Antibody Response to *Pseudomonas aeruginosa* Exoproducts in Cancer Patients

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We analyzed sera from 28 patients with various types of malignancies for the occurrence of antibodies against exotoxin A of *Pseudomonas aeruginosa* and two *Pseudomonas* proteases. A total of 27 of these individuals were colonized or infected with *P. aeruginosa* at one time or another during the study, whereas the remaining patient was colonized with four non-*P. aeruginosa* species of *Pseudomonas*. Sera were obtained from several of these patients before *P. aeruginosa* colonization or infection of these individuals was detected, which provided an opportunity to evaluate their responsiveness to pseudomonal exoproducts as they acquired the organism. Exotoxin A was purified from culture supernatant fluids of strain PA-103, and the two proteases were purified from an isolate of strain JR3, a highly proteolytic strain originally recovered from the sputum of a cystic fibrosis patient. Antibodies to the exotoxin A and the two proteases were detected in these sera, and sera which contained relatively high antibody levels to exotoxin A afforded mice complete protection against lethal challenges with this substance. Statistical analyses showed that patients infected with *P. aeruginosa* had consistently higher antibody levels ($P < 0.005$) to the exoproducts than patients who were colonized with this organism. Also, patients colonized with *P. aeruginosa* possessed significantly higher antibody levels ($P < 0.003$) to these three exoproducts than uninfected, hospitalized patients. Parke-Davis type 1 was the strain most commonly isolated from these patients (46%), but colonization or infection due to this organism usually resulted in the production of low levels of antibody to *Pseudomonas* exoproducts. However, infections with Parke-Davis type 7 organisms were always associated with intermediate- and high-responder sera to exotoxin A. These results indicated that potentially toxic products were elaborated during the course of cancer-related colonization and infection with *P. aeruginosa*.

Cancer patients with numerous types of malignancies often become infected with *Pseudomonas aeruginosa*. A total of 25% of all gram-negative bacillary infections in cancer patients are due to *P. aeruginosa*, the majority occurring in patients with neutropenia (23). Although fatal septicemia is common among cancer patients infected with this organism, lungs are also involved frequently, and localized infections may occur in any organ. Many of these infections are nosocomial and are certainly due in part to the unique nutritional requirements of this organism, which allow it to survive in a variety of moist areas, such as faucets, nebulizers, flower vases, and, in some cases, soaps and germicides (3, 25).

Although many different potential virulence

factors produced by *P. aeruginosa* have been isolated and purified, the role of these components in disease processes is not clear. Among the most closely examined of these factors are the hemolysins, several proteolytic enzymes, a potent exotoxin (exotoxin A), whose mechanism of action is strikingly similar to that of diphtheria toxin, and lipopolysaccharide, which is always associated with gram-negative bacteria. All of these compounds are capable of producing deleterious effects in various *in vivo* and *in vitro* systems (2, 9, 10, 14, 16, 18).

Relatively little is known about the antibody responses of humans to these proposed virulence factors. Pollack et al. (19, 20) have shown that neutralizing and hemagglutinating antibodies to exotoxin A are present in the sera of individuals with *P. aeruginosa* infections. Klinger et al. (11) recently demonstrated that antibodies to exotoxin A and two *Pseudomonas*

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proteases are present in the sera of cystic fibrosis (CF) patients. Diaz et al. (4) have shown that antibodies to *P. aeruginosa* cell wall-associated antigens are present in CF patients, and Pollack and Young (21) have shown that anti-exotoxin A antibodies and anti-lipopolysaccharide antibodies are often present in the sera of patients with *Pseudomonas* septicemia. More importantly, these authors demonstrated that survival is significantly greater in patients who have relatively high levels of both anti-exotoxin A antibodies and anti-lipopolysaccharide antibodies.

In light of the finding that proteases play a prominent role in some models of experimental *P. aeruginosa* infection (4, 18), it would be of considerable interest to determine whether antibodies to these proteases (as well as exotoxin A) are present in the sera of cancer patients who have been infected or colonized with *P. aeruginosa*. All of the patients in the study described below suffered from some type of malignancy, and all but one at one time or another had been colonized or infected with *P. aeruginosa*. An additional aspect of this study was to ascertain whether antibodies against exotoxin A, as detected by a radioimmunoassay (RIA), protected mice against experimental lethal challenges with this substance.

MATERIALS AND METHODS

Sera and handling of *P. aeruginosa*. Serum samples from 28 cancer patients were obtained from the Baltimore Cancer Research Center, Baltimore, Md. The disease states of these patients included chronic myelogenous leukemia, astrocytoma, acute myelogenous leukemia, metastatic carcinoma, brain tumor, acute lymphocytic leukemia, embryonic carcinoma, undifferentiated carcinoma, Hodgkins disease, lymphosarcoma, and adenocarcinoma. A total of 22 of the patients studied were represented by more than one serum sample. *P. aeruginosa* isolates were typed by using Parke-Davis typing antiserum (5). Routine cultures, including rectal, vaginal, skin, and throat swabs, were taken for all patients. In addition, stools, blood, urine, and sputum were examined for the presence of *P. aeruginosa*. During autopsies, biopsies were performed routinely on the lungs, spleens, and livers, and these tissues were examined for the presence of *P. aeruginosa*. If *P. aeruginosa* was found consistently in the lungs, blood, or urine in concentrations greater than 100,000 cells per ml, a patient was considered infected. If *P. aeruginosa* was found in vaginal, rectal, skin, and throat swabs in low numbers (see below) and was present in low numbers in stool and urine samples (less than 100,000 cells per ml), a patient was considered colonized. *P. aeruginosa* isolates were quantitated by clock streaks of swabs or by blood and urine colony counts from pour plates by both methods. Scores of 1+, 2+, 3+, and 4+ refer to growth in various areas of a four-way clock streak on blood agar; growth only in the first quadrant was recorded as 1+, growth in both the first quadrant and the second quadrants was recorded as 2+, and so on.

Exotoxin A and proteases. The purification and characterization of exotoxin A and proteases have been described previously (11). A reference sample of exotoxin A further purified by preparative electrophoresis (12) was provided by Stephen Leppla, U.S. Army Institute of Medical Research, Ft. Detrick, Md. Experiments with this exotoxin A sample gave RIA indices similar to the indices obtained when our toxin preparation was used; however, the absolute counts obtained when the toxin from Leppla was used were usually 10 to 20% higher. Protease I corresponded to DEAE peak I of Morihara et al. (15) and had a molecular weight of 20×10^3 based on its elution volume from Sephadex G-100. Protease II eluted at a molecular weight of approximately 30×10^3 and corresponded to DEAE peak II (elastase) of Morihara et al. (15). Each protease migrated as a single band on polyacrylamide gels (7), and each was immunologically distinct from the other as determined by gel immunodiffusion.

RIA. The solid-phase RIA in microassay trays has been described previously (11). The indices obtained in this study were derived from assays in which both the test sera and the control serum were diluted 1:100. All RIA data were expressed as a ratio of the counts per minute of bound ^{125}I -labeled anti-human immunoglobulin per test serum to the counts per minute of control serum pool 5 for each of the three *P. aeruginosa* exoproducts. The control pool consisted of equal volumes of sera from six randomly selected, uninfected, hospitalized adults and has been described previously (11). In these experiments the ^{125}I -labeled antibody to human immunoglobulin added to each microtiter well had an activity of approximately 5×10^5 cpm.

Mouse protection studies. To determine whether sera from patients containing significant levels of anti-exotoxin A antibodies were indeed protective, serum samples (one each from the low-, intermediate-, and high-responder groups) were tested for the ability to protect mice against lethal challenges with this substance. At 4 h before challenge with the exotoxin, 25- to 30-g Swiss mice each received 0.25 ml of a dilution of patient serum intraperitoneally. The mice were then challenged by intravenous administration of 0.1 ml of exotoxin A in 0.01 M Tris (pH 8.0) containing four 50% lethal doses (3.6 μg).

Statistical analyses. Statistical analyses were performed by comparing means and standard deviations by standard methods (13).

RESULTS

RIA. We examined 10-fold serial dilutions of sera from various cancer patients for antibodies to each of the three purified *Pseudomonas* exoproducts by using the RIA. As previously reported (11), serum samples diluted 1:100 often gave higher binding values than lower dilutions in titrations against any of the three antigens. Further dilutions of sera produced linear decreases in binding. Since most binding curves were approximately linear after this point, 1:100 dilutions were used for all further studies.

Table 1 shows the results obtained with sera from 28 patients. Each patient was assigned a

number from 1 to 28 arbitrarily. As mentioned above, *P. aeruginosa* was isolated eventually from all of the patients except patient 28. Since 22 of the 28 patients were represented by more than one serum sample, the inclusion of all data would have necessitated an extremely cumbersome table; therefore, Table 1 includes only the serum sample with the highest RIA ratios for each patient against each antigen. This table shows that the reactivity to each of the three *Pseudomonas* exoproducts varied widely among

TABLE 1. RIA of sera from cancer patients for antibodies against exotoxin A, protease I, and protease II of *P. aeruginosa*

Patient	Disease ^a	RIA ratio (cpm in test serum/cpm in control pool) ^b		
		Exotoxin A	Protease I	Protease II
1	CML	1.7	3.4	3.2
2	AS	3.4	4.0	3.3
3	AML	6.0	2.3	3.1
4	AML	2.7	3.4	2.8
5	LY	1.0	3.0	3.0
6	AML	2.9	4.6	5.0
7	AML	2.3	3.0	3.3
8	AML	3.9	3.8	5.3
9	AS	4.1	4.1	6.6
10	AS	1.2	1.3	2.6
11	ALL	2.7	2.0	5.3
12	MC	1.2	2.0	2.4
13	HD	2.5	3.0	5.7
14	ALL	1.9	2.2	2.0
15	ALL	3.8	4.2	7.0
16	EC	1.7	2.6	1.8
17	LY	1.6	2.0	1.9
18	AML	0.7	1.6	1.0
19	AML	1.4	2.4	1.2
20	BT	1.7	1.4	1.3
21	MC	4.0	2.1	3.7
22	UC	1.9	1.6	1.6
23	LY	2.3	2.3	2.6
24	LY	2.1	2.1	2.2
25	AML	2.2	3.8	2.1
26	CML	2.1	2.5	2.3
27	AML	1.6	2.8	1.8
28 ^c	AML	0.9	0.6	1.1

^a Abbreviations: CML, chronic myelogenous leukemia; AS, astrocytoma; AML, acute myelogenous leukemia; LY, lymphosarcoma; ALL, acute lymphocytic leukemia; MC, metastatic carcinoma; HD, Hodgkins disease; EC, embryonic carcinoma; BT, brain tumor; UC, undifferentiated carcinoma.

^b By comparing means and standard deviations (13), we found that the antibody levels against all three antigens in both infected and colonized patients (Table 2) were significantly higher ($P < 0.003$) than comparable levels in control sera. Also, antibody levels against these same three exoproducts in infected patients were significantly higher ($P < 0.005$) than levels in colonized patients.

^c *Pseudomonas fluorescens*, *Pseudomonas maltophilia*, *Pseudomonas multivorans*, and *Pseudomonas stutzeri* were isolated from this patient.

the patients; however, if the response was high or low to one antigen, it was generally high or low to the other two. For the mouse protection studies, the RIA values for exotoxin A were divided into three groups. Patient serum ratio values of 0.0 to 1.9 were considered negative or, at best, low responders. Patient RIA ratio values of 2.0 to 3.9 were rated intermediate responders, whereas values of 4.0 or more were considered high. For example, patient 3 was a high responder to exotoxin A, whereas patient 4 was an intermediate responder and patient 20 was a low responder to this exoproduct. Comparing the means and standard deviations of these data, we found that the antibody levels in the sera from infected and colonized patients were significantly higher than the levels in the control sera (confidence limits, 99.7%). Also, the antibody levels in the sera from infected patients were significantly higher than the antibody levels in the sera from colonized patients (confidence limits, 99.5%).

Serology and colonization versus infection status of the patients. The Parke-Davis designations for the isolates are shown in Table 2. Of the 37 different *P. aeruginosa* isolates, 13 were Parke-Davis type 1. This meant that of the 28 patients examined, 13 (46%) had type 1 organisms isolated from them at least once during the study. Although the type 1 organisms were the most common isolates, the majority of these infections or colonizations resulted in low-responder antisera (7 of 13) and intermediate-responder antisera (5 of 13) to exotoxin A. In fact, infections with all of the Parke-Davis types except one resulted in mostly low- or intermediate-responder antisera. The one type that resulted in only intermediate- or high-responder antisera was type 7. Of the five Parke-Davis type 7 isolates, three were isolated from individuals who were intermediate responders (patients 7, 24, and 25), and two were isolated from individuals who were high responders (patients 3 and 21). Therefore, of the three patients whom we classified as high responders to exotoxin A, two were infected with *P. aeruginosa* Parke-Davis type 7 *P. aeruginosa*.

Sequential RIA studies. Because 22 of the 28 patients studied were represented by more than one serum sample, this provided an opportunity to evaluate the responsiveness of these patients to pseudomonal exoproducts over an extended period of time. One general trend in these patients was for the antibody response to a particular antigen to increase after *P. aeruginosa* was isolated, if it had not been isolated previously. However, a considerable amount of fluctuation in the RIA values was evident. Table 3 shows RIA values for three separate patients suffering from *P. aeruginosa* infections, and these values

TABLE 2. *P. aeruginosa* colonization and infection status of cancer patients

Patient	Colonized or infected	Site of isolation	Parke-Davis type isolated
1	Infected	Lung biopsy	1
2	Infected	Sputum, lung biopsy	2
3	Infected	Blood culture	6/7
4	Colonized	Mouth, rectum, gums	1
5	Infected	Sputum, lung biopsy	1
6	Infected	Blood culture, urine culture ^a	4
7	Infected	Blood culture, sputum, lung biopsy	7
8	Colonized	Rectum	2
9	Infected	Urine culture ^a	2
10	Colonized	Rectum, urine culture ^b	5
11	Infected	Blood culture, sputum	4
12	Colonized	Rectum	1/2
13	Infected	Rectum ^c	1
14	Colonized	Rectum, skin	1
15	Infected	Blood culture	4
16	Colonized	Throat, gums, rectum, skin	1/5
17	Colonized	Rectum, gums	1
18	Infected	Blood culture	1
19	Colonized	Gums, nose, rectum	2/6
20	Colonized	Skin	1/4/6
21	Infected	Skin lesion	1/7
22	Infected	Sputum	5
23	Infected	Blood culture	1
24	Colonized	Urine ^b	7
25	Infected	Blood culture, lung biopsy	1/5/6/7
26	Colonized	Urine, vagina ^b	4
27	Infected	Blood culture	6

^a More than 100,000 cells per ml of urine.

^b Less than 100,000 cells per ml of urine.

^c A score of 4+ on a four-way clock streak on blood agar.

indicated an increase in RIA ratios against all three exoproducts after *P. aeruginosa* was isolated. For example, patient 5, who had lymphosarcoma, yielded his lowest RIA ratios at the beginning of the serum studies, before *P. aeruginosa* was isolated. As *P. aeruginosa* was isolated from the sputum in large numbers (4+ on clock streak), the RIA values against the three isoproducts began to increase. The RIA ratio for exotoxin A was highest at the time of the first *P. aeruginosa* isolation (2.0) but decreased to 1.0 at the time of lung biopsy several months later. However, the highest RIA ratios for this patient for proteases I (3.0) and II (3.0) were observed at the time of lung biopsy. A similar phenomenon occurred in patient 11, who was diagnosed as having acute lymphocytic leukemia. In this patient the highest RIA value to exotoxin A (2.7) was found at the time when *P. aeruginosa* was isolated from his sputum in small numbers. However, the RIA ratio to protease II was quite elevated (5.3) at this time. The RIA ratio to exotoxin A decreased significantly when *P. aeruginosa* was isolated from the bloodstream of this patient; however, the RIA index for protease II remained elevated (4.9).

Finally, patient 27 yielded relatively low RIA

indices to all three exoproducts by time of the first serum withdrawal and no isolatable *P. aeruginosa*. After *P. aeruginosa* was isolated from a skin lesion and eventually from the blood, the RIA index for exotoxin A increased from 1.0 to 1.6, and the RIA indices for proteases I and II increased from 0.9 to 2.8 and from 1.3 to 1.9, respectively.

Passive protection against exotoxin A. To determine whether sera from patients containing intermediate and high levels of anti-exotoxin A antibodies were indeed protective, we tested serum samples from three patients (chosen at random, one from each responder category) for the ability to protect mice against lethal challenges with exotoxin A. At 4 h before challenge with the exotoxin, each mouse received 0.25 ml of a dilution of patient serum intraperitoneally. Then each mouse was challenged by intravenous administration of 0.1 ml of exotoxin A containing four 50% lethal doses, as previously described. These data are shown in Table 4. No protection was achieved with the control serum pool or the serum from patient 20, a negative responder to exotoxin A. Partial protection was achieved with serum from patient 4 (two of five mice survived), an intermediate responder to

TABLE 3. RIA ratios against exotoxin A, protease I, and protease II of various sera drawn from different patients at different times

Patient	Disease ^a	Date serum drawn	<i>P. aeruginosa</i> isolated before serum collection	Source	RIA ratio (cpm in test serum/cpm in control pool)		
					Exotoxin A	Protease I	Protease II
5	LY	24 August 1974	No	Sputum (4+ growth) ^b Lung biopsy	0.9	1.4	1.6
		9 December 1974	Yes		2.0	2.4	2.0
		6 January 1975	Yes		1.0	3.0	3.0
11	ALL	2 February 1970	No	Sputum (4+ growth) Blood (>200 colonies per ml), rectum (2+ growth)	2.2	1.5	1.1
		20 February 1970	Yes		2.7	2.0	5.3
		7 March 1970	Yes		1.1	1.6	4.9
27	AML	29 June 1971	No	Skin lesion (4+ growth) Blood (>200 colonies per ml)	1.0	0.9	1.3
		26 July 1971	Yes		1.6	2.8	1.8
		2 August 1971	Yes		1.6	2.6	1.9

^a Abbreviations: LY, lymphosarcoma; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia.

^b Growth scores were determined by clock streaks on blood agar (see text).

exotoxin A, whereas complete protection was obtained with serum from patient 3, a high responder.

DISCUSSION

It has been noted previously (22, 24) that most patients who develop nonbacteremic *P. aeruginosa* infections have preexisting, chronic pulmonary disease or some other form of severe medical disorder; that is, *P. aeruginosa* infections usually occur only in severely debilitated or compromised hosts. This is particularly evident in patients with CF, cancer, and severe burns. Recently, we examined the sera of CF

patients for the production of antibody against the three exoproducts described above (11). In that study, we demonstrated an inverse correlation between the ratio for each of the *Pseudomonas* exoproducts and the clinical (Schwachman) score; that is, the highest ratios occurred in the patients who had the lowest clinical scores.

On the other hand, cancer patients pose an entirely different problem than individuals who have CF. The frequency of *P. aeruginosa* infections is very high among cancer patients, especially at institutions that possess special cancer units (6). This high frequency may be due to the fact that patients with acute leukemia and other

TABLE 4. Passive protection by cancer serum against exotoxin A challenge^a

Serum	RIA index	Dilution	No. of survivors at: ^b							
			Zero time	16 h	24 h	32 h	40 h	48 h	60 h	80 h
Control	1.0	Undiluted	5	0	0	0	0	0	0	0
		1:2	5	0	0	0	0	0	0	0
		1:4	5	0	0	0	0	0	0	0
Patient 3	6.0	Undiluted	5	5	5	5	5	5	5	5
		1:2	5	4	4	4	2	2	2	2
		1:4	5	4	4	2	1	0	0	0
		1:8	5	4	3	1	0	0	0	0
Patient 4	2.7	Undiluted	5	4	4	3	2	2	2	2
		1:2	5	4	3	1	1	1	1	1
		1:4	5	4	3	1	0	0	0	0
		1:8	5	4	3	0	0	0	0	0
Patient 20	0.7	Undiluted	5	1	0	0	0	0	0	0
		1:2	5	0	0	0	0	0	0	0
		1:4	5	0	0	0	0	0	0	0

^a Intraperitoneal administration of 0.25 ml serum, followed 4 h later by intravenous administration of four 50% lethal doses (mouse) of exotoxin A.

^b Each treatment group contained five mice.

bone marrow disorders are usually deficient in neutrophils (25). Neutropenia can also occur less frequently in patients with other forms of cancer and is usually secondary to chemotherapy; hence, it is not as severe or prolonged (25). This may be in part responsible for the fact that the RIA indices in this study were generally lower than the indices observed with CF patients (11). Individuals with neutropenia usually have impaired phagocytic capabilities, and this certainly affects their ability to process an antigenic stimulus. Indeed, despite the fact that the data were collected in the same manner, the RIA indices in this study were generally lower than the indices observed with CF patients. Individuals with CF characteristically have chronic, pulmonary infections with *P. aeruginosa* which can last for many years, but the disease state does not seem to impair the ability of these patients to respond immunologically. In contrast, *P. aeruginosa* infections of cancer patients appear to be of much shorter duration, and this could be another explanation for the lower RIA values against the various *P. aeruginosa* exoproducts found in cancer patients.

A certain degree of fluctuation was present in the data expressing the antibody responses to the three antigens which we tested. This was most likely due to a number of factors, such as the duration of the infection and the immune status of these individuals. Generally, most of the responses shown in Table 1 are responses of low to intermediate capability; this was probably a reflection of the inability of the patients to respond immunologically. One important fact shown by these data is that a significant and detectable level of antibody to one or more of the *P. aeruginosa* exoproducts was present in the sera of almost all of these patients, regardless of whether the patients were merely colonized or actually infected ($P < 0.003$). Of course, the infected patients showed significantly higher antibody responses against the three exoproducts than the colonized patients ($P < 0.005$). This provides at least suggestive evidence that these exoproducts are produced during the course of colonization and infection with this organism.

The serotype distribution of *P. aeruginosa* observed in this study is worth further mention (Table 2). We found that the most common type of *P. aeruginosa* isolated from the cancer patients whom we studied was Parke-Davis type 1. A total of 46% of the patients in this study were either infected or colonized with this type of *P. aeruginosa*. The majority of the *P. aeruginosa* blood isolates (39%) from bacteremic patients in the study of Pollack and Young (21) were also Parke-Davis type 1. This phenomenon resembles the serotype distribution of *P. aeruginosa*

observed in CF patients. Habs type 6 (8) was the most common serotype found in CF patients, and in a study performed in our laboratory, Habs type 6 organisms represented 43% of the *P. aeruginosa* strains isolated from these patients (26). Using the schema of Fisher et al. (5), Baltimore and Mitchell (1) found a preponderance of Parke-Davis types 1, 2, and 6 in 16 strains of *P. aeruginosa* that were isolated from patients with CF. In the study of these authors, Parke-Davis type 1 was the most common type (50%), followed by type 6 (25%) and type 2 (19%). These data are also similar to those of Zierdt and Williams (27), who examined 173 *P. aeruginosa* strains from CF patients and found that Fisher serotypes 1 and 6 comprised the bulk of the monotype strains. Thus, the results of these previous two studies are similar to our results because Parke-Davis types 2 and 6 were also quite prevalent in our study of cancer patients; 18% (5 of 28) of these cancer patients were infected or colonized with Parke-Davis type 2, and 18% (5 of 28) were infected or colonized with Parke-Davis type 6. Therefore, it appears that the strains of *P. aeruginosa* that are most prevalent in CF infections may also be the most important strains in cancer patients. The preponderance of one or two O serotypes isolated from cancer patients (as well as CF patients) suggests that there may be some association between O serotypes and *P. aeruginosa* infections in these patients. However, we do not have any explanation for why infections of cancer patients with Parke-Davis type 7 strains seem to result in high antibody production against exotoxin A.

Table 3 shows the results of a sequential study on three patients over a period of several months. *P. aeruginosa* had not been isolated from these patients at the time that the first serum samples were drawn, but it was isolated by the time that subsequent samples were taken. As was the case for these and other patients from whom *P. aeruginosa* was isolated throughout the entire course of hospitalization, the general trend was for the antibody response to a particular antigen to increase after the isolation of the organism. These data indicate that an increase in the number of organisms during infection is often reflected in the antibody response to certain extracellular virulence factors. Although this was one general trend, fluctuations in the RIA values were evident. These fluctuations were probably due to the continually changing immune status of the patients and antibiotic treatments, which may have eliminated the organism periodically.

Patient 28, a "built-in" control, was not infected with *P. aeruginosa*, but was suffering from infections with other *Pseudomonas* spe-

cies. We detected no response to any of the three *P. aeruginosa* exoproducts in serum samples from this control patient, further establishing the unique association of these exoproducts with this organism.

Vaccines examining either active or passive immunization against *P. aeruginosa* virulence factors would be helpful in studying the concept of prophylaxis in dealing with life-threatening *P. aeruginosa* infections. Our mouse protection experiment (Table 4) showed that antibodies produced against exotoxin A function in preventing death in mice due to lethal challenges with this substance. Although only two of five mice survived when they were given undiluted serum from patient 4 (intermediate responder), death was prolonged in mice that received as high a dilution as 1:8 in this experimental design. This phenomenon appeared to hold true for sera from other patients who were infected with *P. aeruginosa*. Klinger (J. D. Klinger, Ph.D. thesis, University of Texas Health Science Center in San Antonio, 1978) demonstrated that sera containing high levels of anti-exotoxin A antibodies from five different CF patients provided complete protection for mice against 10 50% lethal doses of exotoxin A. Additional experiments may well demonstrate that passive immunization with antibodies to one or more virulence factors of *P. aeruginosa* is useful in prophylaxis designed to prolong or prevent death from infection with this organism in compromised hosts.

The concept of increasing titers against exotoxin A in patients infected with *P. aeruginosa* agrees well with the results of our previous study on CF patients (11) and also the results of Pollack and Taylor (20) and Pollack et al. (19). In addition, anti-exotoxin A produced in rabbits passively protects against lethal *P. aeruginosa* burn infections in mice when the infecting organism is a toxin producer (17) but not when it is atoxigenic. It has also been shown (16) that passive immunization with rabbit antitoxin prevents toxin-mediated protein synthesis inhibition in mice, which occurs in unimmunized animals infected with toxin-producing *P. aeruginosa* strains. Finally, Pollack and Young (21) have shown that antibodies against exotoxin A (as well as *P. aeruginosa* lipopolysaccharide) are present in the sera of patients with *P. aeruginosa* septicemia and that the presence of these antibodies is associated with survival. This is the first report of the production of anti-protease antibodies in cancer patients and also one of the first reports demonstrating that humans infected with *P. aeruginosa* can produce antibodies against exotoxin A that are capable of protecting experimental animals against lethal challenges with that substance.

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