Allograft Colonization and Infections With Pseudomonas in Cystic Fibrosis Lung Transplant Recipients*

David R. Nunley, MD, FCCP; Wayne Grgurich, BS; Aldo T. Iacono, MD; Samuel Yousem, MD; N. Paul Ohori, MD; Robert J. Keenan, MD; and James H. Dauber, MD, FCCP

Objective: To assess the incidence of pseudomonal infection, colonization, and inflammation in the allograft of lung transplant recipients with cystic fibrosis (CF) as compared with recipients with other end-stage lung disease.

Design: Retrospective review.

Setting: University medical center transplant service.

Patients: All patients with CF and chronic pseudomonal infection (n=62) and patients with nonseptic end-stage lung disease (n=52) receiving a double lung transplant between October 1983 and March 1996.

Results: Fifty lung transplant recipients with CF survived beyond postoperative day (POD) 15 and were subject to sequential bronchoscopy with BAL. Forty-four CF lung transplant recipients had *Pseudomonas* isolated from the allograft by median POD 15 as compared with 21 non-CF lung transplant recipients (p<0.001) with isolation at median POD 158 (p<0.0001). Thirteen CF lung transplant recipients had histologic evidence of infection when *Pseudomonas* was isolated as compared with only three of the non-CF lung transplant recipients (p<0.01). These infections occurred earlier in the CF lung transplant recipients (median POD 10 vs 261) (p<0.01). When compared with non-CF lung transplant recipients, CF lung transplant recipients with *Pseudomonas* isolated but without concomitant histologic infection (colonized) were demonstrated to have increased number of polymorphonuclear cells (PMNs) in the BAL fluid recovered from the allograft (17.66±24.94×10⁶ cells vs 3.46±4.73×10⁶) (p<0.05). Non-CF lung transplant recipients who became colonized with *Pseudomonas* also had a greater number of PMNs recovered when compared with non-CF lung transplant recipients who did not have *Pseudomonas* (22.32±34.00×10⁶ cells vs 0.21±0.18×10⁶) (p<0.01). Nine of 32 (28%) lung transplant recipients with CF have died from pseudomonal allograft infections, but this is no greater than 4 of 21 (19%) deaths related to *Pseudomonas* infection in recipients without CF (p=0.34).

Conclusions: Isolation of *Pseudomonas* from the lung allograft occurs more frequently and earlier after transplantation in recipients with CF. While infections related to *Pseudomonas* also occur more frequently in recipients with CF, there is no increase in mortality. There is an intense inflammatory response in the lung allograft associated with the isolation of *Pseudomonas* in recipients with and without CF.

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Key words: cystic fibrosis; lung transplantation; *Pseudomonas*

Abbreviations: BAL= BAL fluid; CF= cystic fibrosis; LRT= lower respiratory tract; OB= obliterative bronchiolitis; PMN= polymorphonuclear cell; POD= postoperative day

Cystic fibrosis (CF) is an autosomal recessive disease that ultimately leads to death from chronic airway infections culminating in respiratory failure. In the adult with CF, most respiratory tract infections are secondary to species of the bacterium *Pseudomonas*. The median age of survival of individuals with CF is between 25 and 29 years. Because of concern that recurrent infections related to *Pseudomonas* would be potentially lethal in conjunction with systemic immunosuppression, lung transplantation was originally thought not to be an option for these patients. However, after early successes in the mid-1980s, CF has become one of the leading
indications for lung transplantation. Regardless, the concern about infections in the posttransplant period remains. A recent report suggests that CF lung recipients have no more infections complications than lung recipients whose transplants were performed for other diseases. However, our observations have raised suspicion that CF lung allograft recipients tend to have more infectious problems related to Pseudomonas than non-CF lung transplant recipients.

We therefore reviewed the course and outcome of our CF lung recipients since the inception of our program to determine the extent of lung infections and associated morbidity and mortality. Specifically, we examined the incidence of Pseudomonas reemergence in the allograft and its association with histologically identifiable infection. Furthermore, we determined the timing of such infections and their impact on survival. Finally, we determined if the isolation of Pseudomonas from the lower respiratory tract (LRT) is associated with evidence of allograft inflammation.

**Materials and Methods**

Between October 1983 and March 1996, 62 double lung transplant procedures were performed at the University of Pittsburgh for patients with end-stage lung disease from CF. Thirty-one were male, and 31 were female. The average age at the time of transplant was 27 years (median, 26 years). Prior to transplantation, all patients were chronically infected with at least one species of Pseudomonas as identified on sputum cultures, and most harbored other organisms, including Haemophilus, Staphylococcus, and Aspergillus. Patients with multiply resistant Pseudomonas (resistant to all agents in at least two of the following antimicrobial classes: β-lactams, aminoglycosides, and quinolones) were excluded from transplantation during the period 1993 to 1996.

Fifty-two double lung transplant recipients receiving allografts for a variety of indications during the same time period as the CF group were utilized for comparison and designated the comparison group. This group consisted of 21 male and 31 female subjects with the mean age at the time of transplant being 38 years (median, 42 years). The pretransplant diagnosis in this recipient group included the following: Eisenmenger's syndrome (n=15), chronic obstructive lung disease (n=14), primary pulmonary hypertension (n=12), silicosis (n=3), pulmonary arteriovenous malformations (n=2), pulmonary fibrosis (n=2), graft vs host disease (n=1), bronchoalveolar carcinoma (n=1), bronchiolitis obliterans (n=1), and chronic thromboembolic disease (n=1). None of these recipients were known to have had Pseudomonas isolated from their lungs prior to transplant. Recipients receiving allografts for bronchiectasis, dysmotile cilia syndromes, or other septic lung disorders were excluded from the comparison group.

Following transplantation, routine immunosuppression was employed utilizing standard regimens consisting of cyclosporine or tacrolimus with azathioprine and corticosteroids (initially IV methylprednisolone and later oral prednisone). For the first 10 to 14 postoperative days (PODs), all CF lung transplant recipients were empirically treated IV with two antipseudomonal antibiotics. The choice of these antibiotics was individualized and based on antipseudomonal sensitivity testing from preoperative sputum and lung explant cultures. The CF lung transplant recipients were not prophylactically treated with aerosolized antibiotics during the postoperative period. Non-CF lung transplant recipients were treated for the first five PODS with clindamycin and ceftazidime unless preoperative sputum or lung explant cultures suggested organisms that required specific antimicrobial coverage. Surveillance bronchoscopy with transbronchial biopsies was performed routinely at a minimum of every 12 weeks during the first year of follow-up, every 16 weeks during the second year of follow-up, and every 36 to 52 weeks afterwards. As determined by the physician, bronchoscopy was additionally performed for any deterioration in clinical status of the recipient manifested by cough, dyspnea, and/or decline in spirometric values. Biopsy specimens were generally taken from either the left or right lower lobe, but occasionally they were taken from other lobes if radiographs demonstrated anomalies in other areas. All biopsy specimens, which were interpreted by a pathologist skilled in lung transplantation, were graded according to established criteria.

BAL was performed as a routine part of each bronchoscopic procedure. If the recipient was receiving mechanical ventilatory support, the bronchoscope was passed via the endotracheal or tracheostomy tube. If the patient was breathing spontaneously, the bronchoscope was passed either through the nose and nasopharynx or the mouth and oropharynx. One percent lidocaine was used as regional anesthesia of the glottic structures. Suction was not applied to the bronchoscope until the tip was wedged into a subsegmental airway—usually in the right middle lobe or lingula. Two hundred milliliters of sterile saline solution was introduced into the airway in 50-mL aliquots. Suction was then employed, and after each aliquot, as much of the saline solution as could be recovered was aspirated from the airway into a sterile suction trap. Once the saline solution lavage fluid was recovered, this BAL fluid (BALF) was processed by an experienced technician. All recovered BALF, including the first recovered sample, was separated into two aliquots of approximately equal volume. One fraction was decanted directly into a 50-mL conical tube and labeled as “fraction 1.” A sterile gauze pad was placed over a tissue sieve atop a 250-mL beaker, and the remaining BALF was decanted through this filter apparatus. This filtered fluid was then decanted from the beaker into a second 50-mL conical tube and labeled “fraction 2.” Both fractions of BALF were then spun in a centrifuge and the cell pellet recovered. This pellet was resuspended in 2 mL of Hanks' balanced salt solution, and a 40-μL aspirate from fraction 2 was placed in a counting vial with 20 mL of solution (Hematol Isotonic Diluent, Azide Free; Fisher Scientific; Pittsburgh). Using the automated particle counter (Coulter Electronics; Miami) and cytocentrifuge (Cytospin; Shandon Instruments; Pittsburgh), a total cell count and differential count were obtained. The 50-mL conical tube labeled fraction 1 with its resuspended pellet was forwarded to a microbiology laboratory where the fluid was plated onto standard agar plates and incubated between 35 and 37°C. Because quantitative bacterial cultures were not consistently performed on BALF throughout the history of our program, it is not possible to know the concentration of Pseudomonas in the LRT of all recipients. Therefore, any concentration of pseudomonal growth was considered significant.

Since our first CF lung transplant procedure in October 1983, the genus Pseudomonas has undergone several reclassifications. In this retrospective review, the term Pseudomonas will be used to denote the following genera of bacteria: Pseudomonas, Xanthomonas, Stenotrophomonas, and Burkholkeria. Special culture
media for the identification of Burkholderia were not consistently utilized throughout the entire history of the lung transplant program.

Definitions

Infection was defined solely on the bases of allograft histology with positive BALF bacterial cultures. With respect to infection, biopsy material was graded as either pneumonia or bronchitis (neutrophilic or lymphocytic). Pneumonia was characterized by neutrophilic airspace infiltration, whereas bronchitis was characterized by mucosal and submucosal infiltration with either lymphocytes (lymphocytic bronchitis) or neutrophils (neutrophilic bronchitis). Since lymphocytic bronchitis may be associated with both allograft rejection and infection,\(^2\) it was necessary to define a threshold for infection in those recipients. It is generally accepted that BALF from a normal, noninfected lung contains <5% polymorphonuclear cells (PMNs).\(^4\) Therefore, those recipients with histologic lymphocytic bronchitis and positive BALF cultures for Pseudomonas were considered as having bacterial bronchitis if there was an increased number of PMNs (≥10%) in the BALF. Those recipients with positive BALF cultures for Pseudomonas but without any histologic criteria for infection were considered to be simply “colonized.”

Statistical Analysis

Data are expressed as mean (±SD) unless otherwise stated. The \(\chi^2\) and Fisher’s Exact Tests were employed for these comparisons between nominal variables utilizing 2×2 contingency tables. The Mann-Whitney U test was employed for comparison of unpaired data, while the Wilcoxon Signed Rank Test was used to compare all paired data. A significant difference was determined at \(p<0.05\).

RESULTS

Of the 62 recipients with CF, 50 survived >15 days and had at least two bronchoscopic procedures. These recipients comprised the study population and were designated the CF group.

Isolation of Pseudomonas From the Lung Allograft

Of the 50 CF lung transplant recipients subjected to sequential bronchoscopy with BAL, 44 (88%) were found to have Pseudomonas in the LRT on at least two occasions. A recipient was considered to have Pseudomonas in the LRT if any concentration of bacterial growth was identified on BALF cultures. By comparison, Pseudomonas was recovered from the LRT in 21 of 52 (40%) of the comparison group recipients (\(p<0.001,\) Fisher’s Exact Test) (Fig 1). In addition, the time to isolation of Pseudomonas in the CF group was shorter than in the comparison group; median POD 15 (range, 2 to 573) as compared with POD 158 (range, 10 to 1,720) (\(p<0.0001,\) Mann-Whitney U) (Fig 2).

Pseudomonal Infections of the Lung Allograft

At the time Pseudomonas was first isolated from the CF lung transplant recipients, there was no histologic evidence of infection on transbronchial biopsy specimens in 29 of 44 (66%), and thus, Pseudomonas appeared to be merely colonizing the allograft. In the remaining 15 recipients (34%), the first appearance of Pseudomonas was associated with histologic evidence of either bronchitis (neutrophilic or lymphocytic bronchial infiltration) (11 recipients) or pneumonia (neutrophilic airspace infiltration) (4 recipients). Of the 11 CF lung transplant recipients with bronchitis, 6 had histologic lymphocytic bronchitis. Of the six recipients with histologic lymphocytic bronchitis, four were concluded to have bacterial bronchitis based on the finding of ≥10% PMNs (10%, 15%, 30%, and 37%) on the differential cell count of the BALF and a positive bacterial culture. Thus, 9 of the 13 CF lung transplant recipients (69%) with evidence of pseudomonal infections had bronchitis, and the remaining 4 (31%) had pneumonia. In contrast, of the 21 recipients in the comparison group who had Pseudomonas isolated from their LRT, only 3 (14%) had concomitant evidence of histologic infection when the organism was first isolated (all with bronchitis). The incidence of infec-
tion (pneumonia and bronchitis) associated with the first appearance of Pseudomonas (13 in the CF group vs 3 in the comparison group) was therefore four times greater in the CF group than in the comparison group (p<0.01, χ²=14.68).

The discovery of Pseudomonas associated with histologic evidence of LRT infection (pneumonia or bronchitis) occurred earlier after transplantation in the CF group as when compared with the comparison group. The median time to development of Pseudomonas infection in the 13 CF lung transplant recipients was POD 10 (range, 2 to 91) as compared with POD 261 (range, 228 to 592) in the comparison group (p<0.01, Mann-Whitney U).

Of the 29 CF lung transplant recipients who were initially colonized with Pseudomonas (ie, having no histologic evidence of infection during the first isolation of the organism), 20 (69%) later developed histologic evidence of either bronchitis or pneumonia associated with Pseudomonas. In contrast, of the 18 recipients in the comparison group who developed Pseudomonas colonization of the LRT, 8 (44%) later developed Pseudomonas-related infections. This difference was not significant (p=0.13, Fisher’s Exact Test). Of the 44 CF lung transplant recipients who had Pseudomonas sequentially isolated from their LRT, 33 recipients developed histologic evidence of at least one Pseudomonas LRT infection at varying times after transplantation (either with the first occurrence of the organism or later after being initially colonized). Of these 33 recipients, 16 (48%) developed two or more histologic infections with Pseudomonas later in their clinical course.

Pseudomonas With Obliterative Bronchiolitis

Obliterative bronchiolitis (OB), identified histologically as dense eosinophilic fibrous scarring of small airways and recognized as a manifestation of chronic allograft rejection, is highly associated with the finding of Pseudomonas in the LRT.7 Yet, at the time of the first isolation of Pseudomonas from the LRT in the CF group, only 1 of the 44 recipients (2%) had a coexisting or pre-existing diagnosis of histologic OB. In contrast, the incidence of coexisting or pre-existing OB in the comparison group at the time of the first isolation of Pseudomonas was 7 of 21 (33%) (p<0.005, Fisher’s Exact Test). Thus, the CF group usually developed Pseudomonas colonization and/or infection independent of chronic rejection, whereas OB and isolation of Pseudomonas were associated in one third of the comparison group recipients.

Death Related to Pseudomonal Infections

At this time, 32 of the 62 CF lung transplant recipients have died. Nine of these deaths were attributed at least in part to infection with Pseudomonas (Table 1). The incidence of Pseudomonas-related death in the comparison group was 19% (4/21) (p=0.34, Fisher’s Exact Test). The median value for postoperative time until death in the nine CF recipients was 121 days (range, POD 16 to 431; mean, 159±50) compared with 617 days (range, POD 96 to 1,063; mean, 598±513) in the comparison group (p=0.12, Mann-Whitney U). Six of the nine (67%) deaths attributed to Pseudomonas in the CF group occurred within the first 180 days after transplant as compared with one of the four deaths (25%) in the comparison group (χ²=2.36, p=0.35). Lastly, death from all causes was analyzed for the first 180 PODs. In the CF group, there were 20 total deaths, 6 of which were attributed in part to infections with Pseudomonas (30%). In the comparison group, there were 10 deaths during the same postoperative period, only 1 of which was attributed to Pseudomonas infection (10%). Once again, the difference in the incidence of death related to Pseudomonas infection was not significant (p=0.37, Fisher’s Exact Test).

Pseudomonas and Allograft Inflammation

To assess the degree of allograft inflammation in both the CF group and the comparison group in whom there was no histologic evidence of infection, the total number of PMNs recovered from BALF was measured. To ascertain whether the mere presence of Pseudomonas in the allograft in the absence of histologic evidence of infection was associated with an inflammatory response, the CF and comparison group recipients with unequivocal histologic pseudomonal infections were excluded from this analysis. Since PMN effluence from the allograft can

| Table 1—Pseudomonas-Related Deaths in CF Lung Transplant Recipients |
|-------------------|-----------------|--------|
| Patient | Cause of Death | POD |
| 1     | *P. aeruginosa* pneumonia | 16 |
| 2     | *P. aeruginosa* cepacia pneumonia and sepsis | 22 |
| 3     | *P. aeruginosa* and adenovirus pneumonia | 31 |
| 4     | *P. cepacia* and empyema with sepsis | 114 |
| 5     | *P. aeruginosa* and *Alcaligenes xylosoxidans* pneumonia | 121 |
| 6     | *Xanthomonas maltophilia* and *Staphylococcus* pneumonia with rejection | 134 |
| 7     | *Pseudomonas* sp pneumonia | 194 |
| 8     | *P. aeruginosa* and *Providencia* pneumonia with chronic rejection | 374 |
| 9     | *Pseudomonas* pneumonia with acute rejection | 431 |
result not only from infections and acute rejection, but also from reperfusion lung injury and surgical manipulation, it was necessary to control for the elapsed postoperative time when comparing the CF group with the comparison group. Therefore, recipients in the CF group having Pseudomonas isolated from BALF were matched with recipients from the comparison group with respect to POD. Recipients were also matched for age and gender. None of the recipients from the comparison group or the CF group had histologic evidence of infections, diffuse alveolar damage, or acute rejection at the POD utilized for comparison. Utilizing these criteria, 25 recipients in the CF group without infection could be matched with 25 recipients from the comparison group and their BALF PMN counts compared. The mean number of PMNs recovered from the BALF of the CF group was \(17.66 \pm 24.94 \times 10^6\) cells (range, 0 to \(106.73 \times 10^6\)). The mean number of PMNs recovered from BALF in the 25 matched comparison group recipients was \(3.46 \pm 4.73 \times 10^6\) cells (range, 0 to \(19.80 \times 10^6\)), which was significantly less than in the CF group \((p < 0.05, \text{Wilcoxon Signed Rank})\). Thus, CF lung transplant recipients who had Pseudomonas isolated from the LRT in the absence of histologic infection or acute rejection demonstrated heightened allograft inflammation, as measured by PMN effluence, compared with the matched comparison group recipients from whom no Pseudomonas was isolated (Fig 3).

We then identified 10 recipients from the comparison group who later in their clinical course became colonized in the LRT with Pseudomonas. None of these 10 had evidence of histologic infection. The mean number of PMNs recovered from BALF when Pseudomonas was first isolated in this group was \(22.32 \pm 34.00 \times 10^6\) cells (range, 0.25 to \(90.25 \times 10^6\) cells). We compared this degree of effluence with 10 other recipients from the comparison group who never had Pseudomonas isolated from their LRT, again matching these recipients for elapsed postoperative time, age, and gender. In those comparison group recipients who never had Pseudomonas colonization, the mean number of PMNs in BALF at the corresponding postoperative time was \(0.21 \pm 0.18 \times 10^6\) cells (range, 0 to \(0.45 \times 10^6\) cells). The PMN effluence from the LRT in the comparison group recipients who developed Pseudomonas colonization (absence of histologic infection) was therefore significantly greater than those comparison group recipients who never had Pseudomonas isolated \((p < 0.01, \text{Wilcoxon Signed Rank})\). Thus, it would appear that lung recipients who developed pseudomonal colonization of the LRT, whether or not they had CF, had a heightened PMN effluence from the allograft compared with recipients who never developed Pseudomonas colonization (Fig 4).

**DISCUSSION**

In the early and mid-1980s, there was great concern over performing lung transplantation on patients with CF due to the theoretical concern of fatal pseudomonal infections occurring in the presence of systemic immunosuppression. Fortunately, these procedures were performed with enough success that CF now comprises one of the major patient groups benefiting from transplantation. In fact, most major transplant centers show no difference in their survival rates for CF lung transplant recipients and all other lung recipients. However, the debate still continues as to whether pseudomonal infections in this group account for a disproportionate degree of morbidity and mortality. Since lung transplantation for the recipient with CF results in the removal of the diseased lungs and their inherent epithelial cell defect, one would expect that the propensity for pseudomonal colonization and infection would also be eliminated. Indeed, it is known that the potential...
difference across the respiratory epithelium that is abnormal in CF patients and a result of impaired epithelial chloride ion transport is corrected following lung transplantation (G. Kurland, MD; personal communication; 1997). Furthermore, the resulting correction of the sodium chloride concentration in airway surface fluid has been demonstrated to be crucial for effective killing of *Pseudomonas aeruginosa* on both CF and normal respiratory epithelium.9 Coinciding with these expectations, a recent publication suggests that CF lung transplant recipients experience no greater infectious complications than other lung recipients using clinical and radiographic criteria.3 Our results suggest, however, that morbidity from pseudomonal infection in patients undergoing transplantation for CF is greater than expected.

Follow-up bronchoscopic examinations revealed *Pseudomonas* in at least two BALF cultures in 88% of our surviving CF lung transplant recipients compared with only 40% in the comparison group. Furthermore, the elapsed postoperative time to isolation of *Pseudomonas* was significantly shorter in the CF lung transplant recipients than in the comparison group. This may not be unexpected if one concludes that the CF lung transplant recipient continues to harbor *Pseudomonas* in the native trachea and sinuses, thus providing a reservoir of organisms that eventually find their way into the LRT. Due to the effects of gravity and an impaired cough reflex, secretions laden with *Pseudomonas* may descend from the upper airway into the LRT and not be quickly cleared, allowing early colonization of the allograft by *Pseudomonas*. Furthermore, studies of the bronchial secretions from native CF airways have demonstrated fragmented IgG and IgA antibodies,1011 the cleavage of which have further been shown to be secondary to elastases synthesized and released by the *Pseudomonas* organism.12 Thus, early colonization of the allograft by *Pseudomonas* may impair host humoral responses allowing for the continuance of the organism in the airway. These mechanisms may also explain why all of our CF lung transplant recipients except one had repetitive isolation of *Pseudomonas* in the absence of OB. OB has not been described prior to POD 60,13 yet by this postoperative date, most of our CF lung transplant recipients already had *Pseudomonas* consistently isolated via BAL from their LRT. Most of the comparison group, however, did not have *Pseudomonas* isolated from their airway until well beyond POD 60. None of the CF patients had “prophylactic” sinus surgery performed prior to transplantation that could have enhanced drainage of secretions containing *Pseudomonas* into the LRT.

Contamination of the bronchoscope as it passes through the upper airway of the CF lung transplant recipient may have artificially increased the isolation of *Pseudomonas*. Although the specificity of bronchoscopically obtained cultures has been debated, and the use of “protected” catheters as an adjunct for increasing the sampling yield of the distal airway has been proposed,14 we believe that these BALF cultures reflect bacterial burden in the LRT for several reasons. First, we did not suction through the bronchoscope until it was placed in the “wedge” position in the allograft. Exclusion of the first recovered aliquot, as is done in some transplant programs, may have provided further protection against contamination arising from the upper airway, but this practice is debatable and not routinely done at our institution. Second, the large number of neutrophils recovered in the BALF would not be expected to arise from the bronchoscope merely passing through the nasopharynx without suctioning the secretions into the channel of the scope. Third, the use of topical lidocaine, considered to be bacteriostatic, in the upper airway, may diminish the accumulation of bacteria on the tip of the bronchoscope. Finally, if the finding of *Pseudomonas* was related to upper airway contamination, then one would expect to find it in all of the CF lung transplant recipients since they all had chronic pseudomonal infection prior to transplantation. This was not the case, as six of our CF lung transplant recipients never had *Pseudomonas* isolated from their LRT. Having accurate colony counts of bacteria cultured from the BALF may have further assisted in discerning infection from contamination. While it has generally been accepted that cultured BALF resulting in 10⁴ cfu defines significant bacterial growth,15 recent investigations have suggested a poor correlation between histologic evidence of pneumonia and quantitative bacterial cultures.1617

Histologic and BAL evidence of either bacterial bronchitis or pneumonia occurred in 13 of 44 recipients in the CF group during the first isolation of *Pseudomonas* and was significantly greater and earlier after transplantation when contrasted with the comparison group. It should be noted that four of our six recipients with lymphocytic bronchitis were concluded to have bacterial bronchitis based on positive BALF cultures for *Pseudomonas* and ≥10% PMNs in the BALF. It is possible that this definition overestimates the actual incidence of infection and rather denotes only those recipients with allograft rejection and coinciding colonization. This distinction is difficult not only on histologic criteria, but also on clinical criteria as well, since recipients with rejection often have similar symptoms as those with infection—ie, productive cough, dyspnea, and declining spirometric values18—and indeed, the two may coexist. However, these four recipients were
concluded to have biopsy specimens most consistent with infection based on an exaggerated number of PMNs in the BALF (10%, 15%, 30%, and 37%) and no other histologic features (i.e., perivascular lymphocytic infiltration) suggestive of more than mild acute cellular radiation. Radiographic data may have provided ancillary evidence of LRT infection in some cases. However, radiographs may not show changes indicating bronchitis, and therefore, they were not utilized for making this diagnosis. With respect to the diagnosis of pneumonia, all four cases of Pseudomonas pneumonia in the CF group occurred very early after transplantation (PODs 2, 4, 6, and 8) when the finding of radiographic infiltrates is very common. Early after this surgery, infiltrates may be secondary to infection, acute rejection, reperfusion injury, or a combination of these. Thus, radiographs were not likely to be helpful in discriminating between pneumonia and other abnormalities.

The fact that the four diagnoses of pneumonia in the CF group occurred so early after transplantation is particularly concerning in light of the nine CF lung transplant recipient deaths in our program related to pseudomonal infections. Six of these nine Pseudomonas-related deaths occurred within the first 180 days following transplant as opposed to only one of four Pseudomonas-related deaths in the comparison group. Thus, it would appear that the allograft is particularly vulnerable to early lethal pseudomonal infections possibly initiated by reperfusion or preservation injury or even high initial levels of immunosuppression. Presumably, the native trachea and sinususes of the CF lung transplant recipient provide a rich inoculum of Pseudomonas for colonization and subsequent infection of the LRT. Even if the first appearance of Pseudomonas in the CF lung transplant recipient is not associated with infection, our series suggests that after the initial colonization, 60% will go on to develop a later pseudomonal LRT infection, whereas only 44% of non-CF lung transplant recipients will do so. These differences were not statistically significant, but they may be subject to a type II error secondary to the small sample sizes. Regardless, the trend for subsequent pseudomonal infections in CF lung transplant recipients is troublesome and suggests that the issue warrants continued monitoring.

The CF lung transplant recipients who had Pseudomonas isolated from their LRT in the absence of histologic evidence of infection were also shown to have a significantly greater influx of PMNs into the allograft as compared with the matched non-CF comparison group recipients. Interestingly, those comparison group recipients who later developed pseudomonal colonization also had greater degrees of PMN efflence when compared with the other comparison group recipients without colonization. Thus, it would appear that the presence of Pseudomonas in the LRT is associated with a heightened inflammatory response in both CF and non-CF lung transplant recipients, but CF lung transplant recipients tend to become colonized and develop an inflammatory response much earlier than their non-CF counterparts. Indeed, it has been shown in children with native CF that LRT inflammation is present in association with bacterial pathogens isolated by BAL despite normal lung function and states of well being. From this observation, the argument has been rightfully made that the native CF lung is in a constant state of infection and inflammation and that the term “colonization” should be abandoned when referring to native CF lung disease. Utilizing this premise, it is therefore possible that those CF and non-CF lung transplant recipients in whom Pseudomonas is isolated from the LRT are already in a perpetual state of infection despite the findings on the biopsy tissue. It is possible that those recipients who we categorize as “colonized” with Pseudomonas all have infection that was simply missed on the biopsy specimen.

Colonization of the native CF lung with Pseudomonas and its subsequent binding with respiratory epithelium provokes a vigorous immune response with increased production of many cytokines, including interleukin-8, a potent neutrophil chemoattractant. The intense infiltration of neutrophils and release of neutrophil mediators such as elastase are thought to be of prime importance in the progressive destruction of bronchial structures and lung matrix that characterizes the progressive destruction of the native CF lung, and efforts are now being employed to quell this inflammatory response in the routine care of CF patients. Although the consequences of neutrophil infiltration into the transplanted lung are not clear, the injurious effect of PMN infiltration in chronic lung disorders is well recognized. In vitro studies have demonstrated that elastases derived from PMNs can degrade the C1 and C3 components of complement and may result in the inactivation of the chemotactic activities of this molecule resulting in ineffective opsonic phagocytosis of Pseudomonas. Furthermore, the C3b receptor on PMNs recovered from BALF in CF patients has been shown to be significantly reduced by both PMN and Pseudomonas-derived elastases. Both of these mechanisms appear to be active in patients with native CF, and they clearly contribute to the failure to eradicate the Pseudomonas organism and promote chronic inflammation.

While acute allograft rejection requiring enhanced immunosuppression therapy may also play an important role in predisposing CF allograft recipients to
infection, most reported series have not demonstrated a greater degree of acute or chronic allograft rejection in CF lung recipients. However, a recent report has suggested an association between OB or refractory acute rejection with pseudomonal infection and high PMN influx with liberation of sufficient quantities of neutrophil elastase to overcome antielastase defenses. Thus, it may be beneficial to prevent or delay pseudomonal colonization of the allograft in these recipients.

It is therefore reasonable to speculate that a scenario not unlike native CF lung disease develops in the CF lung transplant recipient where Pseudomonas first infiltrates and colonizes the airway, ultimately binding to respiratory epithelium. In native CF disease, this binding is thought to be initiated by airway epithelial damage that occurs, in part, from a dysfunctional regulatory membrane protein (CF transmembrane regulator). In lung transplant recipients, early epithelial damage may occur from preservation injury, reperfusion injury, or cellular rejection. Regardless of the cause, Pseudomonas from the native airway may have an opportunity to bind to damaged respiratory epithelium of the allograft, initiating an inflammatory response with resulting impairment of humoral and complement-mediated host immunity and with potential consequences of long-term graft survival.

Given the association between colonization and subsequent infection with Pseudomonas in CF lung transplant recipients, it is possible that prevention or suppression of colonization will reduce morbidity in CF lung transplant recipients. It has been proposed that preoperative sinus surgery and drainage may reduce the burden of Pseudomonas and should be employed routinely in candidates for CF lung transplants. There is continuing debate over the validity of this practice. The use of aerosolized aminoglycoside antibiotics has been shown to reduce the number of colony forming units in the native airways of patients with CF. Also, the use of inhaled colistin (polymixin E) has been shown to delay LRT colonization with Pseudomonas in children with CF. Considering the early colonization, infections, and morbidity associated with Pseudomonas in CF lung transplant recipients, the use of prophylactic aerosolized antibiotics may be one potential strategy allowing for reduced pseudomonal colonization of the allograft in the susceptible early postoperative period.

In summary, we report our experience with 62 CF lung transplant recipients, all of whom were colonized preoperatively with at least one species of Pseudomonas. Pseudomonas colonization of the allograft occurred in most of these recipients, particularly in the early postoperative period. The initial isolation of Pseudomonas was associated with evidence of histologic infection in one third of the cases. Not only do pseudomonal infections occur more frequently in CF lung transplant recipients than in non-CF lung transplant recipients, the propensity for these infections to occur early after transplant appears to cause enhanced morbidity and risk of mortality. Even in CF lung transplant recipients lacking evidence of overt infection with the first isolation of Pseudomonas, there was a tendency toward developing later pseudomonal infections (although this was not significantly different from the comparison group). When Pseudomonas was isolated from both CF and non-CF lung transplant recipients in the absence of any histologically identifiable infection, there was an associated influx of PMNs suggesting a state of heightened allograft inflammation. We speculate that mediators released by these neutrophils may damage the allograft and subsequently affect graft function. The use of prophylactic measures, such as inhaled antibiotics, in an attempt to delay or obviate allograft colonization, warrants further study.

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