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Research Article

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Enhanced Survival in *Pseudomonas aeruginosa* Septicemia Associated with High Levels of Circulating Antibody to *Escherichia coli* Endotoxin Core

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ABSTRACT We studied the relationship between serum antibodies to the cross-reactive endotoxin core of *Escherichia coli* and survival following *Pseudomonas aeruginosa* septicemia. Core glycolipid was purified from the outer cell membrane of a uridine diphosphate galactose 4-epimerase-deficient rough mutant *E. coli* (J5 strain), characterized, and used as the antigen in a quantitative enzyme-linked immunosorbent assay (ELISA) to measure core-specific IgG and IgM antibodies. 43 patients with *Pseudomonas* septicemia, among whom there was a mortality of 42%, were evaluated. Core-specific antibody concentrations in acute sera ranged from 1 to 49 μg/ml in the case of IgG and from 1 to 200 μg/ml for IgM. Core-specific antibodies of both isotypes were higher in patients who survived compared with those who succumbed to their septicemias (mean, μg/ml±SEM, 26±3 vs. 14±4, $P = 0.005$ for IgG, and 55±12 vs. 18±5, $P = 0.009$ for IgM). Although total IgG levels were also higher in acute sera from survivors compared with nonsurvivors (mean, mg/dl±SEM, 1,120±99 vs. 694±119, $P = 0.004$), total IgM levels were virtually identical in the two groups (146±23 vs. 148±48, $P = 0.52$). Conversely, patients with core-specific IgG levels >10 μg/ml at the onset of septicemia had better survival than those with levels <10 μg/ml (79 vs. 14%, $P < 0.001$), and patients with core-specific IgM levels >30 μg/ml had better survival than those with levels <30 μg/ml (81 vs. 44%, $P = 0.01$). In comparison, patients with total IgG levels >1,000 mg/dl also had better survival than those with levels <1,000 mg/dl (82 vs. 42%, $P = 0.01$), while those with total IgM levels >150 mg/dl showed somewhat less improvement in survival compared with those with levels <150 mg/dl (71 vs. 50%, $P = 0.12$). Core-specific IgM was highly correlated with core-specific IgG ($r = 0.52$), but not with type-specific anti-lipopolysaccharide ($r = 0.13$) or anti-toxin A ($r = 0.12$) antibodies, or with total IgG ($r = 0.28$) or IgM ($r = 0.31$). In contrast, core-specific IgG correlated somewhat more closely with type-specific antibodies ($r = 0.36$), and with total IgG ($r = 0.51$) and IgM ($r = 0.52$). Stepwise linear discriminant analysis indicated that type-specific antibody levels were the best predictor of outcome, among those antibodies examined, followed by anti-core IgM. Although anti-core IgG, anti-toxin A, and total IgG levels all correlated individually with survival, none augmented the prognostic power of type-specific antibodies in combination with anti-core IgM, which together predicted outcome accurately 73.5% of the time. Host factors not significantly associated with anti-core antibody levels included rapidly fatal underlying disease, age, sex, leukopenia, and prior treatment with cytotoxic drugs. In contrast, prior steroid therapy was associated with low levels of both core-specific IgG and IgM ($P < 0.05$). These data suggest cross-protective activity against *P. aeruginosa* septicemia of naturally occurring antibodies to the endotoxin core of *E. coli*.

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Anti-core antibodies, particularly of the IgM isotype, appear to augment the more specific protective immunity engendered by antibodies to the O-specific side chains of *Pseudomonas* lipopolysaccharides. This cross-protective immunity likely applies to other Gram-negative pathogens as well.

INTRODUCTION

Lipopolysaccharides (endotoxins) are major cell wall constituents of Gram-negative bacteria. They appear to play an important pathophysiologic role in Gram-negative septicemia, and also serve as targets for host antibodies.

In general, bacterial lipopolysaccharides consist of a highly variable outer region composed of repeating oligosaccharide units ("O-specific side chains"), and a relatively constant core region containing a small number of sugars including the trisaccharide 2-keto-3-deoxyoctonate (KDO)[1] and the biologically active lipid A moiety (1, 2). This core region is sometimes referred to as core glycolipid or endotoxin core. Antibodies to O-specific side chains in the variable outer region of lipopolysaccharides are species- and type-specific, with their protective activity derived from their ability to promote the phagocytosis and killing of infecting organisms by host phagocytes (3). In contrast, antibodies directed toward the lipid A-containing inner core region are broadly cross-reactive among a wide variety of Gram-negative bacteria (4–7) and are thought to act by neutralizing the biological activities of endotoxin (8–11).

Much of our knowledge of the immunochemistry of lipopolysaccharides has been derived from the study of rough mutant strains of *Salmonella* (1) and *Escherichia coli* (12). The lipopolysaccharides from these strains lack O-specific side-chains and varying portions of their outer core structure, yet retain their relatively homogeneous and weakly immunogenic inner core structures. A strategy has evolved over recent years to utilize such rough mutant strains as cross-protective immunogens in Gram-negative septicemia. One such strain, the *E. coli* J5 mutant (13), lacks the enzyme uridine diphosphate (UDP)-galactose 4-epimerase and is therefore unable to incorporate galactose into its lipopolysaccharide. The endotoxin core structure of the J5 mutant consists only of lipid A, KDO, heptose, glucose, and N-acetylglucosamine (13, 14). Immunization of experimental animals with this organism induces cross-protection against homologous and heterologous endotoxins, as well as immunity to live challenge with a variety of Gram-negative bacteria including *Pseudomonas aeruginosa* (15–17). More significantly, administration of serum from persons previously immunized with an *E. coli* J5 whole cell vaccine significantly reduced mortality in patients with Gram-negative septicemia including that caused by *Pseudomonas* (18). However, this study did not identify the protective factor(s) contained in J5 antisera or demonstrate a relationship between levels of circulating core-specific antibodies achieved in patients and subsequent survival.

We therefore prepared purified core glycolipid from the cell wall of the *E. coli* J5 mutant strain and used this material in a quantitative enzyme-linked immunosorbent assay (ELISA) to measure core-specific antibodies. We studied patients with *Pseudomonas* septicemia, reasoning that if antibodies reactive with core glycolipid exerted a protective effect, this would be reflected in increased survival in patients with high levels of these antibodies early in sepsis. Such a relationship was demonstrated, as cross-reactive anti-core antibodies appeared to augment the more specific immunity engendered by antibodies to the O-specific side chains of *P. aeruginosa* lipopolysaccharides (19).

METHODS

Purification of core glycolipid. The *E. coli* J5-RR rough mutant strain was obtained from Dr. Abraham I. Braude, University of California, San Diego, CA. This "double" mutant of *E. coli* 0:111:B4 lacks the enzyme UDP-galactose 4-epimerase and is unable to incorporate exogenous galactose into its lipopolysaccharide structure (13). Bacteria grown overnight at 32°C on tryptic soy agar slants (Difco Laboratories, Detroit, MI) were used to inoculate 4-liter capacity Fernbach flasks containing 1,000 ml of tryptic soy broth (Difco Laboratories). These cultures were incubated, with shaking, at 32°C for 18 h, the cells removed by centrifugation, washed twice with normal saline, and weighed. The bacteria were then suspended at a concentration of 0.2 g/ml in 0.05 M Tris (hydroxymethyl)aminomethane-hydrochloride buffer, pH 9.0, with 0.15 M NaCl, sonicated with a large probe (Sonicator Cell Disruptor, Heat Systems-Ultrasonics, Inc., Plainview, NY) at maximum setting for a total of 10 min. After centrifugation the pellet was resuspended and the sonication repeated. The fragmented cells were suspended at a concentration of 0.4 g/ml in distilled water, heated at 70°C in a water bath, an equal volume of 90% recrystallized phenol (pH 7.0) added, and the mixture incubated at 70°C with constant stirring for 15 min (20). The mixture was then cooled to 4°C, centrifuged at 10,000 rpm for 10 min, the aqueous phase saved, and the phenol phase reextracted with an equal volume of water as described above. The phenol phase and denatured protein at the phenol-water interface were discarded and the lipopolysaccharide-containing aqueous phases combined. The lipopolysaccharide suspension was then placed in a separatory funnel, extracted with anhydrous diethyl ether (2:1 ratio of ether to lipopolysaccharide suspension), and the aqueous phase saved. Water was added to the ether phase

[1] *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; KDO, 2-keto-3-deoxyoctonate; UDP-galactose 4-epimerase, uridine diphosphate-galactose 4-epimerase.

in the amount removed, the mixture shaken, and the aqueous phase removed. The combined aqueous phases were reextracted overnight with additional ether. Excess ether was removed from the final aqueous phase by bubbling nitrogen through the solution. RNase (type II-A, Sigma Chemical Co., St. Louis, MO) was added to the lipopolysaccharide suspension at a concentration of 2 Kunitz units/ml. The mixture was then dialyzed at room temperature against multiple changes of 0.01 M Tris-acetate, pH 7.5, with 0.1 M NaCl and 1.0 mM NaN_3 until the optical denisty of the dialysate, monitored in the 230–300 nm range, reached 0. The lipopolysaccharide suspension was next dialyzed against water in the cold overnight. DNase (type II, Sigma Chemical Co.) was added at a concentration of 2 Kunitz units/ml, and the suspension dialyzed against repeated changes of 0.1 M NaCl with 5 mM $MgSO_4$ and 1 mM NaN_3 until the absorbance of the dialysate reached 0 in the 230–300 nm range. The lipopolysaccharide suspension was then dialyzed against cold water overnight, pronase (type V, Sigma Chemical Co.) added in a concentration of 1 Kunitz unit/100 ml, the solution dialyzed overnight against several changes of 0.01 M Tris-acetate, pH 7.5, followed by dialysis against multiple changes of cold water over several days. The lipopolysaccharide suspension was lypophilized and weighed.

Characterization of purified core glycolipid. Purified *E. coli* J5 core glycolipid contained <1% protein as determined by the Coomassie Blue-binding method of Bradford (21) and corroborated by amino acid analysis. Neutral sugar analysis by reverse-phase chromatography using a cation exchange resin in the Li^+ form with 90% ethanol as eluent (22) revealed a 1:1 ratio of heptose/glucose, trace galactose, and no detectable colitose, a unique sugar contained in the O-specific side chains of the smooth *E. coli* 0111:B4 parent strain. These data confirmed an incomplete core structure corresponding to the Rc chemotype of *Salmonella* (1, 2). Electrophoresis in 15% sodium dodecyl sulfate polyacrylamide gel followed by silver staining (23) revealed a single, broad, fast-migrating band similar in mobility to core structures from other simultaneously run lipopolysaccharides (Fig. 1); regularly spaced slower migrating bands characteristic of intact smooth lipopolysaccharide (23) were absent. The purity and functional integrity of core glycolipid were confirmed in mitogenesis assays using spleen cells from high- and low-responder C3H/FeJ and C3H/HeJ mice, respectively. Mitogenic responses to core glycolipid were comparable to those induced by highly purified lipopolysaccharide from the smooth *E. coli* K235 strain (24). These included high responses by C3H/FeJ spleen cells, low or absent responses by C3H/HeJ spleen cells, and complete abrogation of mitogenic activity following incubation of core glycolipid with polymyxin B.

Measurement of anti-core antibodies. IgG and IgM antibodies to purified core glycolipid were quantified using ELISA (25). Purified *E. coli* J5 core glycolipid was dissolved at a concentration of 25 μg/ml in coating buffer (15 mM Na_2CO_3, 30 mM $NaHCO_3$, 3 mM NaN_3, pH 9.55) and dispensed in 50-μl aliquots into 96-well polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA). After overnight incubation at 4°C, the glycolipid suspension was removed and the wells washed five times with phosphate-buffered saline (PBS)-Tween (0.15 M NaCl, 6 mM Na_2HPO_4, 1 mM KH_2PO_4, 3 mM NaN_3, and 0.5 ml/l Tween-20). 50-μl test samples containing 1:16 and 1:64 dilutions of patients' serum were added to wells, the plates incubated at 4°C for 30 min and then washed five times with PBS-Tween. The final three steps, separated by PBS-Tween washes, were as follows: addition of 50 μl of rabbit anti-human IgG (gamma chain specific, Cappel Laboratories, Cochranville, PA) diluted 1:500 or anti-human IgM (mu chain specific, Cappel Laboratories) diluted 1:1,000, both previously absorbed with whole *E. coli* J5 cells, and incubation at 4°C for 30 min; addition of 50 μl of goat anti-rabbit IgG-alkaline phosphatase conjugate (Sigma Chemical Co.) diluted 1:250 and incubation at 4°C for 30 min; addition of 50 μl of *p*-nitrophenylphosphate substrate (Sigma 104, Sigma Chemical Co.), 1 mg/ml in 10% diethanolamine, pH 9.8, and incubation at 25°C for 60 min. Absorbance was read at 405 nm in a Titertek multiscan micro-ELISA spectrophotometer (Flow Laboratories, Inc. McLean, VA).

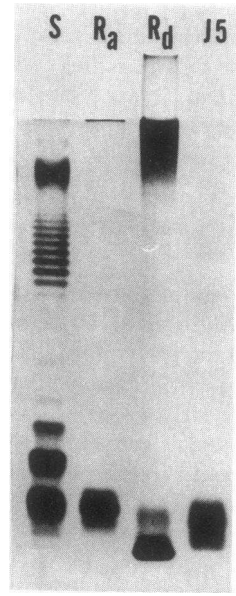

FIGURE 1 Analysis of purified core glycolipids by electrophoresis on 15% SDS polyacrylamide gel. Samples contained 5 μg (S) or 0.2 μg (Ra, Rd, J5) of lipopolysaccharide; gels were stained with silver (23). S: *E. coli* 0111:B4 "wild type" strain. R_a: *E. coli* PL2 rough mutant with complete core structure corresponding to the R_a chemotype of *Salmonella*. R_d: *E. coli* PL2-CL29 rough mutant with incomplete core structure corresponding to the R_d chemotype. J5: UDP-galactose 4-epimerase-deficient mutant derived from the *E. coli* 0111:B4 parent strain; its incomplete core structure corresponds to the R_c chemotype.

The assay was standardized with core-specific immunoglobulin affinity purified from high-titered human plasma on a column consisting of cyanogen bromide-activated Sepharose 4B, diaminodipropylamine spacer, and purified *E. coli* J5 core glycolipid ligand (26). IgG and IgM concentrations were determined in immunopurified material using low-level radial immunodiffusion plates (Hyland Diagnostics Div., Travenol Laboratories, Deerfield, IL). Standard curves generated for anti-core IgG and IgM were linear over a 100- to 1,000-fold dilution range (Fig. 2). Test sample dilutions were used that yielded an absorbance closest to the midpoint of standard curves, and concentrations of specific antibody calculated by least-squares method from standard curves. The sensitivity of the assay was 0.02 μg/ml for IgG and 0.06 μg/ml for IgM. Reproducibility among triplicate samples averaged ±4% and ±4.5% for IgG and IgM isotypes, respectively.

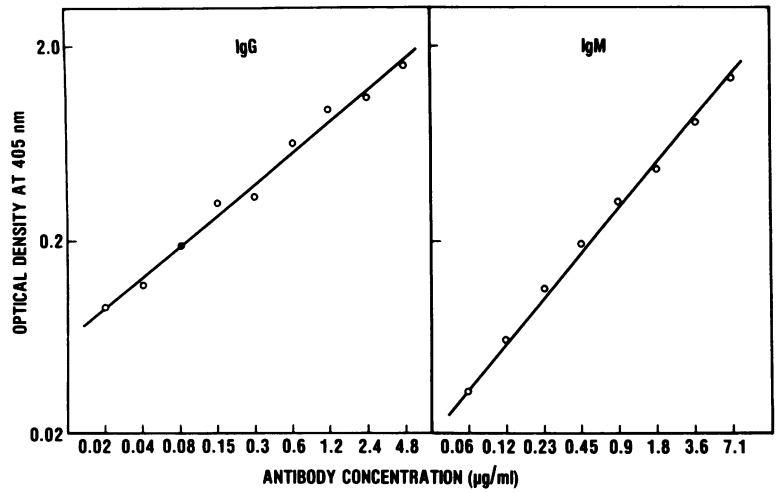

FIGURE 2 Standard ELISA curves for IgG and IgM antibodies to *E. coli* J5 core glycolipid. Standards consisted of affinity-purified anti-core antibodies obtained from high-titered human serum. Points represent means of triplicate samples and curves were calculated by least squares method from logit plots of absorbance vs. antibody concentration.

Patients and clinical specimens. Patients with *P. aeruginosa* septicemia admitted to the medical, surgical, or gynecological services of University of California at Los Angeles Medical Center between 1973 and 1977 were studied (19). The criteria for diagnosis were one or more positive blood cultures for *P. aeruginosa* with signs and symptoms consistent with systemic infection. Criteria used to define the severity of underlying disease were those of McCabe and Jackson (27). Leukopenia was defined as a total leukocyte count <1,000 cells/mm^3. Death was ascribed to *P. aeruginosa* septicemia if a patient died within 72 h of a positive blood culture, and/or if postmortem examination revealed blood vessel invasion by *Pseudomonas* or a primary site of *Pseudomonas* infection and no other obvious cause of death.

Acute sera were obtained simultaneously with initial positive blood cultures and convalescent sera 10 d to 2 wk later; specimens were kept frozen at −85°C before assay. Antibodies to type-specific lipopolysaccharides of patients' infecting *Pseudomonas* strains and toxin A were measured by passive hemagglutination as previously reported (19, 28).

Statistical analyses. Antibody concentrations were compared by paired *t* test in the case of acute and convalescent sera, and by *t* test on sample means in the case of acute sera from survivors and nonsurvivors. The incidence of death and of various host factors was compared in low- and high-antibody groups by chi-square test. Correlations between pairs of variables (i.e., specific antibody and total immunoglobulin levels) were determined using Pearson's correlation coefficient and significance of the correlation coefficient (*r*) determined from a standard table.

Stepwise linear discriminant analysis was performed using program BMDP7M of the BMDP statistical package (29). The purpose of this analysis was to statistically distinguish between those ultimately alive or dead based on a number of discriminating variables (i.e., antibody levels) from all variables studied. The discriminating variables were selected using a "stepwise" procedure which selected the next variable that best distinguished between survival status given the variables already selected. This procedure continued until no additional variables provided any additional discriminating power.

RESULTS

43 patients of an original 52 with *P. aeruginosa* septicemia (19) were analyzed (Table I). Approximately one-third had rapidly fatal underlying disease; infections began at a variety of primary sites, and their mortality was 42%.

Sera from 18 survivors showed a modest overall acute-to-convalescent increase in anti-core antibodies of the IgM but not IgG class (Table II). Although core-specific antibody concentrations in acute sera varied from 1 to 49 μg/ml in the case of IgG, and from 1 to 200 μg/ml in the case of IgM, levels of both core-specific isotypes were significantly higher in patients who subsequently survived compared with those who succumbed to their septicemias (Table III). Although

TABLE I
Study Patients with P. aeruginosa Septicemia

Source	Alive	Dead
Primary	9	8
Urinary tract	5	4
Lung	3	5
Biliary tract	3	0
Gastrointestinal tract	2	0
Other*	3	1
Total	25	18

* Source assumed to be gastrointestinal tract.

TABLE II

Anti-Core Antibody Concentrations in Paired Sera from 18 Survivors of P. aeruginosa Septicemia

Antibody	Acute	Convalescent	P*
	μg/ml		
Anti-core IgG	26±3	27±3	0.47
Anti-core IgM	58±13	70±17	0.08

Data represent mean ± SEM.
* One-tailed paired *t* test.

total IgG levels were also higher in acute sera from survivors compared with nonsurvivors, total IgM levels were virtually identical in the two groups (Table III).

To assess survival as a function of preexisting antibody levels, patients were separated into high and low antibody groups on the basis of arbitrary break-points. The resulting analysis (Table IV) indicated that survival was significantly enhanced in patients with high levels of core-specific IgG and IgM. Although a similar relationship was demonstrated for total IgG, that shown for total IgM was not statistically significant.

Since antibody levels to type-specific lipopolysaccharide immunodeterminants of patients' infecting *Pseudomonas* strains and to toxin A also appear to predict outcome (19), correlations were examined among these previously measured variables and those quantified in the present study. As shown in Table V, core-specific IgM was highly correlated with core-specific IgG, but the IgM isotype did not correlate closely with previously measured type-specific anti-lipopolysaccharide or anti-toxin A antibodies, or with total immunoglobulin levels. In contrast, core-specific IgG levels correlated somewhat more closely with type-specific antibodies, and with total IgG and IgM levels.

Since limited correlations between cross-reactive anti-core and noncross-reactive anti-*Pseudomonas* antibodies suggested their relative independence, multivariate analysis was used to rank them on the basis of their ability to predict outcome. Stepwise linear discriminate analysis revealed *Pseudomonas* type-specific anti-lipopolysaccharide antibody levels to be the best predictor of outcome, followed closely by anti-core IgM. Although other variables examined in the analysis, including anti-core IgG, anti-toxin A, and total IgG levels all correlated individually with survival, none augmented the prognostic power of type-specific antibodies in combination with anti-core IgM, which together predicted outcome accurately 73.5% of the time.

To evaluate the possibility that anti-core antibodies were merely serological markers for other recognized host-related determinants of outcome, we examined some of these risk factors in relation to anti-core antibody levels (Table VI). In the case of anti-core IgM, the incidence of rapidly fatal underlying disease and leukopenia, and the average age of high- and low-antibody groups were virtually identical. On the other hand, patients with low levels of anti-core IgG demonstrated a somewhat higher incidence of rapidly fatal underlying disease and leukopenia, and a greater predominence of males compared with the high antibody group; none of these differences was statistically significant, however. In contrast, the levels of both IgG and IgM anti-core isotypes were significantly lower in patients who had undergone prior steroid therapy.

TABLE III

Circulating Anti-Core Antibody and Total Class-specific Immunoglobulin Levels at the Onset of P. aeruginosa Septicemia in Relation to Subsequent Survival

Antibody or immunoglobulin class	Alive (n = 25)	Dead (n = 18)	P*
Anti-core IgG‡	26±3	14±4	0.005
Anti-core IgM‡	55±12	18±5	0.009
Total IgG§	1,120±99	694±119	0.004
Total IgM§	146±23	148±48	0.52

* *t* test.
‡ Data represent mean ± SEM. Calculated as micrograms per milliliter.
§ Calculated as milligrams per deciliter.

TABLE IV

Survival in Relation to Levels of Circulating Anti-Core Antibody and Total Class-specific Immunoglobulins Present at the Onset of P. aeruginosa Septicemia in 43 Patients

Antibody or immunoglobulin class	Concentration	Survival	P*
		%	
Anti-core IgG‡	<10	14	<0.001
	>10	79	
Anti-core IgM‡	<30	44	0.01
	>30	81	
Total IgG§	<1,000	42	0.01
	>1,000	82	
Total IgM§	<150	50	0.12
	>150	71	

* Chi-square test.
‡ Calculated as micrograms per milliliter.
§ Calculated as milligrams per deciliter.

DISCUSSION

Despite experimental evidence that immunization with the *E. coli* J5 mutant strain provides transferable pro-

TABLE V

Correlation of Anti-Core, Anti-Lipopolysaccharide (Anti-LPS), Anti-Toxin A, and Total Class-specific Immunoglobulin Levels in Acute Serum from 43 Patients with P. aeruginosa Septicemia

	Anti-core IgG	Anti-LPS	Anti-toxin A	Total IgM	Total IgG
			Correlation coefficient		
Anti-core IgM	0.52*	0.13	0.12	0.30	0.28
Anti-core IgG		0.36‡	−0.10	0.52*	0.51*
Anti-LPS			0.37‡	0.35‡	0.39‡
Anti-toxin A				−0.12	0.06
Total IgM					0.45*

* Significant at $P < 0.01$ level.
‡ Significant at $P < 0.05$ level.

tection against the lethal effects of Gram-negative septicemia (10, 15–17), data were heretofore lacking that clearly implicated a specific protective antibody. Likewise, in a recent study demonstrating the efficacy of treatment of Gram-negative bacteremia and shock with human antiserum to the J5 mutant (18), there was no significant relationship between anti-J5 antibody titers achieved in patients and subsequent outcome. On the other hand, a lower incidence of shock and death was previously reported in septic patients with high levels of naturally occurring antibodies to another rough mutant strain, *Salmonella minnesota* Re 595 (30). However, despite similarities, the *E. coli* J5 and *S. minnesota* Re core glycolipids are structurally and immunologically distinct (2), and the cross-reactive antibodies they stimulate have different, albeit overlapping specificities. This is particularly notable in relation to *P. aeruginosa*, against which anti-J5 serum has protective activity (16), while anti-Re serum is nonreactive (5).

In this study, we showed a striking association between high concentrations of circulating antibodies to a well-characterized preparation of *E. coli* J5 core glycolipid present at the onset of *Pseudomonas* septicemia and subsequent survival. Our finding that levels of circulating antibody reactive with J5 core glycolipid are an effective prognostic marker in *Pseudomonas* sepsis provides an important serological link between the known antigenic cross-reactivity of endotoxin core structures (5–7) and the protection recently observed in patients administered antiserum to the rough *E. coli* J5 mutant strain (18). The present study thus identifies an important immunological marker, which correlates with protective immunity in a common form of Gram-negative septicemia.

TABLE VI

Host Factors in Relation to Circulating Anti-Core Antibody Levels Present at the Onset of P. aeruginosa Septicemia in 43 Patients

Host factors	Acute anti-core antibody level			
	Low IgG* ($n = 29$)	High IgG‡ ($n = 14$)	Low IgM§ ($n = 16$)	High IgM‖ ($n = 27$)
		%		
Rapidly fatal underlying disease	43	28	33	31
Age	48¶	54¶	51¶	54¶
Male	50	72	59	75
Leukopenia	43	28	33	31
Steroids	79**	21**	52**	19**
Cytotoxic drugs	36	31	33	31

* <10 μg/ml.
‡ >10 μg/ml.
§ <30 μg/ml.
‖ >30 μg/ml.
¶ Mean age in years; no significant difference between high and low antibody groups at $P < 0.05$ level.
** Significant difference between high and low antibody groups ($P < 0.05$).

Although Ziegler's study demonstrated the clinical efficacy of an immunological approach to the treatment of Gram-negative septicemia, our assay appears to provide a practical means for measuring protective antibody. Although it might be feasible, ultimately, to define protective levels of core-specific antibodies, this may be an unrealistic expectation in view of the complex nature of bacterial virulence and host resistance. This is especially true in the case of opportunistic Gram-negative pathogens like *Pseudomonas*, which tend to infect severely immunocompromised patients. Nevertheless, by measuring serum levels of core-specific antibodies in susceptible individuals or groups of patients, it may be possible to anticipate the need for immunotherapy directed at circulating endotoxin. Similarly, the assay of anti-core antibodies may facilitate the development and standardization of therapeutic J5 antisera or hyperimmune globulin, and help in the assessment of their clinical efficacy.

High serum levels of both IgG and IgM antibodies to J5 core glycolipid correlated with survival in patients with *Pseudomonas* septicemia. Total IgG levels showed a similar association, but total IgM concentrations were virtually identical in survivors and nonsurvivors. These findings suggested a specific protective role for anti-core IgM, while it was more difficult to distinguish the protective activity of core-specific IgG and that of IgG in general.

Since antibodies to type-specific lipopolysaccharide immunodeterminants and toxin A also appear to serve a protective role in *Pseudomonas* septicemia (19), we examined the interrelationship of these antibodies and those directed to endotoxin core. Although core-specific IgG and IgM correlated moderately well, neither had a very high correlation with type-specific antibodies or those directed to toxin A. This was especially true in the case of core-specific IgM, which also correlated poorly with total immunoglobulin levels. Core-specific IgG, on the other hand, corresponded somewhat more closely with total IgG and IgM levels. The lack of strong correlations among these individual antibodies, and between core-specific IgM and total immunoglobulin levels, suggested the likelihood of independent protective activities, perhaps reflecting distinct mechanisms of protection.

The relative influence on outcome of core-specific IgG and IgM, type-specific antibodies, and total immunoglobulin levels, was further examined by stepwise discriminant analysis. This statistical procedure revealed type-specific anti-*Pseudomonas* antibodies to be the best prognostic indicator among those antibodies examined, followed closely by core-specific antibodies of the IgM isotype, which significantly augmented the prognostic power of the more specific antibodies. In contrast, anti-core IgG, anti-toxin A, total IgG and total IgM levels added little to the apparent protective capacity of type-specific antibodies in combination with core-specific IgM. The results of this multivariate analysis were consistent with the prevailing view (9, 31) that type-specific antibodies directed at the outer, variable region of bacterial lipopolysaccharides are more protective, albeit less versatile, than cross-reacting antibodies to endotoxin core. The multivariate analysis also supported the view that anti-core IgM may have greater protective efficacy in Gram-negative septicemia compared with core-specific antibodies of the IgG isotype (18).

Since certain host factors are critical determinants of outcome in Gram-negative sepsis (27) we examined some of these factors in relation to levels of circulating anti-core IgG and IgM present at the onset of *Pseudomonas* septicemia. The relatively small differences in the incidence of these host factors in high- and low-antibody groups, especially in the case of the IgM isotype, suggested that anti-core antibodies were not merely acting as serological markers for these other important risk factors. The single, rather striking exception occurred in the case of prior steroid therapy, which was significantly associated with low serum concentrations of both anti-core IgG and IgM. It is interesting to speculate that there was in fact a cause and effect relationship between these two sets of variables consistent with the reported suppressive effect of corticosteroids on antibody synthesis (32).

Although the data presented here do not prove the protective efficacy in *Pseudomonas* septicemia of antibodies reactive with the endotoxin core of *E. coli*, they strongly suggest that possibility. Immunochemical analyses are needed to confirm and further define the structural basis for the cross-reactivity between *Pseudomonas* and enterobacterial lipopolysaccharide core structures implicit in our observations. Future clinical trials of J5 antiserum or hyperimmune globulin are required to confirm the clinical efficacy of these reagents and to determine whether the antibodies measured by our assay are indeed the protective factors responsible for the therapeutic success of J5 antiserum reported by Ziegler et al. (18). If such clinical trials are accompanied by appropriate antibody determinations that discriminate between anti-core IgG and IgM, the relative importance of these two isotypes might be further clarified. This, in turn, would indicate whether IgG-containing intravenous immune globulins represent an appropriate vehicle for providing clinically effective anti-core antibodies, or whether we must turn to the development of IgM-containing preparations, as suggested by our data.

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REFERENCES

1. Luderitz, O., A. M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of *Salmonella* and related *Enterobacteriaceae*. *Bacteriol. Rev.* 30:192–255.
2. Wilkinson, S. G. 1977. Composition and structure of bacterial lipopolysaccharides. *In* Surface Carbohydrates of the Prokaryotic Cell. I. W. Sutherland, editor. Academic Press, Inc., New York. 97–171.
3. Young, L. S. 1972. Human immunity to *Pseudomonas aeruginosa*. II. Relationship between heat-stable opsonins and type-specific lipopolysaccharides. *J. Infect. Dis.* 126:277–287.
4. Chedid, L., M. Parant, F. Parant, and F. Boyer. 1968. A proposed mechanism for natural immunity to enterobacterial pathogens. *J. Immunol.* 100:292–301.
5. Young, L. S., K. R. Hoffman, and P. Stevens. 1975. "Core" glycolipid of Enterobacteriaceae: immunofluorescent detection of antigen and antibody. *Proc. Soc. Exp. Biol. Med.* 149:389–396.
6. Ng, A., C. H. Chen, C. Chang, and A. Nowotny. 1976. Relationship of structure to function in bacterial endotoxins: serologically cross-reactive components and their effect on protection of mice against some Gram-negative infections. *J. Gen. Microbiol.* 94:107–116.
7. Johns, M. A., S. C. Bruins, and W. R. McCabe. 1977. Immunization with R mutants of *Salmonella minnesota*. II. Serological response to lipid A and the lipopolysaccharide of Re mutants. *Infect. Immun.* 17:9–15.
8. Braude, A. I., H. Douglas, and C. E. Davis. 1973. Treatment and prevention of intravascular coagulation with antiserum to endotoxin. *J. Infect. Dis.* 128(Suppl.):S157–S164.
9. Young, L. S., P. Stevens, and J. Ingram. 1975. Functional role of antibody against "core" glycolipid of Enterobacteriaceae. *J. Clin. Invest.* 56:850–861.
10. Braude, A. I., E. J. Ziegler, H. Douglas, and J. A. McCutchan. 1977. Protective properties of antisera to R core. *In* Microbiology—1977. D. Schlessinger, editor. American Society for Microbiology, Wash., DC. 253–256.
11. Johns, M., A. Skehill, and W. R. McCabe. 1983. Immunization with R mutants of *Salmonella minnesota*. IV. Protective activity of O-specific and R antisera against endotoxin challenge. *J. Infect. Dis.* 147:57–67.
12. Heath, E. C., R. M. Mayer, R. D. Edstrom, and C. A. Beaudreau. 1966. Structure and biosynthesis of the cell wall lipopolysaccharide of *Escherichia coli*. *Ann. NY Acad. Sci.* 133:315–333.
13. Elbein, A. D., and E. C. Heath. 1965. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. I. The biochemical properties of a uridine diphosphate galactose 4-epimeraseless mutant. *J. Biol. Chem.* 240:1919–1925.
14. Fuller, N. A., M. C. Wu, R. G. Wilkinson, and E. C. Heath. 1973. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. VII. Characterization of heterogeneous "core" oligosaccharide structures. *J. Biol Chem.* 248:7938–7950.
15. Ziegler, E. J., H. Douglas, J. E. Sherman, C. E. Davis, and A. I. Braude. 1973. Treatment of *E. coli* and *Klebsiella* bacteriemia in agranulocytic animals with antiserum to a UDP-gal epimerase-deficient mutant. *J. Immunol.* 111:433–438.
16. Ziegler, E. J., J. A. McCutchan, H. Douglas, and A. I. Braude. 1975. Prevention of lethal *Pseudomonas* bacteremia with epimerase-deficient *E. coli* antiserum. *Trans. Assoc. Am. Physicians.* 88:101–108.
17. Braude, A. I., E. J. Ziegler, H. Douglas, and J. A. McCutchan. 1977. Antibody to cell wall glycolipid of gram-negative bacteria: induction of immunity to bacteriemia and endotoxemia. *J. Infect Dis.* 136(Suppl.):S167–S173.
18. Ziegler, E. J., A. McCutchan, J. Fierer, M. P. Glauser, J. C. Sadoff, H. Douglas, and A. I. Braude. 1982. Treatment of gram-negative bacteriemia and shock with human antiserum to a mutant *Escherichia coli*. *N. Engl. J. Med.* 307:1225–1230.
19. Pollack, M., and L. S. Young. 1979. Protective activity of antibodies to exotoxin A and lipopolysaccharide at the onset of *Pseudomonas aeruginosa* septicemia in man. *J. Clin. Invest.* 63:276–286.
20. Westphal, O., O. Luderitz, and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. *Z. Naturforsch.* 7b:148–155.
21. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
22. Boykins, R. A., and T. Liu. 1981. Reverse-phase chromatography of neutral sugars and disaccharides. *In* Biological/Biomedical Applications of Liquid Chromatography III. G. L. Hawk, editor. Marcel Dekker, Inc., New York. 347–361.
23. Tsai, C., and C. E. Frasch. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal. Biochem.* 119:115–119.
24. Skidmore, B. J., D. C. Morrison, J. M. Chiller, and W. O. Weigle. 1975. Immunologic properties of bacterial lipopolysaccharide (LPS). II. The unresponsiveness of C3H/HeJ mouse spleen cells to LPS-induced mitogenesis is dependent on the method used to extract LPS. *J. Exp. Med.* 142:1488–1508.
25. Engvall, E., and P. Perlmann. 1971. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of IgG. *Immunochemistry.* 8:871–874.
26. Ulevitch, R. J., A. R. Johnston, and D. B. Weinstein. 1981. New function for high density lipoproteins. Isolation and characterization of a bacterial lipopolysaccharide-high density lipoprotein complex formed in rabbit plasma. *J. Clin. Invest.* 67:827–837.
27. McCabe, W. R., and G. G. Jackson. 1962. Gram-negative bacteremia. I. Etiology and ecology. *Arch. Intern. Med.* 110:847–853.
28. Pollack, M., and N. S. Taylor. 1977. Serum antibody to *Pseudomonas aeruginosa* exotoxin measured by a passive hemagglutination assay. *J. Clin. Microbiol.* 6:58–61.
29. Morrison, D. F. 1967. Multivariate Statistical Methods. McGraw-Hill Book Co., New York. 130–132.
30. McCabe, W. R., B. D. Kreger, and M. Johns. 1972. Type-specific and cross-reactive antibodies in gram-negative bacteremia. *N. Engl. J. Med.* 287:261–267.
31. McCabe, W. R. 1972. Immunization with R mutants of *S. minnesota*. I. Protection against challenge with heterologous gram-negative bacilli. *J. Immunol.* 108:601–610.
32. Butler, W. T., and R. D. Rossen. 1973. Effects of corticosteroids on immunity in man. I. Decreased serum IgG concentration caused by 3 or 5 days of high doses of methylprednisolone. *J. Clin. Invest.* 52:2629–2640.