA bacteremia in 25 patients who survived and in 17 patients who subsequently succumbed to their infections. Brackets indicate mean titers±SD, and the horizontal line separates high- and low-titered groups used in statistical comparisons of survival (see text).

TABLE VI

Relationship of Survival to AT and ALPS Titers at Time of PA Bacteremia

AT titer*	ALPS titer*	Survival (No. patients)	
		%	
>5	>5	100 (6)	$P = 0.33$‡
≤5	>5	67 (3)	
>5	≤5	75 (8)	$P = 0.10$‡
≤5	≤5	38 (16)	

$P = 0.31$‡ (between 100 (6) and 75 (8))

$P = 0.38$‡ (between 67 (3) and 38 (16))

$P = 0.01$‡ (between 100 (6) and 38 (16))

* Log_2 of reciprocal titer.

‡ One-tailed Fisher exact test.

high survival rates when both were present in high titer, and low survival rates when both were absent.

It is possible that the observed relationship between high antibody titers at the time of bacteremia and subsequent survival reflected the more extensive absorption or consumption of antibodies by circulating bacteria during heavy or fatal bacteremia compared with that which occurred during less intense or nonfatal bacteremia. Because quantitative blood cultures were not obtained, we can neither prove nor disprove an inverse relation between antibody titers and level of bacteremia. Even if we could, however, it would be difficult to ascertain the extent to which the presence of antibody reduced bloodstream invasion and enhanced bacterial clearance, or the extent to which circulating bacteria absorbed antibodies from the blood, thereby reducing their titer. Either of these mechanisms is more probable in the case of ALPS because these antibodies act as opsonins and can be readily absorbed from serum by whole bacteria, whereas AT has no known opsonic properties, cannot be significantly absorbed by intact organisms (34), and would probably not be "neutralized" by the relatively minute amounts of toxin which may circulate, even during heavy bacteremia.[2]

It is also possible that antibody titers to toxin and(or) to type-specific lipopolysaccharide are markers for other unidentified variables related to host defense mechanisms. However, in the case of AT, there were no obvious associations between various host factors and either high or low antibody titers. There were "trends" toward lower mean age and more frequent therapy with cytotoxic drugs in the group with high titers. In contrast, there were suggestions of associations between high ALPS titers and less severe underlying disease, a lower incidence of leukopenia and less frequent use of steroids. However, none of these associations achieved statistical significance.

Further confirmation that the observed association between high antibody titers and survival was independent of other host factors was obtained by analyzing subgroups of patients defined on the basis of two of the most critical host factors: severity of underlying disease and total leukocyte counts. In the case of AT, despite small numbers, the association between high antibody titers and survival was stronger, if anything, among patients with rapidly fatal underlying disease compared with less ill patients. Likewise, high AT titers apparently represented an equal or greater survival factor for leukopenic compared with nonleukopenic patients. In similar fashion, the association between high ALPS titers and survival applied more or less equally to patients in all three McCabe-Jackson prognostic categories. However, in contrast to AT, there was greater survival with high ALPS titers in nonleukopenic patients. This result is not surprising in view of the relationship between ALPS titers and heat-stable opsonizing antibodies against PA and the expectation that opsonization requires functioning phagocytic cells. (41).

These data suggest that in the face of negative host factors, such as inadequate numbers of circulating leukocytes or severe underlying disease, serum antibodies to exotoxin and lipopolysaccharide may assume critical importance. Further, the independent and additive protective activities of the two antibodies suggest separate mechanisms of protection.

The protective effects of active or passive immunization with *Pseudomonas* lipopolysaccharide or ALPS in experimental animal infections are well documented (7–9). However, the clinical efficacy of such immunizations in man is less clear (12–14), probably because many patients who have been immunized are also neutropenic or immunosuppressed. Clinical trials of active PA immunization in cancer patients revealed that bacteremic infections occured in immunized patients who were neutropenic and had low titers of opsonizing antibodies against their infecting PA strain.

[2] Pollack, M., and L. S. Young. Unpublished data.

Such patients may have initially produced antibodies after immunization, but titers subsequently declined in the face of continued immunosuppression (14).

In the case of exotoxin A, passive protection by highly specific AT serum has been demonstrated in experimental mouse burn infections (34). Accompanying this protection were significantly lower levels of bacteremia compared with similarly infected, untreated control animals (34). Furthermore, passive immunization with AT prevented enzymatic inhibition of protein synthesis, presumably resulting from in vivo toxin release, which occurred in the tissues of unimmunized mice infected with toxinogenic PA strains (33). These data derived from experimentally infected animals complement the human serological data presented here in suggesting a significant pathogenic role for exotoxin A as well as the potential clinical efficacy of immunization against it.

Our data indicate that AT and ALPS are present early in the serum of most patients with PA septicemia, increase with survival, and are protective. This suggests that antibodies passively provided or actively engendered to both exotoxin and lipopolysaccharide may have therapeutic or prophylactic potential.

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