Cystic Fibrosis (CF) is an autosomal recessive disease characterized by malnutrition due to pancreatic insufficiency and chronic obstructive pulmonary disease with recurrent respiratory tract infections. In most patients, the characteristic lung disease eventually becomes the predominant problem. Studies of the epidemiology of Pseudomonas aeruginosa in CF have demonstrated the prevalence of this respiratory pathogen and have provided some insights regarding the timing and risk factors in P. aeruginosa-associated pulmonary infections. Previous research on the acquisition of P. aeruginosa-associated pulmonary disease in patients with CF has been cross-sectional in design, did not evaluate the antibody response to P. aeruginosa, or concentrated on older patients. With the ability to diagnose CF by genetic testing at birth, it is now possible to determine the temporal sequence of events that result in P. aeruginosa–associated pulmonary infections. More precise and reliable

Objectives

To evaluate the longitudinal relationship between the production of an antibody response against P. aeruginosa and clinical factors associated with P. aeruginosa pulmonary infections in patients with CF diagnosed in early life.

Design, Setting, and Patients

Serum samples and oropharyngeal cultures (protocol cultures) were obtained at 6-month intervals from April 15, 1985, to April 15, 2000 (or for up to 180 months depending on their enrollment date) from 68 patients at 2 centers in Madison and Milwaukee, Wis, diagnosed through the Wisconsin CF Neonatal Screening Project, a longitudinal cohort study. Additional cultures were obtained at examining physicians’ discretion (all cultures).

Main Outcome Measures

Time to serum IgG, IgA, and IgM antibody titer of at least 1:256 against P. aeruginosa, assessed by enzyme-linked immunosorbent assay using cell lysate, exotoxin A, and elastase as antigens; time to organism isolation from respiratory samples; time to Wisconsin Cystic Fibrosis Radiograph (WCXR) score of 5 or more.

Results

The median time to an antibody titer of at least 1:256 was 17.8, 24.2, and 70.9 months for cell lysate, exotoxin A, and elastase, respectively. The rise of anti–cell lysate and anti–exotoxin A titer to 1:256 or more occurred a mean of 11.9 (P=.001) and 5.6 (P=.006) months, respectively, before the isolation of P. aeruginosa for all cultures and 18.2 (P=.06) and 11.9 (P=.01) months, respectively, before protocol cultures. There was no significant difference between the rise of anti–cell lysate and anti–exotoxin A titer and a WCXR score of 5 or more (P=.24 and .32, respectively). Treatment with long-term, non-Pseudomonas oral antibiotics and integration of CF infants with older, chronically infected patients were associated with a significantly increased risk of P. aeruginosa pulmonary infection.

Conclusions

In CF patients diagnosed through neonatal screening, P. aeruginosa pulmonary infections occurred 6 to 12 months before the organism was isolated from respiratory secretions. The longitudinal monitoring of P. aeruginosa antibody titers, in concert with WCXR score, should facilitate diagnosis and treatment of P. aeruginosa pulmonary infections in young children with CF.
knowledge of these events could be valuable for the development of optimal therapeutic regimens for the treatment of these infections, which account for much of the morbidity associated with CF. Even though drugs are now available for treatment and potential eradication, this approach has been successful in preventing the malnutrition associated with CF.

As part of the Wisconsin CF Neonatal Screening Project, we have studied the longitudinal relationship between clinical indicators of P aeruginosa—associated pulmonary infection, the detection of P aeruginosa by oropharyngeal cultures, and the production of an antibody response in 68 patients who were diagnosed with CF through newborn screening. Our investigation differs from other longitudinal studies correlating the acquisition of P aeruginosa with the production of an antibody response against this organism in that our patients were young children who were free of pulmonary disease at the time of diagnosis with CF through newborn screening. Thus, in our study, a rise in serum antibodies against specific P aeruginosa antigens should be indicative of the initial infection. Our patients were also well nourished throughout the assessment period because of the early nutritional assessment and supplementation. In addition we characterized the antibody response using a statistical model. Using these methods, we have been able to extend our previous assessment of risk factors associated with acquisition of P aeruginosa infections in CF.

**METHODS**

**Study Participants**

The Wisconsin CF Neonatal Screening Project is a longitudinal investigation designed to assess the potential benefits and risks of newborn screening for CF and is described in detail elsewhere. The 2 CF centers and their treatment regimens were described previously. This study consisted of 63 patients diagnosed with CF before age 12 weeks and 5 patients who were diagnosed at ages 13.0, 13.3, 14.4, 20.7, and 280.9 weeks; 13 patients had meconium ileus. To screen for CF, an immunotryptsinogen assay was used from April 15, 1985, to June 30, 1991, and used in combination with DNA analysis for the AF508 CFTR mutation from July 1, 1991, to June 30, 1994. The presumptive diagnosis of CF was confirmed with a positive sweat test revealing a chloride level at or above 60 mEq/L. After consent for participation was obtained from their parents, patients were enrolled at either the Madison or Milwaukee CF Centers (referred to as centers A and B, respectively), and were followed up in an evaluation and treatment protocol that prevented malnutrition. The Wisconsin CF Neonatal Screening Project was approved by the Human Subjects Committee at the University of Wisconsin-Madison and the Research and Publications Committee/Human Rights Board at Children’s Hospital of Wisconsin, Milwaukee.

**Samples of Oropharyngeal Secretions**

Samples of oropharyngeal secretions were obtained and cultured for P aeruginosa every 6 months from April 15, 1985, to April 15, 2000, as part of the longitudinal evaluation protocol. Additional samples were obtained as needed at the request of the examining physician. For each infant or young child who could not cough on instruction, a research nurse depressed the child’s tongue and aggressively swabbed the oropharynx (swab: BD Culturette collection and transport system, Becton Dickinson and Co, Franklin Lakes, NJ) until the child gagged. For children who could cough on instruction, the child was asked to cough and the nurse vigorously swabbed the oropharynx until the child gagged. Expectorated sputum samples (<10% of the specimens) were obtained from patients who could produce such samples. Bacteriological culture methods were similar at the 2 centers. The amounts of P aeruginosa recovered on the initial isolation plates were categorized: x, no colonies; 0, a few colonies to light growth; 1, moderate growth; 2, heavy growth. These groups reflect the approximate number of organisms present in the oropharyngeal or sputum samples and vary by orders of magnitude.

**Quantitation of Anti-Pseudomonas Antibodies**

Serum specimens were obtained at intervals of approximately 6 months, were stored at −80°C, and were then analyzed as coded samples, thus keeping the laboratory workers blinded to the clinical identification. A total of 2134 antibody titers were determined. An antibody capture immunoassay with antigen excess was used to determine the levels of anti-Pseudomonas antibodies. The antigens used were a cell lysate derived from P aeruginosa strains PAO1, FRD1, and UWP100 (isolated from a patient with CF at the University of Wisconsin Hospital and Clinics), purified exotoxin A (List Biological Laboratories Inc, Campbell, Calif), and an elastase toxoid prepared from purified elastase (Nagase and Co Ltd, Tokyo, Japan).

The antigens were diluted in 100 mM of sodium carbonate and 0.02% sodium azide, pH 9.6, for a final protein concentration of 100 ng/well and bound to Costar flat-bottom, high-binding polystyrene enzyme immunosorbent assay/radioimmunosorbent assay/well microtiter plates (Corning Inc, Corning, NY) at 37°C for 2 hours. The plates were washed 3 times in BBS-T (100 mM of boric acid, 150 mM of NaCl, and 0.05% Tween 20, pH 8.5). To saturate excess antigen-binding sites, the plates were blocked with 1% fish gelatin in BBS-T for 16 hours at room temperature. Twofold dilutions (1:4 to 1:8192) of the patient sera in 1% fish gelatin in BBS-T were incubated in the blocked plates for 2 hours at 37°C. The plates were then washed 3 times in BBS-T. Alkaline phosphatase–conjugated ImmunoPure goat anti–human IgG + IgA + IgM (H+L) (Pierce Chemical Co, Rockford, Ill) was used as the secondary antibody and P-nitrophenylphosphate (Pierce) in 10 mM of diethanolamine and 0.5 mM of MgCl₂, pH 9.5, was used as the sub-
strate for alkaline phosphatase. The degree of color development was read at 405 nM using an EL312 Microplate reader and was analyzed using KC3 software (Bio-Tek Instruments, Winooski, Vt).

Two-fold dilutions of sera from an adult male without CF (a physician with 30 years of exposure to P aeruginosa through pediatric pulmonology practice) were included on each plate as a positive control. To control for interfering color that may be present in the lower dilutions of sera, 2-fold dilutions (1:4 to 1:512) of pooled normal human serum from more than 50 Midwestern adults (Sigma Chemical Co, St Louis, Mo) in the absence of antigen were included on each plate. Controls lacking either patient serum or the secondary antibody were also included on each plate. The anti-cell lysate, anti-exotoxin A, or anti-elastase titer was determined as the highest dilution in which the OD405 (optical density) reading was at least 0.2 units. To eliminate any interference due to the color of serum at the lower dilutions, the OD405 reading also had to be 0.1 units above the corresponding dilution of pooled normal human serum in the absence of antigen. For the statistical and graphical analyses, the titer values were expressed as log2.

Clinical Indicators of P aeruginosa–Associated Pulmonary Infections

Chest radiographs were obtained at 6-month intervals up to age 3 years and once per year for children aged 4 years and older, as previously described, and were scored using the Wisconsin Cystic Fibrosis Radiograph (WCXR) Scoring system. This system provides a sensitive, objective method for longitudinal and quantitative chest radiology in children with CF. Our analyses show that, with this scoring system, very mild, reversible abnormalities are distinguished as scores less than 5. Additional radiographs were obtained at the discretion of the patient’s physician. Data on the presence and magnitude of cough, other signs of respiratory infection, and the use of antibiotics were obtained at all protocol visits; oral antibiotics given routinely included trimethoprim-sulfamethoxazole and cephalosporins. Cough severity was assessed by parents and reported to investigators at visits according to 4 categories: 0, no cough; 1, rare cough; 2, cough in the morning or with postural drainage; or 3, frequent (>10 times per day), productive, or paroxysmal cough.

Statistical Analysis

To characterize the shape and timing of the antibody response directed against P aeruginosa, we used least-squares isotonic regressions to fit a monotone increasing, decreasing, or flat curve to the titer level as a function of age. The significance of the nonflatness of each curve was assessed by comparing the R² value based on a non-parametric bootstrap under the null hypothesis of flatness and assuming Gaussian errors. We then used an exact binomial test to evaluate whether the number of increasing or decreasing curves was significantly greater than what would be expected under the flat null hypothesis. We then performed a piecewise linear regression of the antibody titers (cell lysate, exotoxin A, and elastase) on age for each patient. The regression function was constrained to be nondecreasing and to consist of at most 3 phases: an initial plateau, a linear rise, and a final plateau. The number of phases and the boundary points between the phases were not determined a priori but were estimated from the data. This estimation was accomplished by converting the regression model into a linear complementarity problem and solving using the Lemke method, which is a pivoting algorithm similar to the Simplex algorithm for linear programming.

To determine the longitudinal relationships between P aeruginosa infection and antibody response, the distribution of the times to first antibody titer 1:256 or higher (cell lysate, exotoxin A, and elastase), time to first culture positive for P aeruginosa (with infection severity of 0, 1, or 2), and time to first significant clinical event (coughing, WCXR score ≥5) were summarized with Kaplan-Meier estimates of the cumulative distribution function to account for right-censoring of the data. Comparisons between the time-to-event distributions were made with a paired log-rank test using a robust variance estimate since many individuals typically experienced both events being compared.

To assess the association between antibody response and patient characteristics and risk factors, the effects of risk factors on the time when antibody titers (cell lysate, exotoxin A, and elastase) were first 1:256 or greater was evaluated with the Andersen-Gill proportional hazards model to account for baseline and time-dependent covariates. Eleven baseline covariates (treatment received at CF centers A or B, population per square mile according to ZIP code, mother’s education in years beyond high school, genotype [homozygous AF508 vs other genotypes and heterozygous AF508 vs other genotypes], estimated median annual income [in units of $10000] based on ZIP code of parents’ residence, marital status of mother, meconium ileus status, definite or probable pancreatic sufficiency vs definite or probable pancreatic insufficiency, sex, and residence in an urbanized area) were considered in this model. Also considered were 8 time-dependent covariates (use or nonuse of long-term [>30 days] non-Pseudomonas oral antibiotics, total number of days hospitalized, total duration of infections in days, total count of antibiotic prescriptions for infections, first-degree relatives with CF [parents and siblings] vs no first-degree relatives with CF, total number of upper respiratory tract [presumably viral] infections, treatment at center B older hospital prior to June 1, 1990, and treatment by the single physician at center B prior to June 1, 1990).

The effect of life history covariates on the time to the first titer of 1:256 or greater was assumed to occur in the interval between the first titer of 1:256 or greater and the most recent prior titer.
less than 1:256. A step-down regression selection procedure was performed beginning with a full model consisting of all potentially significant covariates. The least significant covariates were removed 1 at a time until the remaining covariates were significant at the .10 level. The proportionality assumption for the Andersen-Gill model was assessed by looking at the smoothed plot of the Martingale residuals vs time. For these statistical analyses we used SAS version 6.12 (SAS Institute, Cary, NC) and S-Plus version 3.4 (Mathsoft Inc, Seattle, Wash) software packages.

RESULTS

Patient Characteristics

TABLE 1 summarizes the baseline characteristics of the study population. When the presumptive diagnosis of CF was confirmed with a positive sweat test result, the median age at diagnosis was 6.8 weeks. Four patients had a false-negative newborn screening result and were diagnosed at ages 6.9, 7.9, 20.7, and 280.9 weeks on the basis of family history or clinical symptoms. Chest radiographs were obtained for 57 patients within 1 week of diagnosis; 30% of these had WCXR scores of less than 2.0 (indicating a normal appearance or minimal changes), 40% had a score of 2.0 to less than 5.0 (indicating reversible, mild abnormalities), and 30% had a score of 5.0 or greater (indicating irreversible lung damage). For all patients, the initial chest radiographs showed median and mean (SD) WCXR scores of 3.36 and 4.32 (3.7), respectively. These scores indicate that the majority of patients had no irreversible lung damage when they entered the study. None of the patients received antimicrobial agents specific for *P aeruginosa* (ie, ciprofloxacin, parenteral anti-*Pseudomonas* drugs, or aerosolized tobramycin) before the organism was isolated from an oropharyngeal culture.

Shape and Timing of the Antibody Response

Sufficiently complete data for least-squares isometric complete data were available for 52 (cell lysate), 51 (exotoxin A), and 51 (elastase) patients. The numbers of patients with significantly increasing curves were 41 (cell lysate), 42 (exotoxin A), and 34 (elastase). This number was significantly larger than the 2.5% expected under the flat null hypothesis (*P* < .001 for all 3 antigens). In contrast, the numbers of patients with significantly decreasing curves were 3 (cell lysate), 3 (exotoxin A), and 2 (elastase). None of these numbers was significantly larger than the 2.5% expected under the flat null hypothesis (P = .14 for cell lysate; P = .14 for exotoxin A; and P = .37 for elastase). Thus the curves are significantly monotone increasing. Representative responses are shown in FIGURE 1 and the characteristics of the response for each antigen are given in TABLE 2. Most responses were characterized by an initial plateau, rise, and final plateau (51%, 56%, and 55% of patients for cell lysate, exotoxin A, and elastase, respectively). Distinct differences were noted in the response to the 3 antigens. The midpoint of the responses to the cell lysate preparation and exotoxin A occurred approximately 10 months before the midpoint of the elastase response and 11 to 14 months, respectively, before the beginning of the final plateau of the elastase response. The magnitude of the cell lysate and exotoxin A responses was approximately 2 doubling dilutions greater than the response to elastase. In 5 patients, the cell lysate and exotoxin A titers rose to 1:256 or greater before age 9 months; however, this response was not due to the presence of maternal antibody against *P aeruginosa*, because each of these patients had earlier titers ranging from 1:8 to 1:32.

Longitudinal Relationships of Indicators of *P aeruginosa*-Associated Pulmonary Infections

To assess the longitudinal relationship of the different events associated with acquisition of *P aeruginosa*-associated pulmonary infections, we plotted the percentage of patients with a specific event vs the time of first acquisition of that event using Kaplan-Meier analysis (FIGURE 2). All 68 were used in each Kaplan-Meier plot. We studied as potential early indicators the first report of significant cough (category ≥2) during the day as reported by the parents, the first pulmonary infection or course of antibiotics, a WCXR score of 5 or greater, the first report of a significant antibody response against the cell lysate, exotoxin A, and elastase antigens, and the first isolation of *P aeruginosa* from an oropharyngeal culture. A category 2 cough was defined as cough occurring in the morning or with postural drainage. A significant antibody response was defined as a titer of 1:256 or greater and was considered indicative of an infection caused by *P aeruginosa*; for the majority of our patients this represented a 64-fold increase.
crease in titer over the initial titer obtained at the time of CF diagnosis.

The median time to a cough of category 2 or greater was 4.33 months and for a WCXR score greater than or equal to 5 was 24.02 months. The median times to a titer of 1:256 or greater were 17.8, 24.2, and 70.9 months for cell lysate, exotoxin A, and elastase, respectively. The median time to the first colony (magnitude categories ≥0, ≥1, or ≥2) was 29.8, 101.6, and 132.7 months, respectively, for all cultures and 36.3, 102, and 135 months, respectively, for the protocol-only cultures. One culture-positive patient did not develop a titer of 1:256 or greater to cell lysate, 3 did not develop a titer of 1:256 or greater to exotoxin A, and 11 did not develop a titer of 1:256 or greater to elastase. Sixteen patients remained culture negative throughout the study but titers of 1:256 or greater were detected for cell lysate in 14 of these patients, for exotoxin A in 11 patients, and for elastase in 7 patients.

As shown in Figure 3, a significant difference was observed between the first detection of a titer of 1:256 or greater for 
$P. aeruginosa$ antigens and the first isolation of the organism from an oropharyngeal culture at a magnitude of 0 or greater (all cultures). Titers to cell lysate and exotoxin A were detected a mean of 11.9 and 5.6 months before the first isolation of $P. aeruginosa$ ($P<.001$ and $P=.04$, respectively). In contrast, the titer to elastase was detected a mean of 41.1 months after the first isolation of the organism ($P=.003$). For the protocol-only cultures, the titers to cell lysate and exotoxin A were detected 18.2 and 11.9 months, respectively, before the first isolation of the organism ($P<.001$ for cell lysate and $P=.006$ for exotoxin A). The rise of anti–cell lysate and anti–exotoxin A titers occurred before, or coincided with, the first isolation of $P. aeruginosa$ in 63% and 54% of the patients, respectively.

We also compared the difference in timing of a WCXR score greater than or equal to 5 and the first detection of a titer of 1:256 or higher for cell lysate and exotoxin A and the first isolation of $P. aeruginosa$. There was no significant difference between these outcomes for cell lysate and exotoxin A ($P=.24$ and $P=.32$, respectively). However, like the detection of a titer of 1:256 or higher for cell lysate and exotoxin A, the WCXR score greater than or equal to 5 first occurred 3.8 months before the first isolation of $P. aeruginosa$ ($P<.001$). These observations indicate that radiographic evidence of irreversible lung damage occurred before $P. aeruginosa$ was first isolated by culture and that the radiographic detection of lung pathology coincided in time with the production of an antibody response directed against cell lysate and exotoxin A.

**Figure 1.** Representative Monotone Increasing Curves of the Antibody Response

The y-axis scale shows the magnitude of the antibody response expressed as the log 2 of the negative reciprocal of the titer. Open circles indicate titer values; lines indicate the fitted curves.
revealed that treatment at an integrated CF clinic and increased use of aerosols were significant risks while the maternal level of education provided a beneficial effect.

For our assessment of risk factors associated with production of anti–P aeruginosa antibodies, we used life history information to evaluate the relationship between the development of an antibody titer greater than or equal to 1:256 and the baseline characteristics (eg, genotype) and longitudinal health care experience of the study patients. This analysis was limited to development of cell lysate and exotoxin A titers greater than or equal to 1:256 because these titers correlated well with clinical indicators of pulmonary disease.

The final results from a stepdown regression model selection are presented in Table 3. Martingale residual plots indicated that the proportionality assumption was valid. Patients followed up in center B had a significantly higher risk of developing titers greater than or equal to 1:256 for cell lysate and exotoxin A than patients followed up at center A. Long-term (>30 days) use of oral non-Pseudomonas antibiotics was also associated with a higher risk of developing titers greater than or equal to 1:256 for cell lysate and exotoxin A. We also found that as the parents’ income increased, the risk of developing an exotoxin A titer greater than or equal to 1:256 decreased; this beneficial effect was not associated with developing a titer greater than or equal to 1:256 against cell lysate. The apparently mild decreased risk of developing a titer greater than or equal to 1:256 for cell lysate associated with an increased number of viral respiratory tract infections is likely to be a confounding covariate rather than a beneficial risk factor. Similarly, the apparent relationship of cell lysate and exotoxin A titers to “original physician” (1985-1990) in center B may reflect a confounding effect. The other variables that were considered, as listed in the “Statistical Analysis” section, did not show any significant relationships with acquisition of a high titer.

Table 2. Characterization of the Anti-Pseudomonas Antibody Response*  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cell Lysate</th>
<th>Exotoxin A</th>
<th>Elastase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial titer</td>
<td>4.3 (2.7)</td>
<td>2.6 (2.1)</td>
<td>1.8 (1.4)</td>
</tr>
<tr>
<td>Maximum titer attained</td>
<td>10.6 (2.2)</td>
<td>10.1 (1.9)</td>
<td>8.3 (2.4)</td>
</tr>
<tr>
<td>Magnitude of rise in titer</td>
<td>6.2 (2.5)</td>
<td>7.5 (2.3)</td>
<td>6.4 (2.5)</td>
</tr>
<tr>
<td>Slope of the rise in titer</td>
<td>0.5 (0.6)</td>
<td>0.8 (0.8)</td>
<td>0.5 (0.4)</td>
</tr>
<tr>
<td>Age, mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of initial plateau</td>
<td>18.4 (27.0)</td>
<td>20.4 (25.6)</td>
<td>26.7 (27.2)</td>
</tr>
<tr>
<td>Midpoint of rise in titer</td>
<td>30.8 (27.5)</td>
<td>29.4 (26.0)</td>
<td>40.6 (27.1)</td>
</tr>
<tr>
<td>Beginning of final plateau</td>
<td>43.2 (32.3)</td>
<td>38.5 (29.6)</td>
<td>54.6 (31.6)</td>
</tr>
<tr>
<td>Duration of rise in titer, mo</td>
<td>24.8 (22.7)</td>
<td>18.1 (18.7)</td>
<td>28.0 (23.2)</td>
</tr>
</tbody>
</table>

*Titers are expressed as the log of the negative reciprocal. Four or more consecutive serum samples were obtained from 53, 54, and 47 patients for quantification of anti-cell lysate, anti-exotoxin A, and anti-elastase antibodies, respectively.

Figure 2. Kaplan-Meier Survival Analysis Characterizing the First Isolation of Pseudomonas aeruginosa, First Incidence of Clinical Indicators of Infection, and Production of an Antibody Titer

A, Time to first isolation of P aeruginosa from an oropharyngeal culture in all screened patients. B, Time to when serum antibody titer to cell lysate, exotoxin A, and elastase reached 1:256 or greater. C, Time to first occurrence of infection requiring treatment with antibiotics, first parental report of significant cough, and first report of WCXR (Wisconsin Cystic Fibrosis Radiograph) score greater than or equal to 5. Small cross-marks indicate censored events.

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COMMENT
Recognizing the importance of *P aeruginosa* in the morbidity and mortality of CF as well as the new opportunities for presymptomatic diagnosis through neonatal screening,\(^1\) we have focused on methods for improved detection of *P aeruginosa*-associated respiratory tract infections. Our studies are being pursued under the assumption that early identification of *P aeruginosa* is essential to achieve success in treating or eradicating this organism before irreversible lung disease occurs. It has been especially advantageous to evaluate our study patients longitudinally with a systematic protocol for collecting data prospectively.\(^1,3\) Since our patients were generally diagnosed with CF through newborn screening (median age of confirmed diagnosis = 6.8 weeks) and the majority had negative *P aeruginosa* cultures and WCXR scores less than 5 at the time of entry into the study, they were considered to be free of detectable *P aeruginosa* at that time. The observation that the mean magnitude of the titers for the initial plateau for cell lysate, exotoxin A, and elastase of 1:16 or less confirms this assumption and conclusion (Table 2). Thus, this investigation differs significantly from previous longitudinal studies assessing *P aeruginosa* acquisition and production of an anti-*P aeruginosa* antibody response\(^1,11,12,18,20,25,27,44\) in which the patients were investigated after a CF diagnosis based on clinical indicators or were followed up for a short time. Additionally, as a result of newborn screening and early nutritional intervention, the patients in our study were well nourished.\(^1,30\)

We characterized the production of serum antibodies directed against *P aeruginosa* in patients with CF and detected a temporal hierarchy in the development of antibodies against specific *P aeruginosa* antigens: exotoxin A before cell lysate before elastase. Similarly, Hollsing et al\(^27\) observed that antibodies against exotoxin A and phospholipase C were produced before antibodies against elastase and alkaline protease. This temporal difference in antibody response to specific antigens could reflect the ability of the tested antigens to act as immunogens or alternatively represents a sequential production of these antigens during the establishment of *P aeruginosa*-associated pulmonary infections.\(^43-48\)

Specifically, *P aeruginosa* converts from a nonmucoid to a mucoid state, modifies the composition of lipopolysaccharide, produces decreased amounts of elastase, and loses the ability to produce flagella during the establishment of the infection. It has been postulated that mucoid strains are more difficult to eradicate, and this phenomenon is a hallmark of progressive CF lung disease.\(^44\) Thus, early detection of *P aeruginosa* is essential for successful chemoprophylaxis and treatment of infections.

Figure 3. Kaplan-Meier Analysis for All Culture-Screened Patients

Comparison of the longitudinal relationships for first acquisition of *Pseudomonas aeruginosa* from an oropharyngeal culture, first occurrence of cell lysate or exotoxin A titer greater than or equal to 1:256, and first report of a WCXR (Wisconsin Cystic Fibrosis Radiograph) score greater than or equal to 5. Small cross-marks indicate censored events.

### Table 3. Estimates of Significant Risk Factors Associated by Survival Analysis With Antibody Titer ≥1:256

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cell Lysate</th>
<th>Exotoxin A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Center B</td>
<td>2.57 (1.34-4.91)</td>
<td>.004</td>
</tr>
<tr>
<td>Original physician†</td>
<td>0.53 (0.26-1.10)</td>
<td>.09</td>
</tr>
<tr>
<td>Oral antibiotic use‡</td>
<td>1.87 (1.01-3.45)</td>
<td>.046</td>
</tr>
<tr>
<td>Parents’ estimated income§</td>
<td>. . .§</td>
<td>. . .§</td>
</tr>
<tr>
<td>Upper respiratory tract infections¶</td>
<td>0.95 (0.90-0.99)</td>
<td>.04</td>
</tr>
</tbody>
</table>

\(^*\)OR indicates odds ratio; CI, confidence interval.

† Treatment was provided by a single physician at center B prior to June 1, 1990.

‡ Use of long-term (>30 days) oral non-*Pseudomonas aeruginosa* antibiotics.

§ Estimated from the median annual income (in units of $10 000) for the ZIP code of the parents’ residence.

¶ Not significant, so did not remain in the final model.

∥ Total number of upper respiratory tract infections; these were assumed to be of viral etiology.
of a \(P\) aeruginosa–associated pulmonary infection in CF patients.\textsuperscript{45–48} In a previous study, we showed that the magnitude of the elastase response is predictive of a more advanced infection.\textsuperscript{3} Thus, this temporal hierarchy in development of antibodies to specific \(P\) aeruginosa antigens may reflect the development of lung disease. It is noteworthy that patients with CF younger than 9 months developed cell lysate and exotoxin A titers of 1:256 or higher before \(P\) aeruginosa was isolated from an oropharyngeal culture. This finding agrees with recent observations on the early development of \(P\) aeruginosa–associated pulmonary infections from serial bronchoscopy studies.\textsuperscript{17}

Comparing the temporal relationships of several clinical indicators of \(P\) aeruginosa–associated pulmonary infections revealed that a rise in antibody titers to 1:256 or greater directed against exotoxin A and cell lysate occurred after the first report of significant cough and correlated with a WCXR score greater than or equal to 5. Even though \(P\) aeruginosa frequently had not been isolated from an oropharyngeal culture at this time, the combination of a rise in antibody titer to 1:256 or greater and these clinical indicators of pulmonary infection indicates the presence of the organism. Because of their noninvasive nature, oropharyngeal cultures have been recommended and are routinely used for diagnosis of \(P\) aeruginosa–associated respiratory tract disease.\textsuperscript{49} However, because conflicting results have been obtained regarding the predictability of oropharyngeal cultures in the diagnosis of \(P\) aeruginosa–associated disease,\textsuperscript{12,14,18,25} the ability to detect an antibody titer against exotoxin A and cell lysate suggests that an enzyme-linked immunosorbent assay to detect antibodies directed against specific \(P\) aeruginosa antigens, in conjunction with a WCXR score greater than or equal to 5, could be used to diagnose \(P\) aeruginosa–associated pulmonary infections in young patients with CF.

The diagnostic strategy of longitudinal antibody titer monitoring would be better for children than recurrent bronchoscopy. Additionally, the diagnosis of \(P\) aeruginosa–pulmonary infection could be made 6 to 12 months before the organism is isolated from oropharyngeal and perhaps bronchoalveolar lavage cultures in a majority of patients. Other investigators have suggested the utility of serum antibody titers against the organism for the diagnosis of \(P\) aeruginosa–associated pulmonary infections.\textsuperscript{24,27} The potential presence of \(P\) aeruginosa in lower respiratory tract infections has implications for both the time at which treatment is begun and also for the choice of antimicrobial agents that are used. For example, treatment with non–\(P\) aeruginosa antibiotics could either predispose a patient to a \(P\) aeruginosa lung infection, as our data suggest (Table 3), or could exacerbate an undiagnosed lung infection caused by the organism. In support of this conclusion, Holby and colleagues\textsuperscript{44,50,51} have shown that early intervention with appropriate antibiotics can eradicate nonmucoid \(P\) aeruginosa from the lungs of patients with CF.

Assessment of factors that can potentially increase the risk of \(P\) aeruginosa–associated pulmonary infection was possible in this study because we have available in our database life history information collected prospectively from the date of CF diagnosis. Our previous evaluations of potential risk factors for \(P\) aeruginosa–associated pulmonary disease\textsuperscript{3} were based solely on positive cultures and were limited by the sensitivity of isolation of the organism from oropharyngeal secretions. In addition, it is obvious from the data reported herein that the organism may not be isolated until after respiratory disease supervenes. Therefore, our current evaluation of covariates using titers greater than or equal to 1:256 as the outcome event is much better than our earlier studies using only oropharyngeal cultures as the outcome event. Our previous research, however, identified 20 potential factors that we focused on with respect to \(P\) aeruginosa–associated pulmonary infection risk.

The results of the stepwise regression model used in this study confirm the previous finding of a center effect: from 1985 to 1990 (when half the screened patients were diagnosed), center B used an old clinic setting with a small waiting room that mixed young patients diagnosed through screening with older infected patients with CF, as described in detail previously; also, there was only 1 physician and limited handwashing facilities. In contrast, center A employed a segregated clinic with isolated waiting areas and 3 physicians. Our finding in this study of a strong center effect with significantly more risk of \(P\) aeruginosa–associated pulmonary infection among the patients followed up in an integrated clinic provides more evidence for the concept of cross-infection, ie, person-to-person transmission of the organism, and the potential value of segregated clinics. This strategy of care is used with good results in Denmark and should be considered by every region embarking on neonatal CF screening programs. Indeed, clinics dedicated specifically to infants and young children with CF offer many advantages as an integral component of the follow-up system needed for a successful newborn screening program.\textsuperscript{1} Both nutritional and pulmonary care can be delivered in a highly effective fashion when very specialized treatment plans are provided to young children with CF, rather than integrating them with older patients.\textsuperscript{1} Communication and psychosocial advantages also emerge in such a setting.\textsuperscript{53}

The other significant risk factor we found was long-term (>30 days) non-\(Pseudomonas\) antibiotic administration taken orally as a prophylactic measure in a manner that has become common for many CF centers.\textsuperscript{30,54–56} Other studies with less precise outcome measures have also suggested this risk.\textsuperscript{37} In addition, a recent controlled trial involving 209 children (average age <15 months), 119 of whom completed continuous therapy (either daily cephaloxin or placebo), demonstrated a significantly increased risk of cultures positive for \(P\) aeruginosa. Our study also shows that long-term use of non-\(Pseudomonas\) antibiotics is an especially significant risk for young CF patients. Indeed this is the first epidemiologic in-
vestigation, to our knowledge, to link the oral antibiotic risk factor directly to *P. aeruginosa*-associated pulmonary infection, as opposed to colonization by the organism identified by culturing respiratory secretions. Because the risk of long-term, non-*Pseudomonas* oral antibiotics in young children with CF might outweigh the potential benefits, we recommend a more prudent approach based on a careful comprehensive determination of each patient's respiratory status, especially in the care of infants and young children with CF. The longitudinal monitoring of *P. aeruginosa* antibody titers should be helpful in this ongoing assessment.

The implications of the Wisconsin CF Neonatal Screening Project are profound for the treatment of young children with this genetic disorder. Early diagnosis through neonatal screening provides an opportunity for a transformation in treatment strategy. Instead of hospitalizations and medical interventions for sick patients who experience delayed diagnosis, screening allows presymptomatic identification of most patients with CF and the potential for prospective evaluation and preventive care. It has been demonstrated conclusively that malnutrition can be prevented in association with screening. Similarly, since the CF lung is free of pathology at birth, early diagnosis coupled with careful follow-up of the respiratory symptoms (especially cough), infection status, and chest radiographs could provide better outcomes, as already suggested by some observational studies. We recommend that longitudinal *P. aeruginosa* serology also become part of the respiratory care follow-up, especially using exotoxin A titers. In addition, therapeutic trials with anti-*Pseudomonas* antibodies are needed in young children with CF when they first show an increased anti-*P. aeruginosa* antibody titer.

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No one can be a great thinker who does not recognize, that as a thinker it is his first duty to follow his intellect to whatever conclusions it may lead.

—John Stuart Mill (1806-1873)