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Research Article

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ABSTRACT Chronic respiratory infection with *Pseudomonas aeruginosa* is a leading clinical problem among patients with cystic fibrosis. Because antimicrobial agents are usually ineffective in eradicating these infections, additional therapeutic or prophylactic measures should be considered. In this study, an experimental guinea pig model of chronic *Pseudomonas aeruginosa* bronchopneumonia was utilized to determine whether active immunization with lipopolysaccharide (LPS) *P. aeruginosa* antigen may favorably influence the course of this infection. Experimental pneumonia was established by tracheobronchial instillation of suspensions of microscopic agar beads, which were impregnated with viable *P. aeruginosa*. After 4 wk of infection, the geometric mean (reciprocal) passive hemagglutinating Pseudomonas antibody titer was 185±1.3, and lungs contained 16.8±4 × 10^3 colony-forming units Pseudomonas/ml of lung homogenate. Pseudomonas immunization, given prior to a 4-wk infection, resulted in significantly higher passive hemagglutinating titers (474±1.4; $P < 0.05$), lower numbers of viable Pseudomonas in lung tissues (2.4±0.6 × 10^3; $P < 0.01$), and reduced histopathology in lungs. In contrast, providing Pseudomonas immunization to animals 2 wk after pulmonary infection was established, offered no apparent benefit. Likewise, no protection was afforded by prophylactic immunization with a non-Pseudomonas LPS antigen (*Escherichia coli* J5 vaccine). Using a Raji cell assay, modified to detect circulating immune complexes in vaccinated and infected guinea pig sera, there was no evidence that active immunization increased the frequency of circulating immune complexes in infected guinea pigs. It is concluded that prophylactic immunization with Pseudomonas LPS antigen may confer protection from subsequent Pseudomonas bronchopneumonia, but that immunization during established infection is not beneficial.

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INTRODUCTION

Cystic fibrosis (CF)[1] is the most common fatal hereditary disease of the Caucasian race (1). In recent years, the leading cause of death among CF patients has become respiratory failure, almost always in conjunction with chronic and progressive bacterial bronchitis. In 70–80% of these patients, *Pseudomonas aeruginosa* is isolated as the predominant pathogen in sputum (2–4). Repeated hospitalizations for intensive parenteral antimicrobial therapy have not been successful in eradicating Pseudomonas from lungs (2, 4–6), and it is clear that respiratory deterioration correlates with duration and severity of Pseudomonas bronchitis (4–7). The predictable bacteriology of infected sputum among CF patients, plus the lack of effective antibiotic therapy for Pseudomonas lung infections, has prompted interest in utilization of immunotherapy or immunoprophylaxis with Pseudomonas antigens for such patients (2, 8). Recent reports that active immunization with lipopolysaccharide (LPS) Pseudomonas antigen confers specific protection from experimental Pseudomonas pneumonia (9), apparently by producing high levels of opsonic antibody in areas

[1] *Abbreviations used in this paper*: AGG, aggregated gammaglobulins; CF, cystic fibrosis; cfu, colony-forming units; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PHA, passive hemagglutinating antibody; SDG, sodium deoxycholate.

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of inflamed respiratory tissues (10), has heightened interest in this approach.

There are several concerns regarding the use of active Pseudomonas immunization for cystic fibrosis patients, however. First, chronically infected CF patients have permanent lung damage and it may be impossible to arrest or reverse the disease at this stage (1–3, 8). Second, chronically infected CF patients routinely develop elevations in Pseudomonas antibodies that appear inadequate to eradicate localized Pseudomonas infections in the lung (5–8, 11). Thus, further elevations in humoral antibody, raised by active immunization, might not offer benefit. Third, circulating immune complexes are seen at increased frequency among infected CF patients (12–15), and it is possible that these complexes result from the presence of Pseudomonas antigen with elevated titers of Pseudomonas antibodies. Such complexes, if deposited in lung tissues, might predispose to hypersensitivity lung disease (12, 13), and further immune stimulation by vaccine would be contraindicated in that setting. Fourth, the peculiar mucoid slime coating frequently associated with Pseudomonas in the CF lung (1–7, 16), may act as an antiphagocytic and antiopsonic substance (17, 18), rendering antibodies to somatic antigen less effective. Finally, the unpleasant side effects associated with lipopolysaccharide bacterial vaccine (8), have impeded rapid acceptance of such vaccines by clinicians, even for pilot investigations.

Since progressive lung infection with Pseudomonas is currently the leading clinical problem among CF patients and since little progress has occurred in either preventing or treating this disease, it would be appropriate to consider innovative and aggressive approaches to management of this problem. The present study of active Pseudomonas LPS immunization in an experimental model of chronic Pseudomonas lung infection is meant to address some of the concerns raised above and to serve as a preclinical trial. The results of this study reveal markedly different results when Pseudomonas immunization is provided prophylactically rather than after an infection has been established in the lung.

METHODS

Animals. Hartley strain guinea pigs weighing 400 g were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Animals were housed in standard cages, and fed guinea pig chow (Ralston-Purina Co., St. Louis, Mo.), cabbage, and water.

Pseudomonas aeruginosa. The strain of *P. aeruginosa* used for the majority of these studies has been described in detail elsewhere (9, 19). This organism was originally isolated from a patient with sepsis and was provided to our laboratory by Dr. Mike Fisher and Dr. Carl Heifetz, Parke-Davis and Co., Detroit, Mich. The strain is a Fisher immunotype 4 (20) and is hereafter designated as strain P-4. Strain P-4 has been demonstrated to be serum resistant to guinea pig serum and to produce relatively small amounts of exotoxin A, and large amounts of protease (9). Previous studies in guinea pigs demonstrated considerable virulence for this strain in acute lung challenge experiments (9, 19). Two additional nonmucoid strains of *P. aeruginosa* were obtained from sputum cultures of cystic fibrosis patients at Children's Hospital Medical Center, Boston, Mass. These isolates were designated as strain P-1 and P-258, and were found to be Fisher immunotypes 6 and 1, respectively (20).

Chronic Pseudomonas pneumonia. Recent studies in rats have indicated that by incorporating viable Pseudomonas into microscopic agar beads prior to intratracheal instillation, a chronic purulent Pseudomonas bronchopneumonia can be established (21). We have modified this model for guinea pigs. An overnight growth of *P. aeruginosa* was prepared as previously described (9). A final suspension of 2×10^9 colony-forming units (cfu) per ml phosphate-buffered saline (PBS) was prepared and 1 ml was mixed with 4 ml melted tryptic soy agar. For control studies, 1 ml sterile PBS was added to agar. Mixtures were quickly poured into 50 ml of warm mineral oil (50°C) (E. R. Squibb & Sons, Inc., Princeton, N. J.) and stirred by magnetic bar so that microscopic agar beads would form (21). Suspensions were further stirred in an ice bath for 5 min, and the oil-bead slurry was then transferred to 50-ml plastic centrifuge tubes (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.). Sodium deoxycholate (SDC) dissolved in PBS was used to wash mineral oil from agar beads. An equal volume of 0.5% SDC was added to tubes containing oil-bead slurries. Tubes were shaken by hand, then spun at 500 *g* for 8 min. Oil and SDC were carefully decanted from agar bead pellets, and beads were washed again, using 0.25% SDC. Agar beads were finally washed twice in PBS, and a final bead suspension made in 10 ml PBS. Microscopic examination of bead suspensions revealed a mean bead diameter of 138 μm (range, 115–184 μm).

A modification of a previously described method for creating experimental pneumonia in guinea pigs was used (9, 19). Guinea pigs were anesthetized with intraperitoneal pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.) and the trachea exposed via midline incision. Syringes were loaded with 0.3-ml aliquots of agar bead suspensions and direct instillations of beads into the lower respiratory tract were carried out via tracheal punctures. These inocula were larger in volume than in previous studies (21) and were designed to create bilateral lung infections, rather than the unilateral infections described in rats (21).

Groups of animals were electively killed at timed intervals after experimental infection. Surgically removed lungs were then evaluated for quantities of viable *P. aeruginosa* and for extent of histopathologic damage (9, 19). The trachea was cannulated and the left mainstem bronchus ligated. The left lung was resected distal to the ligature, placed in a commercial grade blender (Waring Products Div., New Hartford, Conn.), and homogenized. Quantitative cultures of lung homogenate were carried out using the serial-dilution, pour-plate technique (9, 19). Additionally, colonies were routinely selected from these plates and identified as *P. aeruginosa* using standard microbiological methods (22). Right lungs were perfused via the trachea with 10% buffered formalin under 10 cm hydrostatic pressure and then immersed in identical fixative. After 72 h in fixative, lung sections were prepared for histologic exam, as previously described (9, 19).

Lung sections were coded by Dr. Pennington and examined in blind fashion by a member of the pathology department, Dr. Hickey, who graded each lung for degree of lung in-

flammation and bronchiolar or bronchial wall destruction. A series of criteria were used to establish an overall score for lung histopathology. These criteria included: (*a*) number of bronchi or bronchioles with 20 or more polymorphonuclear leukocytes in the lumen per 20 consecutive bronchi or bronchioles encountered (range, 0–11); (*b*) number of bronchi or bronchioles with focal wall destruction, also per 20 counted (range, 0–3); and (*c*) the number of neutrophil containing microabscesses per 20 consecutive oil power (×1,000) fields observed (range, 1–22). These numbers were added for each lung specimen and the mean value for these sums was regarded as the histopathologic score for each study group.

Vaccination and serology. The *P. aeruginosa* vaccine used for active immunization was a heptavalent LPS antigen preparation, containing Fisher type 1 through 7 somatic antigens, and has been described in detail, elsewhere (23). The vaccine was supplied by Dr. Fisher and Dr. Brackett (Parke-Davis and Co., Detroit, Mich.) in glass vials, each containing 0.85 mg of LPS antigen, suspended in buffered isotonic saline and preserved with 0.01% thimerosal. Prior studies in guinea pigs have established that six intramuscular injections of this preparation, each containing 50 μg of LPS antigen/kg body wt, given over a 2-wk period, result in fourfold elevations of passive hemagglutinating antibody, significant elevations in Pseudomonas-specific opsonic antibodies, and protection against acute Pseudomonas pneumonia (9, 10). This vaccine regimen was again used for the present study.

For selected studies, active immunization was carried out with the J-5 mutant of *E. coli* 0111 (24). This isolate was obtained from Dr. Elias Menkes, and originally from Dr. Melvin Marks. The J5 vaccine was prepared as a heat-killed, whole-cell antigen, as previously described (24). J-5 immunization consisted of six intramuscular injections each containing 1 mg antigen, given over 2 wk.

Blood specimens were collected from guinea pigs by cardiac aspiration at various times before, during, and after vaccination and experimental infections. Sera were stored at −70°C. Humoral immune response to vaccination was monitored using passive hemagglutinating antibody (PHA) titers. Serum PHA antibodies to Pseudomonas LPS antigens, as defined by the Fisher-Devlin-Gnabasik system (20), were determined as previously described (25). Briefly, fresh erythrocytes were coated with types 1–7 LPS Pseudomonas antigen and reacted with twofold dilutions of serum, in microtiter plates. The PHA titer was expressed as the reciprocal of the highest serum dilution that resulted in 1 + erythrocyte agglutination. Duplicate serum specimens were incubated overnight at room temperature with an equal volume of 0.2 M 2-mercaptoethanol to reduce macroglobulins (26). PHA titers were then determined on the reduced sera, as before. The PHA titers for various study groups were expressed as reciprocal geometric mean values. PHA titers were also determined in sera from J-5 vaccinees, using sheep erythrocytes sensitized with J-5 antigen.

Immune complexes. The Raji cell assay as described by Theofilopoulus et al. (27), was modified to detect circulating immune complexes in guinea pig sera. Guinea pig gammaglobulins (N. L. Cappel Laboratories, Cochranville, Pa.) were aggregated using bis-diazobenzidine (28, 29). Guinea pig gammaglobulins were dissolved in borate-buffered saline, pH = 8.5 at 10 mg/ml concentration. To 1 ml, 0.25 ml of a 1:10 dilution of BDB stock was added drop-wise at 0°C. The mixture was kept at 0°C for 15 min, then dialyzed vs. PBS at 4°C for 48 h. The aggregated gammaglobulins (AGG) so formed were used to obtain the standard curve in the Raji cell assay. The immunoglobulin fraction of rabbit anti guinea pig gammaglobulin was purchased from Dako Antibodies, Accurate Chemical and Scientific Corp., Westbury, N. Y. It was radiolabeled with I^{125} using chloramine T (30) to a specific activity of 0.2 μCi/μg, and used at a concentration of 0.5 mg/ml in PBS with 1% human serum albumin (Hyland Division, Travenol Laboratories, Costa Mesa, Calif.). The amount of complexlike material present in tested sera was expressed as microgram AGG equivalent per milliliter of serum. Values of 10 μg AGG equivalents/μl or less were considered negative (27), and values >20 were considered definite positives. All positive sera were confirmed by repeat assay.

Study design. This study was carried out in two phases. The first phase was a prophylactic study in which equal numbers of guinea pigs received either a full course of Pseudomonas vaccination, or else a series of sterile isotonic saline injections, followed 5–7 d after the final vaccine or saline injection by lung challenge with an agar-bead Pseudomonas inoculation. Vaccinees and controls were then killed after 4 wk of infection and compared for numbers of viable Pseudomonas in the lung, degree of pulmonary histopathology, and immunologic response, including hemagglutinating antibodies and immune complex formation. The second phase of the study was designed to determine the effect of active Pseudomonas vaccination of animals during an established Pseudomonas lung infection. For this phase, animals were infected using Pseudomonas in agar beads, as before. 2 wk after Pseudomonas bronchopneumonia was established, one group of infected animals underwent a 2-wk regimen of Pseudomonas vaccination, while a control group received saline placebo, as before. After a full 4 wk of infection, vaccinees and controls were killed and compared for the parameters, as described above. In each experiment, animals were included in which sterile agar beads were used for lung challenge rather than beads containing Pseudomonas.

RESULTS

Response to agar bead induced chronic Pseudomonas pneumonia. It was initially important to validate the agar bead model as appropriate for immunologic studies of chronic Pseudomonas bronchopneumonia. Although morphologic evidence exists to document that Pseudomonas enclosed in agar beads may eventually grow free of this matrix and invade lung tissues (21), there is no documentation that the host immune system will detect and respond to this experimentally induced infection. Initial work was thus carried out to ensure that the agar bead model for chronic Pseudomonas pneumonia would result in a host immune response similar to that described among infected CF patients. Pseudomonas strain P-4 was used for these initial studies.

Viable Pseudomonas were easily detected in lung tissues up to 6 wk after infection (Table I). The initial infectious load immediately after lung challenge was 110×10^3 cfu Pseudomonas/ml lung homogenate, which gradually decreased in numbers of bacteria over the 6-wk observation period. A vigorous humoral immune response to lung infection was observed that also decreased over this period (Table I). During the first 4 wk of infection, the majority of PHA antibody was reduced by 2-mercaptoethanol, thus was putatively of the IgM class. After 6 wk of infection, however, a considerable portion of PHA antibody appeared to be

TABLE I
Bacteriologic and Immunologic Parameters during Chronic Pseudomonas Pneumonia

Parameter	Weeks after infection			
	0 (Base line)	3	4	6
Number studied	6	7	16	12
Cfu ($\times 10^3$)				
Pseudomonas per ml homogenate	110±18*	27.7±7	16.8±4	1.7±0.8
PHA titer‡	<4	561±1.2	185±1.3	95±1.1
	(<4)	(4)	(6)	(39)

* Mean values±SEM.

‡ Serum PHA to Fisher type 4 Pseudomonas antigen, expressed as reciprocal geometric mean±SEM. (Values in parentheses are titers after 2-mercaptoethanol reduction.)

IgG (Table I). A group of 16 animals was studied after 4 wk of infection to determine if the magnitude of PHA titer bore a direct relationship to number of viable Pseudomonas in lung tissues. A correlation coefficient of $r = 0.696$ was calculated ($P < 0.01$), indicating that the host did respond with higher PHA titers in the presence of greater numbers of Pseudomonas in the lung.

Histologic examination of lung tissues 4 wk after animals received Pseudomonas containing agar-beads, showed markedly abnormal pulmonary architecture, resembling those findings described previously for lung tissues from infected patients with cystic fibrosis (31). The Pseudomonas containing beads were always surrounded by one or more layers of neutrophils with occasional macrophages admixed (Fig. 1). These cells were occasionally seen to have invaded the substance of the bead and died therein. Neutrophils were also seen scattered in the mucosa and bronchial wall. Beyond the layers of inflammatory cells surrounding the bead the response was primarily a mixed macrophage-lymphocytic infiltrate (Fig. 1). These cells were always present and formed the predominant component of the inflammation in the bronchial wall and surrounding lung parenchyma. The bronchioles varied with respect to the degree of damage caused by the Pseudomonas containing beads and their attendant tissue reactions. Most showed mucosal damage with loss of lining cells and reactive mucosal thickening. Occasional airways demonstrated focal necrosis that extended through the mucosa into the smooth muscle layers of the wall (Fig. 2). Where damage to the bronchiole had been extensive there was a marked, concentric, fibrotic reaction around the airway. In contrast were the histologic findings for animals receiving sterile agar beads. These beads elicited only a mild macrophage response, with mild edema and rare neutrophils seen in the adjacent mucosa (Fig. 3). The mucosa and walls of airways were intact.

Thus, both immunologic and histopathologic responses occurred in this model that resembled those conditions described in cystic fibrosis patients, and it appeared that our guinea pig model offered an acceptable system in which to monitor the immunologic, bacteriologic, and histopathologic effects of active Pseudomonas immunization for chronic lung infection.

Prophylactic immunization. Pseudomonas vaccination routinely resulted in fourfold or greater elevations of passive hemagglutinating antibody in guinea pigs (Table II). 4 wk of Pseudomonas pneumonia resulted in serum PHA titers equivalent to those titers resulting from 2 wk of active vaccination from LPS antigen (Table II). After 1 mo of Pseudomonas pneumonia, animals that had received vaccine prior to the onset of chronic infection had significantly higher PHA titers ($P < 0.05$, two-tailed Student's t test), lower numbers of viable Pseudomonas in lung tissues ($P < 0.01$), and lower histopathology scores ($P < 0.05$), than animals not prevaccinated (Table II). The statistical analysis for the differences in cfu per milliliter lung was repeated, using cfu values expressed as $\log_{10}$. Again, vaccinees had significantly fewer viable Pseudomonas (mean±SEM of 3.07±0.16), than animals not prevaccinated (3.92±0.16), with a $P < 0.001$. The individual PHA titers for vaccinees after 4 wk of infection, did not correlate either directly or inversely with the number of viable Pseudomonas in lung tissues ($r = 0.09$). Thus, for vaccinees, higher PHA titers did not necessarily indicate greater numbers of Pseudomonas remaining in lungs, contrasting with the correlation noted for unvaccinated animals. It was concluded that prophylactic vaccination with LPS Pseudomonas antigen offered the host a more favorable outcome from chronic pulmonary infection than observed among the unvaccinated group.

Immunization during infection. Animals mounted a vigorous systemic humoral immune response after

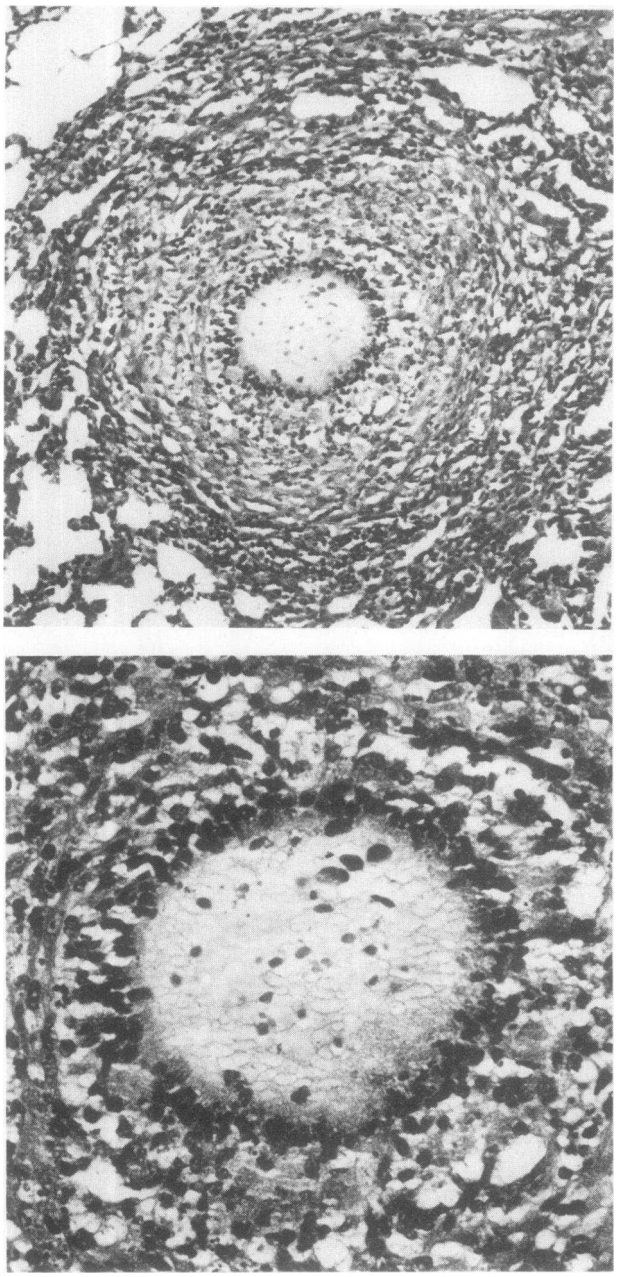

FIGURE 1 Guinea pig lung 4 wk after intratracheal instillation of agar beads containing *P. aeruginosa*. Tissues stained with hematoxylin-eosin and magnified to ×125 (A) and ×312 (B). Note agar bead layered and invaginated with polymorphonuclear leukocytes. Lymphocyte-macrophage inflammatory reaction is seen in more peripheral surrounding tissues.

only 2 wk of Pseudomonas bronchopneumonia (Table III). The PHA titers after 2 wk of pneumonia were significantly higher than those titers produced by 2 wk of LPS vaccine exposure ($P < 0.001$) (Tables II and III). In general, the nonvaccinated animals in this study harbored fewer viable Pseudomonas per milliliter of lung tissue than did the nonvaccinated animals in the prophylactic study, and this was unexplained by any phenomenon other than experimental variability. Infected groups of animals that received active LPS immunization during the final 2 wk of their 4-wk infection, had higher PHA titers ($P < 0.02$), and higher numbers of viable Pseudomonas in lungs ($P > 0.10$), than concomitantly infected groups not receiving vaccine (Table III). Tissue histopathology was similar among these groups. It was concluded that active vaccination of animals with established Pseudomonas bronchopneumonia offered no advantage to the host, despite enhanced availability of humoral antibodies.

Additional prophylactic studies. To establish that the beneficial effects of prophylactic vaccination with LPS Pseudomonas antigen in this model would not be limited to a single Pseudomonas isolate or immunotype, further challenge studies were carried out. Groups of animals were again vaccinated over a 2-wk period, followed by experimental infection with either Pseudomonas strain P-1 or strain P-258, in agar beads. An additional study group was prevaccinated with *E. coli* 0111 J-5 mutant vaccine, then infected with Pseudomonas strain P-1. Control groups were infected using isotonic saline. All groups were killed after 4 wk of infection and studied, as before. Significant pulmonary protection was afforded to the Pseudomonas vaccinees against strain P-1 and to a lesser extent, against P-258 pneumonia, as reflected by superior intrapulmonary clearance of viable bacteria and less tissue damage (Table IV). In contrast, the J-5 vaccinees were no better served by prophylactic immunization than

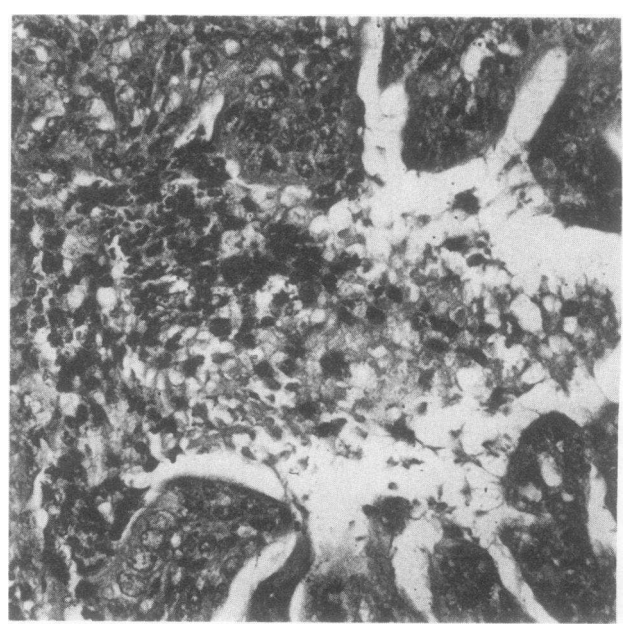

FIGURE 2 Guinea pig lung (hematoxylin-eosin stain, ×312), 4 wk after infection. Note degenerating bead in bronchiolar lumen with local polymorphonuclear leukocyte reaction. Partial obliteration of bronchial wall has occurred.

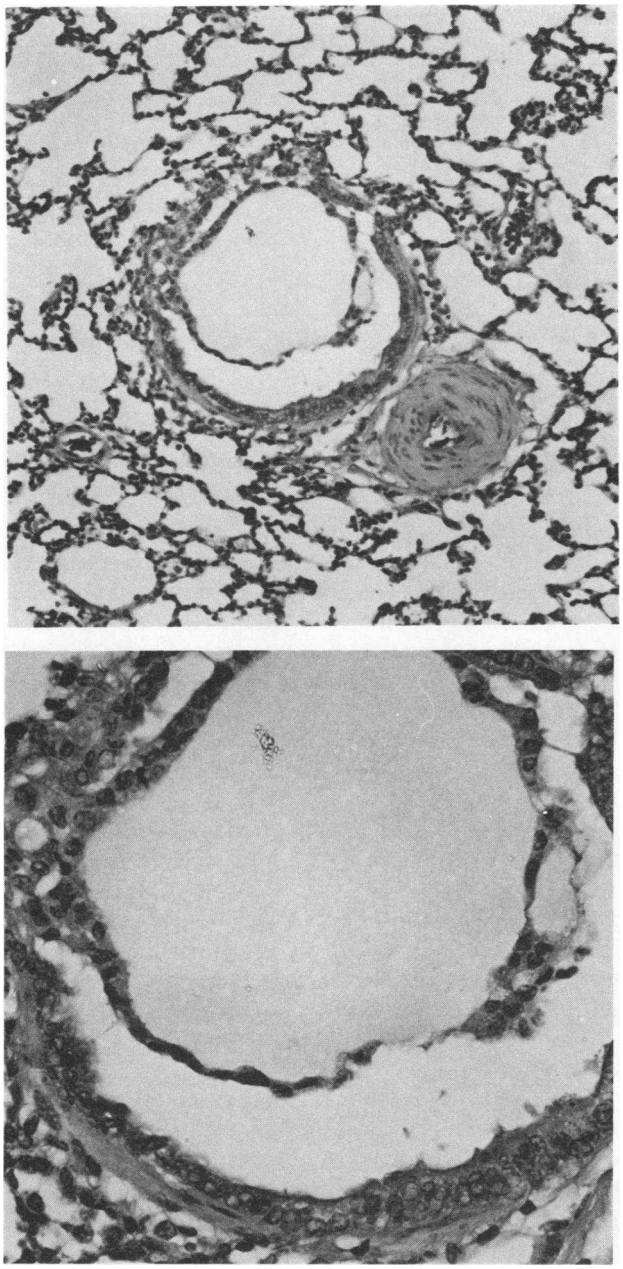

FIGURE 3 Guinea pig lung 4 wk after intratracheal instillation of sterile agar beads. Tissues stained with hematoxylin-eosin and magnified to ×125 (A) and ×312 (B). Note that agar bead in bronchiolar lumen is minimally coated by macrophages and that inflammatory reaction in surrounding lung tissues is sparse. The bronchiolar wall is intact.

the controls, suggesting the necessity for immunospecific vaccination to achieve Pseudomonas protection in the lung.

Circulating immune complexes. Considerable interest has developed in whether chronic Pseudomonas lung infection in CF patients might predispose to immune complex disease (12–15). In this study, sera were analyzed for circulating immune complexes to determine whether Pseudomonas infection and active LPS vaccination might result in their appearance. It was of particular relevance to determine whether vaccination might potentiate the formation of immune complexes in the chronically infected host. Specimens from 14 normal guinea pigs and 4 animals challenged with sterile agar beads were assayed, and in no case were circulating immune complexes detected (range, 2.8 to 10 μg AGG equivalent/ml). Among 50 sera from Pseudomonas strain P-4 infected and/or vaccinated

TABLE II
Immunologic, Bacteriologic, and Histopathologic Response to Prophylactic Pseudomonas Immunization

Study groups*	PHA titer‡	Cfu (×10³) Pseudomonas/ml lung	Histopathology score
Vaccine only (n = 26)	167±1.2 (3)	—	—
Infection only (n = 16)	185±1.3§ (6)	16.8±4.0‖ (6.8–54)¶	17.6±1.9§
Vaccine, then infection (n = 19)	474±1.4§ (34)	2.4±0.6‖ (0.1–4.5)	9.4±0.9§
Sterile agar bead, only (n = 8)	<4	<0.1	2.0

* Vaccine given over 2 wk. Infection or sterile bead challenges, 4 wk before death.
‡ Serum passive hemagglutinating antibodies to Fisher type 4 Pseudomonas antigen, expressed as reciprocal of geometric mean±SEM. (Values in parentheses are titers after 2-mercaptoethanol reduction.)
§ $P < 0.05$, two-tailed Student's t test.
‖ $P < 0.01$, two-tailed Student's t test.
¶ Range.

TABLE III
Immunologic, Bacteriologic, and Histopathologic Response to Pseudomonas Immunization during Established Infection

Study groups*	PHA titer*	Cfu (×10³) Pseudomonas/ml lung	Histopathology score
Infection only, 2 wk (n = 14)	798±1.5 (7)	—	—
Infection only, 4 wk (n = 8)	83±1.3§ (7)	6.6±0.4‖ (1.9–14)¶	18.5±2.1‖
Infection 2 wk, then vaccine (n = 9)‡	322±1.5§ (7)	7.6±2.0‖ (2.3–20)	14.3±2.7‖

* See footnote Table II.
‡ Vaccine given over final 2 wk of 4-wk period of infection.
§ $P < 0.02$, two-tailed Student's t test.
‖ No significant difference, two-tailed Student's t test.
¶ Range.

TABLE IV

Prophylactic Vaccination with Type-Specific and Cross-Protective Vaccines for Chronic Pseudomonas Pneumonia

Study groups	Strain P-1				Strain P-258			
	Number in group	PHA titer*	Cfu‡	Histo-pathology score	Number in group	PHA titer	Cfu	Histo-pathology score
Controls	9	<4	36:0±4.8	14.7±2.1	6	<4	0.65±0.29	9.6±2.8
J-5 vaccine	10	90±1.7	32.4±1.5	13.9±2.9	—	—	—	—
Pseudomonas vaccine	12	120±1.1	11.3±2.7§	5.4±1.0‖	6	112±1.4	0.18±0.11	4.6±1.3

* Serum passive hemagglutinating antibodies to Fisher type 6 (strain P-1) or type 1 (strain P-258) Pseudomonas antigen, or J5 antigen; as reciprocal geometric means±SEM.

‡ Colony forming units ($\times 10^3$) Pseudomonas per milliliter lung; as mean±SEM.

§ Different from control, $P < 0.02$, two-tailed Student's t test.

‖ Different from control, $P < 0.05$, two-tailed Student's t test.

animals, 7 were found to contain immune complexes in significant amounts (range, 42 to 550 μg AGG equivalent/ml). Detectable complexes occasionally resulted from LPS vaccination alone (1/6) or lung infection alone (4/24). There were no circulating immune complexes detected in a group of 10 animals given LPS vaccine, then infected for 4 wk. In 10 animals with an established Pseudomonas pneumonia of 2 wk duration, an additional 2 wk of vaccination resulted in immune complexes in two animals. There was no evidence that active vaccination resulted in any further increase in circulating immune complexes, beyond that normally found among infected animals.

An attempt to correlate the magnitude of Pseudomonas PHA antibody titers with presence of immune complexes was made. Animals with circulating immune complexes had a mean PHA titer of 1,675±3.0, whereas animals without complexes had a mean PHA titer of 226±1.4 ($P < 0.001$, two-tailed Student's t test). Similarly, an attempt to correlate circulating complexes with greater numbers of viable Pseudomonas in lung tissues was made for animals in whom these values were available. Animals with complexes had a mean cfu per milliliter of lung homogenate of 73×10^3, whereas those without complexes had a mean value of 39×10^3. This difference did not reach statistical significance.

DISCUSSION

There has been considerable debate regarding the potential risks and benefits of immunizing CF patients against *P. aeruginosa*. Although rationale can be marshalled both to defend or to refute the use of Pseudomonas immunization among populations of CF patients, there is scant clinical data to support these rationales. The humoral immune system among CF patients appears to respond vigorously to Pseudomonas antigen (5, 8, 11). Additionally, in vitro studies have established that specific Pseudomonas antibodies enhance the bactericidal efficiency of phagocytic cells (10, 32, 33). In certain patient groups, the presence of these antibodies correlates with improved outcome from serious Pseudomonas infections (34). Recent data, in fact, demonstrate increased survival and intrapulmonary killing among actively immunized guinea pigs acutely infected with Pseudomonas (9, 19), indicating that specific pulmonary protection can result from active Pseudomonas vaccination. On the other hand, the single reported trial of LPS Pseudomonas vaccine for CF patients was unable to demonstrate clinical benefit (8). In that study, the 12 vaccine recipients, aged 12 to 30 yr, were chronically infected with Pseudomonas before vaccination and continued to harbor Pseudomonas, thereafter. Thus, there exists a valid concern that chronic Pseudomonas infection of the lung may respond to immune interventions quite differently from acute infections. The present study is designed to address this issue.

CF is exclusively a human disease, and the difficulty in mimicking the clinical problems of CF in an animal model is well known. In this study, the agar bead method for establishing chronic Pseudomonas bronchopulmonary infection in guinea pigs provided a model in which viable Pseudomonas and specific Pseudomonas antibodies could co-exist in the host for a period of weeks. Host immunologic response in this model appeared to resemble that described among CF patients, with particular importance ascribed to the direct relation between extent of Pseudomonas infection in the lung and the magnitude of antibody response. This has clearly been an established finding among the CF population (5–7, 11). The animal model used in this study was, therefore, successful in mimicking the combination of high Pseudomonas antibody levels and poor clearance of infection from the lung.

Two methods for active immunologic intervention in the management of Pseudomonas infection in CF patients must be considered. These are immuno-

prophylaxis and hyperimmunization. The majority of older CF patients have already become infected with Pseudomonas. In this setting, the rationale for use of Pseudomonas vaccine would be to hyperimmunize the patient with an established infection, in hopes of boosting antibody titers to potentially more effective concentrations. In younger CF patients, however, there may exist the opportunity to provide a prophylactic immunization, at a time when destructive Pseudomonas lung infection has not occurred. Established lung infections in CF patients may induce defects in mucociliary clearance, and also in local antibody response in bronchial secretions (8, 35, 36). Thus, early vaccination prior to Pseudomonas infection of the respiratory tract might be particularly valuable. The recent observation in vaccinated guinea pigs that a sudden increase in bronchial inflammation results in a rapid local influx of Pseudomonas-specific opsonic antibodies (10), also suggests that availability of circulating Pseudomonas antibody prior to an initial episode of Pseudomonas bronchitis, might be desirable. Finally, the epidemiologic observations that Pseudomonas infection tends to occur among CF pateints after 5 yr of age (37), and that only 38% of CF patients under age 10 yr have acquired Pseudomonas in their sputum (38), indicate that a number of candidates for active prophylaxis exist. Prior studies have confirmed that children between 4 and 14 yr will respond immunologically to LPS Pseudomonas vaccines (8). The present study indicates that prophylactic immunization of guinea pigs using a LPS Pseudomonas vaccine results in significantly greater intrapulmonary killing of Pseudomonas and less extensive lung damage during a subsequent chronic lung infection. This beneficial effect was observed with three separate challenge strains of Pseudomonas, encompassing the three most frequently encountered Fisher immunotypes (types 1, 4, 6) isolated from CF patients (39). As suggested by previous work (10), pulmonary protection depended upon type-specific Pseudomonas immunization, with no benefit afforded using a cross-protective *E. coli* mutant (J-5) vaccine. In contrast to the prophylactic studies, the usefulness of active Pseudomonas immunization for established Pseudomonas lung infection could not be demonstrated.

The potential for the immune response to Pseudomonas vaccine to result in adverse effects was also considered in this study. Recent work has described circulating immune complexes in 50 to 80% of chronically infected CF patients (14, 15), and it has been suggested that deposition of these immune complexes in the lung may result in hypersensitivity lung disease (12, 13). Of importance in this study was that active vaccination did not appear to further increase the chance of developing circulating immune complexes during lung infection. Thus, while immune complex-induced hypersensitivity lung disease may occur in the CF patient, there was no evidence from this study that prophylactic Pseudomonas vaccination predisposes to this condition.

A final consideration for the use of active immunization among CF patients is whether opsonic antibodies can be effective in the presence of the mucoid coated strains of Pseudomonas so often isolated from these patients. It has been proposed that this extracellular polysaccharide slime may be antiphagocytic (17) and inhibit opsonins (18). We have used nonmucoid strains of Pseudomonas in the present study to more closely duplicate the early phase of lung colonization and infection among CF patients, when the majority of isolates are nonmucoid (4, 6, 16). However, based upon recent studies in our laboratory (40), we would not consider mucoid coating of Pseudomonas to necessarily preclude the effectiveness of opsonic antibodies in the lung.

It would appear from the present findings that a clinical trial of prophylactic Pseudomonas immunization of uninfected CF patients with cell-wall derived Pseudomonas antigens might offer potential benefit. Whether the polyvalent LPS vaccine used in this study, or newer and potentially less toxic cell wall preparations (19, 36), would be best suited for clinical use is unknown. Finally, whether the addition of toxoid materials derived from Pseudomonas exotoxin A or protease (11, 36) might further protect the CF patient should be considered.

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