INFECTION AND IMMUNITY, Mar. 1983, p. 1072-1079
0019-9567/83/031072-08$02.00/0

Vol. 39, No. 3

Protection Against *Pseudomonas aeruginosa* Infection in a Murine Burn Wound Sepsis Model by Passive Transfer of Antitoxin A, Antielastase, and Antilipopolysaccharide

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Received 4 November 1982/Accepted 1 December 1982

The protective capacity of passively transferred immunolgobulin G (IgG) fractions from antitoxin (AT-IgG), antielastase (AE-IgG), and antilipopolysaccharide (ALPS-IgG) against *Pseudomonas aeruginosa* infection was evaluated in a murine burn wound sepsis model. Complete protection was afforded by homologous ALPS-IgG against intermediate challenge doses (10 50% lethal doses) of *P. aeruginosa* PA220, whereas AT-IgG and AE-IgG offered no significant protection ($P < 0.5$). The simultaneous transfer of AT-IgG or AE-IgG with ALPS-IgG gave no additional protection above that seen with ALPS-IgG alone. The transfer of ALPS-IgG did not dramatically alter bacterial multiplication in the skin at the site of infection. However, bacteremia and infection of the liver were prevented. In parallel experiments, AT-IgG or AE-IgG did not significantly alter either the course of the infection or the number of bacteria seen in the blood, liver, or skin when compared with controls. ALPS-IgG administered 24 h before infection, at the time of infection, or 4 h postinfection provided complete protection. Even when ALPS-IgG was transferred at a time when the infection was well established locally in the skin (8 h postinfection), highly significant protection ($P > 0.999$) was obtained. Protection afforded by ALPS-IgG was serotype specific. These results indicate that antibody to lipopolysaccharide is of critical importance for protection against *P. aeruginosa* challenge in a relevant animal model.

Pseudomonas aeruginosa is a major cause of morbidity and mortality in compromised patients. Patients who are debilitated due to neutropenia (43, 50), cystic fibrosis (14), or burn wounds (29) are especially susceptible to *P. aeruginosa* infections. Antibiotic therapy has met with only limited success due to the increasing occurrence of resistant strains (25, 42). A further complication to the treatment of burn patients is the emergence of *P. aeruginosa* strains resistant to silver sulfadiazine, routinely used as a prophylactic agent (4). These findings illustrate the need for immunotherapeutic agents to prevent or treat *P. aeruginosa* infections.

Although the pathogenesis of *P. aeruginosa* infections has been intensively studied, the identification of the principal protective antigen(s) has not been determined. This is at least partially due to the fact that *P. aeruginosa* causes a variety of different disease syndromes. Furthermore, *P. aeruginosa* can elaborate a number of products which may enhance virulence to a varying degree dependent upon the disease syndrome (17). Both extracellular and somatic antigens have been implicated as virulence factors (15, 17).

Of the extracellular products, toxin A (18) and elastase (28) have been most thoroughly studied as to their potential role in disease. Toxin A is produced during infections with *P. aeruginosa* in humans (7, 39). Increased survival in patients has been found to correlate with high antitoxin titers (6, 40). Furthermore, mutants of *P. aeruginosa* specifically deficient in toxin A display a reduced virulence in nonlethal animal models (31, 51). Attempts to protect animals in experimental burn wound sepsis models by antitoxic immunity have met with equivocal results. Passively transferred antitoxin was found to afford little protection against highly virulent challenge strains (33, 46). The active immunization of mice with a Formalin toxoid (32) but not a glutaraldehyde toxoid (32, 48) conferred a modest level of protection, even though both toxoids elicited comparable levels of antitoxin. This discrepancy has not been resolved but may be caused by the different adjuvants used during active immunization (10, 32).

Antibody to elastase during human infections with *P. aeruginosa* has been demonstrated, indicating in vivo production (7). Purified elastase has been shown to be dermonecrotic (23) and to

cause severe damage when applied to traumatized mouse corneae (22). Passively transferred antielastase has been shown to provide protection against challenge with a highly proteolytic strain of *P. aeruginosa* (34).

Antibody to somatic antigens of *P. aeruginosa* have been shown to be protective in animal studies. Lipopolysaccharide (LPS) confers type-specific immunity (9, 19, 37, 38, 44). Similarly, nontoxic high-molecular-weight polysaccharide antigens, immunologically related to LPS, also confer type-specific immunity (37, 38).

The present study was conducted to determine the ability of passively transferred antibody to elastase, toxin A, and LPS to protect mice against lethal *P. aeruginosa* infections in a burn wound sepsis model. We chose to employ immunoglobulin G (IgG)-enriched preparations derived from hyperimmune rabbit sera for intravenous transfer to avoid the adverse effects which can result from colloid overload by the transfer of whole sera (33). Furthermore, IgG appears to be of primary importance in protection against *P. aeruginosa* infections (1, 20).

MATERIALS AND METHODS

Bacterial strains and growth conditions. *P. aeruginosa* PA220 was a gift of B. Wretlind, Karolinska Institute, Stockholm, Sweden. *P. aeruginosa* M2, SBI-C, and SBI-N were kindly provided by I. A. Holder, Shriners Burns Institute, Cincinnati, Ohio. *P. aeruginosa* PA103 was a gift from B. H. Iglewski, University of Oregon Health Sciences Center, Portland, Ore. Cultures were routinely grown in the deferrated Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy broth dialysate (TSBD) medium described by Björn et al. (3). *P. aeruginosa* PA220, M2, SBI-C, and SBI-N produced elastase, as detected by elastin degradation (31), and toxin A, determined in an adenosine-diphosphate ribosyl transferase assay (8). The challenge inoculum for the burn wound sepsis model was prepared by incubating 10 ml of TSBD medium with 0.1 ml of an overnight culture. The culture was grown at 37°C with vigorous shaking until mid-log phase (absorbancy at 540 nm = 0.28 − 0.30). The culture was washed in phosphate-buffered saline (PBS), diluted in PBS, and stored on ice until used.

Extracellular antigens. Toxin A was purified by a modification of previously described methods (24). TSBD medium was prepared by ultrafiltration with an Amicon H10P10 hollow-fiber cartridge. The filtrate was treated with Chelex 100 (Bio-Rad Laboratories, Richmond, Calif.) for 12 h at 4°C. The Chelex was removed by filtration, and the medium was supplemented with glycerol (1% final concentration) and monosodium glutamate (0.05 M). A mid-log-phase culture of PA103 (2 liters) was added to 190 liters of medium and grown in a Giovanola fermentor (Giovanola Frères S.A., Monthey, Switzerland). The culture was grown at 32°C with vigorous agitation and aeration ($PO_2 \geq 50\%$) for 11 h. The culture was harvested by centrifugation, and the cell-free supernatant (4°C) was dialyzed against 4 volumes of distilled water using the H10P10 cartridge. DEAE (2 kg) was added and stirred for 16 h at 4°C. Toxin A was eluted batchwise from the DEAE and further purified by DEAE and hydroxyapatite column chromatography as previously described (24). The final product migrated as a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with M_r = 66,000. The 50% lethal dose (LD_{50}) upon intraperitoneal injection into 18- to 20-g outbred Swiss Webster mice was 0.24 μg. Purified elastase was purchased from Nagase Biochemicals Ltd., Kyoto, Japan. Elastase migrated as a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and possessed an LD_{50} of 125 μg upon intraperitoneal injection into 18- to 20-g mice.

Purification of LPS. LPS was purified from *P. aeruginosa* PA220 cells by the hot phenol-water method of Westphal et al. (49). The extracted material was subjected to three rounds of ultracentrifugation at 100,000 × *g* for 3 h and lyophilized. The purity of the final preparation was confirmed by measuring the absorption spectrum from 200 to 350 nm. LPS contained no detectable protein as measured by the method of Lowry et al. (26), and less than 1% nucleic acids as determined by absorption at 260 nm.

ELISA. Enzyme-linked immunosorbent assays (ELISAs) for the detection of IgG antibody to LPS, toxin A, and elastase were performed in the following manner. Microtiter plates (Linbro; Flow Laboratories, Hamden, Conn.) were coated with 200 μl of LPS (50 μg/ml), toxin A (5 μg/ml), or elastase (50 μg/ml) by incubation at 37°C for 3 h. For coating, toxin A and elastase were suspended in 0.1 M sodium carbonate (pH 9.6), whereas LPS was suspended in 0.005 M sodium phosphate (pH 7.2). Plates were stored at 4°C until needed. The ELISAs were performed as previously described (11) except that all volumes were reduced to 200 μl and the absorbance at 405 nm was measured in a Titertek Multiscan (Flow Laboratories). Titers were expressed as the reciprocal of the dilution of antiserum which gave half-maximal absorption.

CHO cell cytotoxicity assay. The Chinese hamster ovary (CHO) cell cytotoxicity and cytotoxicity-neutralizing assay was performed essentially as previously described (8). The minimal cytotoxic dose per 2×10^4 CHO cells for toxin A and elastase was 2 and 800 ng, respectively. Neutralization assays were performed by testing twofold serial dilutions of IgG against 4 minimal cytotoxic doses of toxin or 2 minimal cytotoxic doses of elastase.

Antisera. Antisera to toxin A, elastase, and LPS were prepared in rabbits. For toxin A and elastase, the initial immunization (day 0) consisted of 50 μg of protein in 0.5 ml of PBS mixed with an equal volume of Freund complete adjuvant (FCA) and was given intramuscularly (i.m.). At days 7, 14, 21, and 28, 100 μg of protein was given i.m. in Freund incomplete adjuvant, and the rabbits were exsanguinated at day 35. For LPS, the initial injection administered i.m. consisted of 10 μg of LPS in 0.5 ml mixed with an equal volume of FCA. At day 14, 20 μg of LPS was administered. At days 28, 42, and 56, 50 μg of LPS was given i.m., mixed with an equal volume of aluminum hydroxide (1-ml total volume). Rabbits were exsanguinated at day 70.

Isolation of IgG from antisera. IgG was isolated from hyperimmune sera by repeated precipitation with ammonium sulfate (30). After three rounds of fraction-

ation, the IgG was dissolved in PBS (original serum volume) and extensively dialyzed against PBS at 4°C. IgG fractions from antielastase and antitoxin were then subjected to two rounds of adsorption with 2×10^{10} CFU of the challenge strain as follows. Mid-log-phase cells were washed in PBS and suspended in the IgG fraction for 1 h at 37°C. Cells were removed by centrifugation, and the process was repeated. The adsorbed material was then filter sterilized, lyophilized, and stored at 4°C. IgG fractions were reconstituted in sterile distilled water immediately before use. The ELISA titers for antitoxin-IgG (AT-IgG), antielastase-IgG (AE-IgG), and anti-LPS-IgG (ALPS-IgG) were 2,500, 1,200, and 1,450, respectively, for their homologous antigen. AT-IgG and AE-IgG neutralized 256 and 320 μg of their respective antigens per ml as measured in the CHO cell cytotoxicity assay. AT-IgG and AE-IgG possessed anti-LPS ELISA titers of less than 1. Normal rabbit IgG (NR-IgG) possessed ELISA and CHO cell cytotoxicity-neutralizing titers of less than 1 in all assay systems.

Mouse burn wound sepsis model. The model was performed as described by Stieritz and Holder (47) with slight modification. The animals (18- to 20-g Swiss Webster outbred mice) were anesthesized in an atmosphere of methoxylfluorane (Penthrane; Abbott Laboratories, North Chicago, Ill.). The animals were then subjected to a 10-s ethanol burn over a 2-cm^2 area of the back. The challenge organisms (in 0.5 ml of PBS) were immediately injected subcutaneously into the burned area. The number of viable organisms contained in a given challenge dose was quantitated by plating onto TSBD agar plates. The animals were observed for 5 days postchallenge. Controls receiving only PBS routinely survived.

Passive transfer and duration of IgG in mice. Sterile IgG (0.2 ml) was transferred via the tail vein. Control experiments showed that up to 0.4 ml could be transferred without any noticeable adverse effects. The duration of passively transferred rabbit IgG in mice was determined as follows. Each mouse received 0.2 ml of AT-IgG intravenously (ELISA titer, 2,500). At various intervals, blood was obtained from three mice and pooled, and the serum antitoxin titer was determined in ELISA, using the initial antitoxin-IgG as a reference standard. Titers were expressed as the reciprocal of the serum dilution which gave half-maximal absorption of the standard.

Bacterial quantitation in tissues and blood. Groups of mice were challenged with approximately 100 CFU of PA220 immediately after traumatizing. At the indicated times postchallenge, groups of three mice were sacrificed for bacterial quantitation. Blood collected from each mouse was immediately diluted 10-fold in cold PBS. A full-thickness skin specimen (approximately 1.5 cm^2) was obtained from the burned area, weighed, and homogenized in 10 ml of PBS for 2 min at top speed with a Sorvall Omnimixer (Du Pont Co., Newtown, Conn.). The liver was also removed and homogenized in PBS. Samples were placed on ice, diluted in PBS, and plated on TSBD agar plates.

Statistical analysis. The mean lethal dose was determined by the method of Reed and Muench (41). *P* values were calculated by the chi-square method.

Serological typing. The O antigen serotype for all strains was based on the scheme described by Homma (16). Antisera were purchased from Toshiba Kagaku Kogyo Co. Ltd., Tokyo, Japan. Slide agglutinations were performed as previously described (16).

RESULTS

To evaluate the optimal time for antibody administration, we determined the duration of intravenously administered rabbit AT-IgG in the serum of recipient mice (Fig. 1). The half-life of AT-IgG in the serum was approximately 55 h. Even after 96 h posttransfer, approximately 30% of the transferred antibody was still present in the serum. Since the mean time to death when PA220 or M2 were employed as challenge strains at doses ranging from 10 to 100 LD_{50} was between 36 and 42 h, antibody was administered 24 h before challenge. In this manner, not only would high levels of antibody remain in the serum during the critical period of infection, but also the diffusion of antibody into the tissues (presumably also to the burn site) would have taken place.

Initial studies to gauge the protective capacity of the various IgG preparations were carried out with PA220 as the challenge strain at a dose of approximately 10 LD_{50} (10^2 bacteria; 1 LD_{50} is <10 organisms). Passively transferred AT-IgG or AE-IgG afforded no significant protection ($P < 0.5$) when mortality rates were compared with those of animals which received only NR-IgG (Table 1). In contrast, all mice which received ALPS-IgG survived in both experiments. When, in additional experiments, the amount of AT-

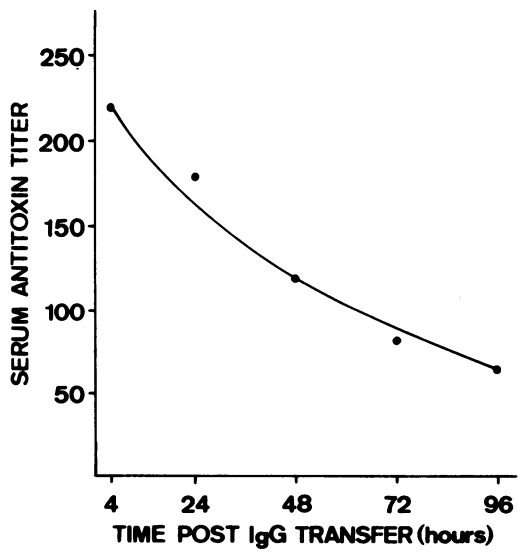

FIG. 1. Duration of passively transferred rabbit AT-IgG in mice. Each mouse received 0.2 ml of AT-IgG intravenously at time 0. At the times indicated, three mice were sacrificed, and the serum was collected and pooled. Titers were determined by ELISA, using the rabbit AT-IgG as a reference standard.

TABLE 1. Protective effect of passively transferred IgG against *P. aeruginosa* PA220 challenge[a]

Expt	IgG transferred[b]	Mortality[c]	*P*
1	NR-IgG	16/18	
	AT-IgG	14/18	<0.5
	AE-IgG	15/18	<0.5
	ALPS-IgG	0/12	>0.999
2	NR-IgG	10/10	
	AT-IgG	9/10	<0.5
	AE-IgG	10/10	<0.5
	ALPS-IgG	0/10	>0.999

[a] Challenge dose contained approximately 1×10^2 CFU of *P. aeruginosa* PA220.
[b] IgG (0.2 ml) was transferred intravenously 24 h before challenge.
[c] Number of mice dead/total.

IgG or AE-IgG transfused was doubled to 0.4 ml, the mortality rates as compared with those of mice receiving NR-IgG were still not statistically significant (mortality rates of 100, 90, and 90%, respectively).

To accurately assess the degree of protection against PA220 infection, we determined the LD_{50} for mice receiving various IgG preparations (Table 2). More than 50% of the animals in groups which received NR-IgG, AT-IgG, or AE-IgG died at the lowest challenge dose tested in both experiments (18 to 20 bacteria). In contrast, all mice in experiment 1 which received ALPS-IgG survived the highest challenge dose tested (2.5×10^5 bacteria). In experiment 2, the LD_{50} was calculated to be 2×10^6 bacteria, corresponding to a 100,000-fold increase in the LD_{50} compared with groups which received either NR-IgG, AT-IgG, or AE-IgG. Thus, only ALPS-IgG was capable of providing a substantial level of protection against PA220 challenge. Although we were unable to demonstrate a significant protection by the transfer of AT-IgG or AE-IgG alone, it was of interest to determine whether the level of protection afforded by ALPS-IgG could be enhanced by the cotransfer of either AT-IgG or AE-IgG (experiment 3). The addition of AT-IgG or AE-IgG to ALPS-IgG did not result in further protection.

To better understand the mechanism by which ALPS-IgG mediates its protective effect and to determine whether AE-IgG or AT-IgG, although not offering absolute protection, could alter the course of infection, we quantitated the number of *P. aeruginosa* in the blood, liver, and skin at the site of infection at various times postchallenge (Fig. 2). Regardless of the IgG preparation transferred, the challenge organisms rapidly multiplied in the skin during the first 26 h postinfection. However, although multiplication continued between 26 and 48 h postinfection in mice receiving either AT-IgG, AE-IgG, or NR-IgG, the number of viable bacteria in the skin of mice receiving ALPS-IgG decreased over this same time period. Bacteremia and colonization of the liver were first noted at 26 h in animals which received AT-IgG, AE-IgG, or NR-IgG. However, no *P. aeruginosa* could be detected either in the blood or in the liver at any time during the infection in mice which received ALPS-IgG.

Prior studies have shown that the virulence of different strains of *P. aeruginosa* varies greatly in the burn wound sepsis model (33). Since in the above experiments only PA220 was used, we felt it necessary to test the protective capacities of AT-IgG and AE-IgG when another challenge strain was used. *P. aeruginosa* M2 was selected due to the fact that, like PA220, it produces elastase and toxin A and is virulent in this model. Again, as with PA220, AT-IgG and AE-IgG were found to offer no statistically significant protection ($P < 0.5$) against M2 challenge as compared with mice which received only NR-IgG. Furthermore, AT-IgG or AE-IgG did not significantly reduce the number of organisms in the skin, blood, or liver after challenge with M2 (data not shown).

The above experiments demonstrated that high levels of protection against PA220 challenge could only be obtained when mice re-

TABLE 2. Mean lethal dose of *P. aeruginosa* PA220 for mice receiving passively transferred IgG

IgG transferred[a]	Mean lethal dose[b]		
	Expt 1	Expt 2	Expt 3
NR-IgG	<18	<20	10
AT-IgG	<18	<20	ND[c]
AE-IgG	<18	<20	ND
ALPS-IgG	$>2.5 \times 10^5$[d]	2×10^6	0.9×10^6
ALPS-IgG + AT-IgG	ND	ND	0.9×10^6
ALPS-IgG + AE-IgG	ND	ND	0.62×10^6

[a] Each IgG preparation (0.2 ml) was transferred 24 h before challenge. For combinations of IgG, equal volumes (0.2 ml) were mixed, and 0.4 ml was transferred.
[b] Expressed as the number of viable PA220. Groups of six mice were used for each challenge dose.
[c] ND, Not determined.
[d] Highest dose tested (all mice survived).

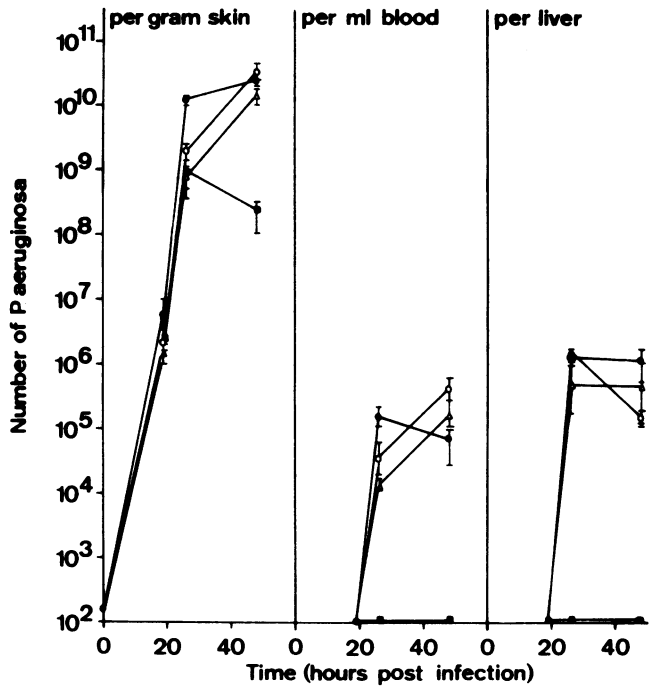

FIG. 2. Quantitation of bacteria in the skin (site of infection), blood, and liver ± the standard error of the mean after challenge with 10^2 *P. aeruginosa* PA220 (time 0). Each mouse received at 24 h before infection 0.2 ml of either AT-IgG (○), AE-IgG (△), NR-IgG (●), or ALPS-IgG (■). Groups of three mice were sacrificed at each timepoint.

ceived ALPS-IgG which was produced against purified PA220 LPS. It was of interest to determine the protective capacity of antibody raised against a given LPS (PA220, serotype G) when the challenge was with a different strain of the same serotype (SBI-C and SBI-N, serotype G) or of a different serotype (M2, serotype B). Therefore, the following experiment was performed. Groups of mice received either NR-IgG or ALPS-IgG and were subsequently challenged with the above four strains (Table 3). Mice which received antiserotype G LPS were completely protected when the challenge was of the same serogroup (PA220, SBI-C, and SBI-N, all of which agglutinated with the transferred antisera). However, the mortality for M2 (serotype B), which did not agglutinate with the transferred antisera, was 100% in groups which received either NR-IgG or anti-serotype G LPS. Therefore, although protection within a given serogroup was complete, it was serospecific.

In all prior experiments, IgG was transferred 24 h before challenge. It was of interest to determine whether ALPS-IgG could still provide good levels of protection when given at or after the challenge (Table 4). In both experiments, 100% mortality was recorded for mice receiving NR-IgG. ALPS-IgG transferred 24 h before infection, at the time of infection, or 4 h after infection afforded complete protection. When given 8 h postchallenge, highly significant protection was obtained ($P > 0.999$), and the mortality rate was only in the range of 10 to 20%. The protective capacity of antibody administered 24 h after challenge was substantially diminished. These results show that ALPS-IgG, even when administered after the infection was established, still give reproducibly significant protection.

DISCUSSION

In the present study, we used a murine burn wound sepsis model to evaluate the ability of passively transferred AT-IgG, AE-IgG, and ALPS-IgG to protect against fatal *P. aeruginosa* infection. Passive, rather than active immunization, was selected for comparing the efficacy of these antibodies due to the following: (i) differences in the immunogenicity of these three antigens (native elastase and stable toxin A toxoids) are poor immunogens in mice unless administered with potent adjuvants, whereas LPS is a good immunogen (8, 32, 37); (ii) adjuvants, such as muramyl dipeptide, have been shown to evoke nonspecific immunity to *P. aeruginosa* (10); and (iii) passive immunity has been shown to be an effective means of conferring protection against *P. aeruginosa* (9, 33, 46).

TABLE 3. Protective effect of passively transferred anti-serogroup G LPS against challenge by serogroup G and serogroup B *P. aeruginosa*[a]

Challenge strain (O serogroup)	Agglutination with ALPS-IgG (serogroup G)	IgG transferred[b]	% Mortality[c]
PA220 (G)	+	NR-IgG	90
		ALPS-IgG	0
SBI-C (G)	+	NR-IgG	80
		ALPS-IgG	0
SBI-N (G)	+	NR-IgG	80
		ALPS-IgG	0
M2 (B)	−	NR-IgG	100
		ALPS-IgG	100

[a] The challenge dose consisted of 10^2 organisms of each indicated strain.

[b] The IgG preparation (0.2 ml) was transferred intravenously 24 h before challenge.

[c] Groups of 10 mice.

TABLE 4. Protective effect of ALPS-IgG administered pre- and postchallenge against *P. aeruginosa* PA220 infection[a]

Expt	IgG administered	Time of IgG administration[b] (h)	Mortality	*P*
1	NR-IgG	−24	10/10	
	ALPS-IgG	−24	0/10	>0.999
	ALPS-IgG	0	0/10	>0.999
	ALPS-IgG	+4	0/10	>0.999
	ALPS-IgG	+8	2/10	>0.999
	ALPS-IgG	+24	5/10	>0.999
2	NR-IgG	−24	10/10	
	ALPS-IgG	−24	0/10	>0.999
	ALPS-IgG	0	0/10	>0.999
	ALPS-IgG	+4	0/10	>0.999
	ALPS-IgG	+8	1/10	>0.999
	ALPS-IgG	+24	8/10	0.555

[a] The challenge dose was 1×10^2 *P. aeruginosa* PA220.

[b] The time of antibody administration relative to the time of challenge (0).

We were unable to demonstrate an appreciable degree of protection against lethal infections by two virulent strains of *P. aeruginosa* by the transfer of AT-IgG or AE-IgG. Neither AT-IgG nor AE-IgG altered the course of infection or consistently decreased bacterial numbers in the skin, blood, or liver.

In contrast, serotype-specific ALPS-IgG was found to be highly protective against challenge with several virulent strains. ALPS-IgG administered before infection afforded complete protection against a PA220 challenge dose of approximately 10 LD_{50}. Under similar circumstances, the LD_{50} could be increased by as much as 100,000-fold. It is important to note that high levels of protection could still be obtained even when ALPS-IgG was administered during advanced stages of the infection. Therefore, preexisting ALPS-IgG antibody is not essential for protection against lethal infections.

Some understanding as to how ALPS-IgG mediates its protective effect was obtained from experiments designed to monitor the growth and spread of the bacterial challenge. ALPS-IgG had little effect on the bacterial growth in the skin during the initial phase of the infection, with all groups having a comparable bacterial load at the challenge site 26 h postinfection. Although this bacterial load was sufficient to result in pronounced bacteremia in all non-ALPS-IgG-treated groups, the blood and liver of ALPS-IgG-treated mice remained sterile. Since the bacterial numbers in the skin were comparable in all groups at the time bacteremia was first noted, we feel that the bloodstream of ALPS-IgG-treated mice was invaded to some extent but that the ALPS-IgG acted as an opsonin to rapidly clear the bacteria. In this manner, established bacteremia and organ colonization would be prevented. The opsonic activity of antibody directed against *P. aeruginosa* LPS has been well documented (44, 45, 52) and appears to play an important role in recovery from *P. aeruginosa* infections (5, 40, 53).

Results from vaccine trials using LPS-containing cell extract vaccines have been encouraging (2, 21). However, a high rate of adverse reactions in certain patient populations has restricted their usage (12, 35). Since the above vaccines contained appreciable amounts of non-LPS material (13, 27), the contribution of such material to the reactions noted cannot be ignored. Highly purified LPS, such as that used in the present study, has not been tested for reactivity in humans. Alternatives to the use of native LPS as a vaccine exist and include detoxified LPS (44) and high-molecular-weight polysaccharides antigenically related to LPS (36–38). Immunization with polysaccharides affords type-specific immunity in a mouse intraperitoneal challenge model (36, 37). The protective capacity of either detoxified LPS or polysaccharides has not yet been evaluated in an animal model relevant to human disease.

ACKNOWLEDGMENTS

We thank E. Zaugg, J. Schlup, and D. Pavlovic for excellent technical assistance and V. Rupp for secretarial help.

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